A screening study investigating the presence of Emerging Contaminants within the Ohio River Basin

The Ohio River Valley Water Sanitation Commission
(ORSANCO)

and

United States Environmental Protection Agency
Office of Research and Development
National Risk Management Research Laboratory

A screening study investigating the presence of Emerging Contaminants within the Ohio River Basin

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Executive Summary

Background: Recent improvements in analytical detection capabilities and the availability of these methods to researchers via commercial labs have now enabled water resource managers to conduct studies such as this on broader spatial scales and on a wider range of compounds. Analytes are now being detected and quantified in the part per trillion (ng/L) range. In 2002, ORSANCO's Research Committee identified Contaminants of Emerging Concern (CECs) as a top research priority. Research has demonstrated there are many sources of CECs to the environment, including wastewater treatment plants (WWTPs), confined animal feeding operations (CAFOs), industrial discharges, etc. It has been shown that WWTPs are not currently designed to remove these chemicals to the very low levels, nor are they required by regulatory agencies to do so. Therefore, we anticipated finding detectable levels of CECs in the Ohio River at locations below possible sources and potentially at background areas.

To date, little information is available regarding possible human health risk, however, it is more generally accepted that risks have been demonstrated for aquatic species exposed to these compounds. Understanding possible ecological risks was considered a primary purpose for the Commission's research efforts on CECs. In 2005, ORSANCO collaborated with USEPA to conduct a pilot study on the Ohio River. This study targeted a limited list of endocrine disrupting chemicals which included steroid hormones and alkylphenolic compounds. Methods employed during the pilot study enabled researchers to detect the presence of steroid hormones, but quantification was not possible. It was determined that future efforts should include a broader list of chemicals and provide reportable quantities.

Study Overview: In September and October, 2009, single grab samples were collected from 22 locations on the mainstem Ohio River and the lower reaches of tributaries. Target analytes included 158 compounds considered to be contaminants of emerging concern or emerging contaminants.

- 118 - Pharmaceutical and Personal Care Products (PPCPs)
- 27 - Hormones and Sterols
- 13 - Perfluorinated Compounds (PFCs)

This study was designed to generate data as preliminary survey by the Commission and results would be used to guide future actions.

Practical Limitations of Study:

Though this study utilized the state of the art analytical approaches, practical limitations to the study could restrict the data utility and conclusions that can be drawn. The analytical laboratory methods employed are research or draft EPA methods. For PFCs, no EPA standard

method for surface waters is currently available. However, draft EPA methods 1694 (PPCPs) and 1698 (Hormones and Steroids) have been released to the public as single-laboratory validated methods. It should be noted that the lab that worked with EPA to develop the draft methods (1694 and 1698), the single lab that has been validated, and the lab utilized for this study are one and the same.

Due to the spatial and temporal limits of the study approach, it may not be possible to quantify loadings, identify or characterize sources, or characterize downstream fate and transport. The collection of single grab samples does not allow for temporal characterizations or statistical analysis at individual sites.

Data Use:

This report will be used by ORSANCO Committees, Sub-Committees, Advisory Committees and Workgroups. These groups will provide advice and guidance to the Commission regarding warranted future studies or actions. Additionally, ORSANCO and EPA staff will collaborate on a more thorough data summary/evaluation and report.

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1.0 Introduction

Marked improvements in analytical detection capabilities over the last 15 years has led to the quantitative and qualitative characterization of a variety of organic chemicals occurring at trace levels in surface waters, treated wastewater effluents, drinking water supplies, and fish tissue (Boyd and Furlong 2002; Kolpin et al. 2002; Galloway et al. 2005; Zimmerman 2005; Benotti et al. 2006; Lower Columbia River Estuary Partnership 2007). Compounds considered to be *Contaminants of Emerging Concern* (CECs), such as pharmaceuticals and personal care products (PPCPs), alkylphenols (APs), steroid hormones, and perfluoroalkyl compounds (PFCs), in U.S. waterways have been of increasing public concern (Richardson, 2008). The extent and persistence of their occurrence in surface waters of the United States remains unclear. Though there are many sources of these contaminants, research has focused on such sources as waste water treatment plants (WWTPs) and concentrated animal feeding operations (CAFOs). Kolpin et al. (2002) surveyed 139 surface waters downstream of major municipal, industrial, or agricultural centers across 30 states for 95 organic contaminants including natural and synthetic steroids. They found that steroid hormones were present at varying levels in 86% of the streams surveyed.

A national survey conducted by U.S. EPA's National Exposure Research Laboratory (NERL) of 50 WWTP aqueous effluents, including several along the Ohio River, found 26% of all surveyed effluents contained levels of estrogenic endocrine disrupting activity at levels high enough to induce vitellogenesis in laboratory-exposed male fathead minnows (Lazorchak & Smith 2004). However, these were grab samples and subject to changes in composition depending on variations in influent characteristics, therefore these results may not provide an accurate estimate as to the temporal and spatial extent of the problem. Regardless, these studies provided valuable information about the presence of CECs in surface waters, however; the longitudinal occurrence and concentrations of these compounds within a large river system are unknown. Identifying CECs and their concentrations in surface waters of the Ohio River and its tributaries is essential to protecting public health and aquatic life.

ORSANCO's research strategy for CECs originally was, and continues to be, focused on screening for these compounds within the Ohio River basin to document the occurrences and potentially shape future studies. The detection of CECs was expected when designing this study due to the analytical capabilities at such low concentrations (ng/L or parts per trillion). However, at present, limited evidence is available to conclude that the occurrences of these compounds in the environment pose a causal risk to human health. While pharmaceuticals have been found in U.S. drinking waters, there is no evidence of human health risk from consumption of these waters (AwwaRF Study Project #3085; USEPA www.epa.gov/ppcp/faq.html#iftherereareindeed).

Furthermore, there are no state or national water quality standards established for the compounds targeted in this study (Section 2.3) in U.S. surface waters. However, there are USEPA Provisional Health Advisories for PFOS and PFOA in Drinking Water (200 ng/L and 400 ng/L, respectively) and a New Jersey Department of Environmental Protection drinking-water guidance value for PFOA (40 ng/L). It should also be noted that water utilities are not designed, nor mandated to monitor or treat these chemicals.

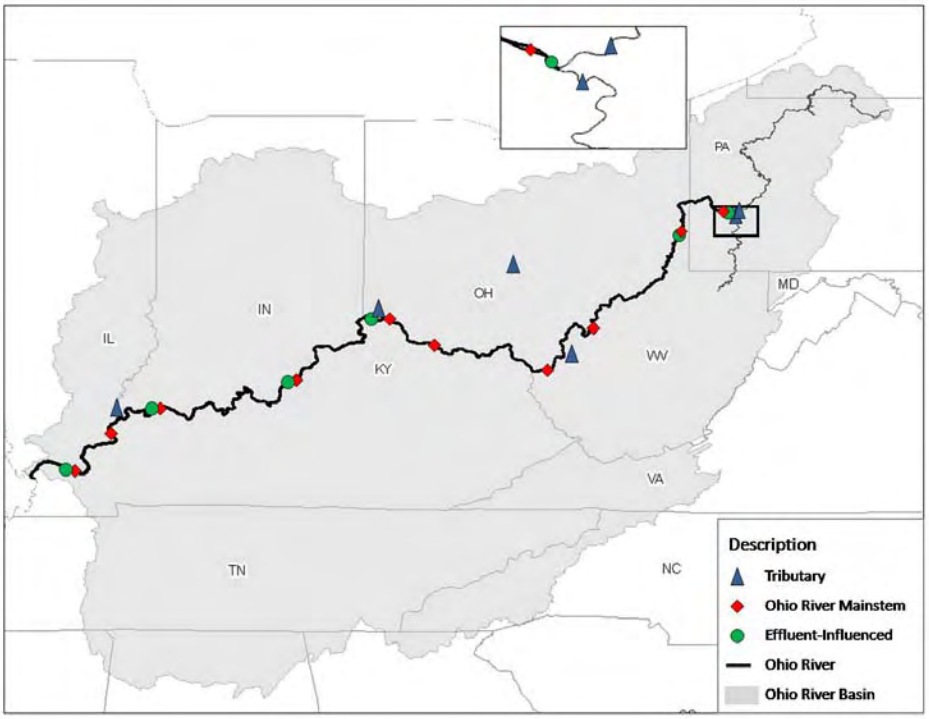
The primary focus of this study is to document the occurrence and concentrations of CECs in the mainstem of the Ohio River and six of its tributaries for the purpose of expanding current knowledge and guiding future studies.

2.0 Methods

2.1 Study Design

Twenty-two locations were targeted for sampling. Sampling sites were located on the mainstem upstream of major urban areas, located in visible effluent plumes (effluent influenced; EI) or fell within areas of the river that had relatively few local upstream (potential) sources (Table 1; Figure 1). Additionally, tributaries were sampled to characterize other sources to the mainstem.

Figure 1: Map showing sampling locations



Effluents were chosen based on discharge volume and those with easily identifiable surface discharges were given priority. By selecting effluents with surface discharges, we were able to sample close to the end-of-pipe, which represents a 'worst-case-scenario' for aquatic organisms in the receiving waters. Tributaries were selected based on a cursory examination of upstream landuse characteristics, upstream industry/urban concentrations or other suspected potential influences.

It should be noted that when samples were collected in effluent plumes the samples were collected close to the end of the pipe in the mainstem Ohio River. Samples were not taken directly from the effluent flow. Additionally, some effluents appeared to be more fully mixed than others as some were surface discharges and some were submerged.

All other sites were established so as to provide a representative longitudinal distribution and were generally situated upstream of major urban areas or in areas of the river with few immediate upstream influences.

Table 1: Sample numbers and general location information.

Sample Number	Location	River	River Mile*	Date
1	Tributary	Allegheny	9.5	9/14/2009
2	Tributary	Monongahela	5.0	9/14/2009
3	Ohio River Mainstem (EI)	Ohio	3.1	9/14/2009
4	Ohio River Mainstem	Ohio	9.7	9/14/2009
5	Ohio River Mainstem (FB)	Ohio	462.9	9/16/2009
6	Ohio River Mainstem	Ohio	86.7	9/14/2009
7	Ohio River Mainstem (EI)	Ohio	91.3	9/14/2009
8	Ohio River Mainstem	Ohio	220.3	9/15/2009
9	Tributary	Kanawha	4.1	9/15/2009
10	Ohio River Mainstem	Ohio	306.8	9/15/2009
11	Tributary	Scioto	105.8	10/20/2009
12	Ohio River Mainstem	Ohio	394.9	9/15/2009
13	Ohio River Mainstem (FB)	Ohio	220.3	9/15/2009
14	Ohio River Mainstem	Ohio	462.9	9/16/2009
15	Ohio River Mainstem (EI)	Ohio	478.0	9/16/2009
16	Tributary	Mill Creek	0.2	9/16/2009
17	Ohio River Mainstem	Ohio	600.5	9/21/2009
18	Ohio River Mainstem (EI)	Ohio	612.2	9/21/2009
19	Ohio River Mainstem	Ohio	791.5	9/21/2009
20	Ohio River Mainstem (EI)	Ohio	791.8	9/21/2009
21	Tributary	Wabash	0.8	9/21/2009
22	Ohio River Mainstem	Ohio	889.1	9/21/2009
23	Ohio River Mainstem	Ohio	934.4	9/22/2009
24	Ohio River Mainstem (EI)	Ohio	935.9	9/22/2009
25	Tributary (FB)	Scioto	105.8	10/20/2009
26	PFC Field Spike 1 (Low)	Scioto	105.8	10/20/2009
27	PFC Field Spike 2 (High)	Scioto	105.8	10/20/2009

* - Tributary river miles are assigned by the number of miles upstream of the confluence with the Ohio River proper.

FB - Field blank taken at this location

EI - Effluent influenced

2.2 Sample Collection

All locations were sampled in September (2009) with the exception of one location (sample 11) that was sampled later in mid-October (Table 1). All water samples (with the exception of sample 11) were collected from the bow of the boat as it faced the upstream direction. Sample 11 was collected on a shallow tributary that was accessed without a boat. Personnel collecting the sample waded into the water and collected a sample by submerging the sampling device upstream of their position.

A water sampling device was fabricated using aluminum and stainless steel materials capable of holding two new/clean 2.5-liter amber glass bottles (silanized; without preservative; Figure 2). The 2 bottles were secured to the sampling device using stainless steel spring-loaded chains. Bottles were quickly submerged in an upright position to a depth of 3 feet below the water's surface and allowed to completely fill. Once full, the sampling device was raised to the surface, amber bottles were unsecured from the sampling device, and then the water was transferred to all the appropriate sample bottles (Figure 3). The sample bottles contained appropriate preservatives according to each analytical method specification and immediately capped. All sample bottles from each location were then placed into a cooler with wet ice. Samples were maintained at <4°C following sample collections and during overnight shipment to the contractual laboratory for analyses. In the event that samples collected and shipped the same day did not have sufficient time to cool down to 4°C, the lab cooled the samples prior to processing. All samples were received by the laboratory within holding time limitations (7 days). Due to the sensitivity of the analytical analyses (in parts per trillion) and the uncertainty of potential contamination by the samplers, nitrile gloves and face masks were worn during all sample collections. At each location, the sampling device was decontaminated (using methanol), allowed to dry, and new amber bottles were installed and readied for use at the next sampling site.

Figure 2. Sampling device.

Figure 3. Sample transfer.

2.3 Target Analytes

Target analytes included 158 contaminants of emerging concern (PPCPs, hormones/sterols, and PFCs; Table 2).

Table 2. Analytes targeted for Ohio River emerging contaminants screening study.

Compound	Category	Use(s)	Other name/Abbr.
Perfluorinated compounds (PFCs): AXYS Analytical MLA-060			
PFBA	Manufacturing	Stain/greaseproof coating	Perfluorobutanoate
PFPeA	Manufacturing	Stain/greaseproof coating	Perfluoro-n-pentanoic acid
PFHxA	Manufacturing	Stain/greaseproof coating	Perfluorohexanoic acid
PFHpA	Manufacturing	Stain/greaseproof coating	Perfluoroheptanoic acid
PFOA	Manufacturing	Stain/greaseproof coating	Perfluorooctanoic acid
PFNA	Manufacturing	Stain/greaseproof coating	Perfluorononanoic acid
PFDA	Manufacturing	Stain/greaseproof coating	Perfluorodecanoic acid
PFUnA	Manufacturing	Stain/greaseproof coating	Perfluoroundecanoic acid
PFDoA	Manufacturing	Stain/greaseproof coating	Perfluorododecanoic acid
PFBS	Manufacturing	Stain/greaseproof coating	Perfluorobutane sulfonate
PFHxS	Manufacturing	Stain/greaseproof coating	Perfluorohexanesulfonate
PFOS	Manufacturing	Stain/greaseproof coating	Perfluorooctanesulfonate
PFOSA	Manufacturing	Stain/greaseproof coating	Perfluorooctanesulfonic acid
Pharmaceuticals and personal care products (PPCPs): Draft EPA Method 1694			
Acetaminophen	Pharmaceutical	Analgesic & fever reducer	Paracetamol
Albuterol	Pharmaceutical	Bronchodilator	Salbutamol
Alprazolam	Pharmaceutical	Anti-anxiety	
Amitriptyline	Pharmaceutical	Antidepressant	
10-Hydroxy-amitriptyline	Metabolite	Amytriptyline metabolite (antidepressant)	
Amlodipine	Pharmaceutical	Calcium channel blocker	
Amphetamine	Pharmaceutical	Stimulant	
Atenolol	Pharmaceutical	Hypertension, angina	
Atorvastatin	Pharmaceutical	Lowers blood cholesterol	
Azithromycin	Pharmaceutical	Antibiotic	
Benzoylecgonine	Metabolite	Cocaine metabolite	
Benztropine	Pharmaceutical	Acetylcholine blocker	
Betamethasone	Pharmaceutical	Steroid, anti-inflammatory	

Compound	Category	Use(s)	Other name/Abbr.
Bisphenol A	Manufacturing	Plastics, synthetics	BPA
Caffeine	Pharmaceutical	Stimulant	
Carbadox	Pharmaceutical	Veterinary antibiotic - fights parasites	
Carbamazepine	Pharmaceutical	Anticonvulsive, mood stabilizer	
Cefotaxime	Pharmaceutical	Antibiotic	
Cimetidine	Pharmaceutical	Antacid	
Ciprofloxacin	Pharmaceutical	Antibacterial	
Clarithromycin	Pharmaceutical	Antibiotic	
Clinafloxacin	Pharmaceutical	Antibiotic	
Clonidine	Pharmaceutical	Hypertension, ADHD	
Cloxacillin	Pharmaceutical	Antibiotic	
Cocaine	Pharmaceutical	Stimulant	
Codeine	Pharmaceutical	Analgesic, antitussive	Methylmorphine
Cotinine	Metabolite	Nicotine byproduct	
DEET	Manufacturing	Insect repellant	
Dehydronifedipine	Metabolite	Nifedipine metabolite	
Desmethyldiltiazem	Metabolite	Diltiazem metabolite (calcium channel blocker)	
Diazepam	Pharmaceutical	Anti-anxiety	
Digoxigenin	Pharmaceutical	Steroid	
Digoxin	Pharmaceutical	Cardiac Glycoside	
Diltiazem	Pharmaceutical	Calcium channel blocker	
1,7-Dimethylxanthine	Pharmaceutical	Bronchodilator	Theophylline
Diphenhydramine	Pharmaceutical	Antihistamine	
Enalapril	Pharmaceutical	Hypertension	
Enrofloxacin	Pharmaceutical	Antibiotic	
Erythromycin-H2O	Pharmaceutical	Antibiotic	
Flumequine	Pharmaceutical	Antibiotic	
Fluocinonide	Pharmaceutical	Steroid, anti-inflammatory	
Fluoxetine	Pharmaceutical	Antidepressant	
Fluticasone propionate	Pharmaceutical	Steroid, asthma	

Compound	Category	Use(s)	Other name/Abbr.
Furosemide	Pharmaceutical	Edema, congestive heart failure	Frusemide
Gemfibrozil	Pharmaceutical	Antihyperlipidemic	
Glipizide	Pharmaceutical	Anti-Diabetic	
Glyburide	Pharmaceutical	Anti-Diabetic	Glibenclamide
Hydrochlorothiazide	Pharmaceutical	Diuretic	HCTZ, HCT, HZT
Hydrocodone	Pharmaceutical	Antitussive, analgesic	
Hydrocortisone	Pharmaceutical	Synthetic cortisol (arthritis)	
Ibuprofen	Pharmaceutical	Anti-inflammatory	
2-Hydroxy-ibuprofen	Metabolite	Ibuprofen metabolite	
Lincomycin	Pharmaceutical	Antibiotic	
Lomefloxacin	Pharmaceutical	Antibiotic	
Meprobamate	Pharmaceutical	Anti-anxiety	
Metformin	Pharmaceutical	Anti-Diabetic	
Methylprednisolone	Pharmaceutical	Steroid, anti-inflammatory	
Metoprolol	Pharmaceutical	Beta receptor blocker (hypertension)	
Miconazole	Pharmaceutical	Antifungal	
Naproxen	Pharmaceutical	Anti-inflammatory	NSAID
Norfloxacin	Pharmaceutical	Antibiotic	
Norfluoxetine	Metabolite	Fluoxetine metabolite (antidepressant)	
Norgestimate	Pharmaceutical	Hormonal contraceptives	
Norverapamil	Pharmaceutical	Calcium channel blocker	
Ofloxacin	Pharmaceutical	Antibiotic	
Ormetoprim	Pharmaceutical	Antibiotic	
Oxacillin	Pharmaceutical	Antibiotic	
Oxolinic Acid	Pharmaceutical	Antibiotic	
Oxycodone	Pharmaceutical	Analgesic	
Paroxetine	Pharmaceutical	Antidepressant	Paxil
Penicillin G	Pharmaceutical	Antibiotic	
Penicillin V	Pharmaceutical	Antibiotic	
Prednisolone	Metabolite	Prednisone metabolite (steroid)	

Compound	Category	Use(s)	Other name/Abbr.
Prednisone	Pharmaceutical	Steroid (immunosuppressant)	
Promethazine	Pharmaceutical	Antihistamine	
Propoxyphene	Pharmaceutical	Analgesic, antitussive	Dextropropoxyphene
Propranolol	Pharmaceutical	Beta receptor blocker (hypertension)	
Ranitidine	Pharmaceutical	Antacid	
Roxithromycin	Pharmaceutical	Antibiotic	
Sarafloxacin	Pharmaceutical	Antibiotic	
Sertraline	Pharmaceutical	Antidepressant	
Simvastatin	Pharmaceutical	Hypercholesterolemia	
Sulfachloropyridazine	Pharmaceutical	Antibiotic	
Sulfadiazine	Pharmaceutical	Antibiotic	
Sulfadimethoxine	Pharmaceutical	Antibiotic	
Sulfamerazine	Pharmaceutical	Antibiotic	
Sulfamethazine	Pharmaceutical	Antibiotic	
Sulfamethizole	Pharmaceutical	Antibiotic	SMX
Sulfamethoxazole	Pharmaceutical	Antibacterial	
Sulfanilamide	Pharmaceutical	Antibacterial	
Sulfathiazole	Pharmaceutical	Sulfa drug	
Anhydrochlortetracycline [ACTC]	Pharmaceutical	Antibiotic	
Anhydrotetracycline [ATC]	Pharmaceutical	Antibiotic	
Chlortetracycline [CTC]	Pharmaceutical	Antibiotic	
Demeclocycline	Pharmaceutical	Antibiotic	
Doxycycline	Pharmaceutical	Antibiotic	
4-Epianhydrochlortetracycline [EACTC]	Pharmaceutical	Antibiotic	
4-Epianhydrotetracycline [EATC]	Pharmaceutical	Antibiotic	
4-Epichlortetracycline [ECTC]	Pharmaceutical	Antibiotic	
4-Epioxytetracycline [EOTC]	Pharmaceutical	Antibiotic	
4-Epitetracycline [ETC]	Pharmaceutical	Antibiotic	
Isochlortetracycline [ICTC]	Pharmaceutical	Antibiotic	
Minocycline	Pharmaceutical	Antibiotic	

Compound	Category	Use(s)	Other name/Abbr.
Oxytetracyclin [OTC]	Pharmaceutical	Antibiotic	
Tetracycline [TC]	Pharmaceutical	Antibiotic	
Theophylline	Pharmaceutical	COPD, asthma	Dimethylxanthine
Thiabendazole	Pharmaceutical	Fungicide and parasiticide	TBZ
Trenbolone	Pharmaceutical	Steroid	
Trenbolone acetate	Pharmaceutical	Refined form of Trenbolone	
Triamterene	Pharmaceutical	Diuretic, hypertension, edema	
Triclocarban	Personal Care Product	Disinfectant	TCC
Triclosan	Personal Care Product	Disinfectant	
Trimethoprim	Pharmaceutical	Antibiotic	
Tylosin	Pharmaceutical	Antibiotic	
Valsartan	Pharmaceutical	High blood pressure, congestive heart failure	
Verapamil	Pharmaceutical	Calcium channel blocker	
Virginiamycin	Pharmaceutical	Antibiotic	
Warfarin	Pharmaceutical	Anticoagulant	
Hormones and sterols: Draft EPA Method 1698			
Androstenedione	Hormone	Androgenic hormone	
Androsterone	Hormone	Androgenic hormone	
Desogestrel	Pharmaceutical	Used in hormonal contraceptives	
17 alpha-Dihydroequilin	Metabolite	Horse estrogenic hormone	
Equilenin	Hormone	Horse steroid hormone	
Equilin	Hormone	Horse estrogenic hormone	
beta-Estradiol 3-benzoate	Hormone	Estrogenic hormone	
17 alpha-Estradiol	Hormone	Estrogenic hormone	
17 beta-Estradiol	Hormone	Estrogenic hormone	
17 alpha-Ethinyl-Estradiol	Hormone	Estrogenic hormone	E2
Estriol	Hormone	Estrogenic hormone	
Estrone	Hormone	Estrogenic hormone	
Mestranol	Hormone	Estrogenic hormone	

Compound	Category	Use(s)	Other name/Abbr.
Norethindrone	Pharmaceutical	Used in hormonal contraceptives	Norethisterone
Norgestrel	Pharmaceutical	Progestin used in hormonal contraceptives	
Progesterone	Hormone	Estrogenic hormone	P4
Testosterone	Hormone	Androgenic hormone	
Coprostanol	Sterol	Cholesterol metabolite	
Epicoprostanol	Sterol	Cholesterol metabolite	
Cholesterol	Sterol	Steroid metabolite	
Cholestanol	Sterol	Cholesterol metabolite	
Desmosterol	Sterol	Steroid metabolite	
Ergosterol	Sterol	Fungal sterol	
Campesterol	Sterol	Plant sterol	
beta-Sitosterol	Sterol	Plant sterol	
Stigmasterol	Sterol	Plant sterol	
beta Stigmastanol	Sterol	Plant sterol	

2.4 Analytical Methods

After proper preservation, samples were sent to the contract laboratory, AXYS Analytical, for extraction and analysis. The following three methods were used to measure three classes of target analytes.

- AXYS Method MLA-060 for PFCs (Appendix 1)
  AXYS used its own internal method, MLA-060, for PFC analysis. Thirteen PFCs were analyzed by high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). The MLA-060 did not describe sample handling methods including sample collection, storage, and preservation so the field personnel used the sample container that had been sent to ORSANCO from AXYS and followed AXYS' instruction (no preservatives and ice chilling). Since this sample handling method was deviated from the EPA's SOP EMAB-113-0 "Sample Collection Protocol for PFCs in Surface and Well Water", the EPA researcher recommended ORSANCO to prepare two levels of field spikes and ship them to AXYS for analysis in order to evaluate possible loss during the sample storage and transport.
- AXYS Method MLA-075 for PPCPs (Appendix 1)
  This method is technically equivalent to the EPA Draft Method 1964 released in 2007. The Method 1964 is a single laboratory validated method. On-going precision and recoveries (OPRs) were calculated and demonstrated in the MLA-075. A total of 118 PPCP analyte were measured using HPLC–MS/MS.
- AXYS Method MLA-068 for Hormones/Sterols (Appendix 1)
  AXYS' internal method MLA-068 is technically equivalent to the EPA draft Method 1698 which is a single laboratory validated method. A total of 27 hormones and sterols were analyzed by gas chromatography–high resolution mass spectrometry (GC–HRMS).

3.0 Data Validation

3.1 Quality Assurance Review

Independent quality assurance (QA) reviews of the analytical data and final data report were performed by USEPA and ORSANCO QA managers.

3.1.1 USEPA Memo

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL RISK MANAGEMENT RESEARCH LABORATORY
CINCINNATI, OH 45268

OFFICE OF
RESEARCH AND DEVELOPMENT

EPA Independent Review of the Ohio River Data Packages

May 20, 2010

MEMORANDUM

Subject: Data Validation Report for the Screening Study Investigating the Presence of Emerging Contaminants within the Ohio River Basin

From: Marc Mills, Environmental Engineer
National Risk Management Research Laboratory, Office of Research and Development, USEPA

To: Erich Emery, Project Manager
Ohio River Valley Water Sanitation Commission

CC: Laurel Staley, QA Manager, LRPCD
Dennis Timberlake, Chief, SSMB
National Risk Management Research Laboratory, Office of Research and Development, USEPA

The quality assurance (QA) review of the analytical data of surface water samples collected from 22 locations within the Ohio River Basin has been completed. These samples were analyzed for perfluorinated alkyl compounds (PFCs), pharmaceuticals and personal care products (PPCPs), and hormones and sterols (H&S). The analyses were performed by Axys Analytical Services, Ltd. (Axys) located in Sidney, BC Canada in accordance with the Axys Method MLA-060 Rev 09 for PFCs; Axys Method MLA-075 for PPCPs, modification to USEPA Draft Method 1694 *"Pharmaceuticals and Personal Care Products in Water, soil, Sediment and Bio-solids by HPLC/MS/MS"*; and Axys Method MLA-068 for H&S, modification to Draft Method 1698 *"Steroids and Hormones Water, soil, Sediment and Bio-solids by GC/HR/MS"*. The entire sample analyses were validated following the specification of the methods cited, the Axys' internal methods, the USEPA National Guidelines for Organic Data Review, and the USEPA's *Guidance for Labelling Externally Validated Laboratory Analytical data for Superfund Use, EPA 540-R-08-05, January 2009*.

The following samples were evaluated in this validation report:

Table 1: Sample number and dates of collection, extraction and analysis

				PFCs Analysis		PPCPs Analysis		Hormones and Sterols Analysis	
ORSANCO Sample No.	Axys Sample No.	Collection Dates	Sample Reception Dates	Extraction Dates	Analysis Dates	Extraction Dates	Analysis Dates	Extraction Dates	Analysis Dates
1	L13550-1	9/14/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/3/09
2	L13550-2	9/14/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/4/09
3	L13550-3	9/14/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/4/09
4	L13550-4	9/14/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/4/09
5	L13550-5	9/16/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/5/09
6	L13550-6	9/14/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/4/09
7	L13550-7	9/14/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/4/09
8	L13550-8	9/15/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/4/09
9	L13550-9	9/15/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/4/09
10	L13550-10	9/15/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/5/09
11	L13813-1	10/20/09	10/22/09	10/28/09	10/30/09	10/27/09	11/5/09	10/29/09	11/5/09
12	L13550-11	9/15/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	9/21/09	10/2/09
13	L13550-12	9/15/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/5/09
14	L13550-13	9/16/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/5/09
15	L13550-14	9/16/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/5/09
16	L13550-15	9/16/09	9/17/09	9/28/09	9/30/09	10/2/09	10/7/09	10/29/09	11/5/09
17	L13603-1	9/21/09	9/23/09	9/30/09	10/1/09	10/2/09	10/6/09	9/25/09	10/2/09
18	L13603-2	9/21/09	9/23/09	9/30/09	10/1/09	10/2/09	10/6/09	9/25/09	10/2/09
19	L13603-3	9/21/09	9/23/09	9/30/09	10/1/09	10/2/09	10/6/09	9/25/09	10/2/09
20	L13603-4	9/21/09	9/23/09	9/30/09	10/1/09	10/2/09	10/6/09	9/25/09	10/2/09
21	L13603-5	9/21/09	9/23/09	9/30/09	10/3/09	10/2/09	10/6/09	10/29/09	11/4/09
22	L13603-6	9/21/09	9/23/09	9/30/09	10/1/09	10/2/09	10/6/09	9/25/09	10/3/09
23	L13603-7	9/22/09	9/23/09	9/30/09	10/1/09	10/2/09	10/6/09	10/6/09	10/19/09
24	L13603-8	9/22/09	9/23/09	9/30/09	10/1/09	10/2/09	10/6/09	10/6/09	10/19/09
25	L13813-2	10/20/09	10/23/09	10/28/09	10/30/09	10/27/09	11/5/09	10/29/09	11/5/09
26	L13811-1	10/20/09	10/22/09	10/28/09	10/30/09	–	–	–	–
27	L13811-2	10/20/09	10/23/09	10/28/09	10/30/09	–	–	–	–

DATA QUALIFICATIONS

The following comments refer to the laboratory performance in meeting the Quality Control Specifications outlined NRMRL Quality Assurance Project Plan (QAPP) QAID #:521-Q7-0 "Sampling and Analysis of Contaminants of Emerging Concern within the Mainstream of Ohio River and Its Tributaries" As specified in the QAPP, all samples were collected, handled, and analyzed in accordance with the QAPP and the specification of the Axys Method MLA-060 Rev 9 for PFCs; MLA-075 for PPCPs, modification to USEPA Draft Method 1694; and MLA-068 Rev 3 for H&S, modification to USEPA Draft Method 1698; and the *Guidance for Labelling Externally Validated Laboratory Analytical data for Superfund Use, EPA 540-R-08-05, January 2009*. Some of the data quality elements were qualified using the reviewer's professional judgment.

The conclusions presented herein are based on the information provided for the review.

Condition of Samples upon Receipt

All of the samples were received intact and were stored by the laboratory at 4°C while waiting for extraction. None of the data were qualified on the basis of sample preservation.

Holding Time

USEPA has no established holding times for the PFC, PPCP, and H&S target analytes. The analytical methods for PPCPs and H&S recommend the extraction of samples within 7 days of sample collection to avoid the potential of losses. Due to field and shipping logistics, all of the samples were extracted on different dates for each of the target analyte group. The 7 day method recommended holding time was exceeded for all analyses, therefore all data should be considered qualified based on holding times.

Sample Preparation

Samples were prepared and extracted in accordance with the Axys Methods MLA-060 Rev 9, MLA-075, and MLA 068 Rev 3. PFCs were extracted using solid phase extraction (SPE) technique as specified in the method. Acid and basic PPCPs were extracted separately with SPE cartridges as specified by the method. Each of the 5 PPCP groups was analyzed separately using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Samples for H&S analyses were extracted using SPE and then extracts were derivatized for instrumental analysis. Samples were prepared and analyzed for PFCs, each of the 5 PPCP groups, hormones, and sterols separately. No data was qualified due to sample preparation

Instrument Performance

The frequency of system performance checks were met for all instruments used for the PFC, PPCP, and H&S analyses. For PFCs and PPCPs, the sample extracts were analysed using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Each target and labelled compound peak met the signal-to-noise ratio (S/N) criterion of > 3:1 and the retention time limits. Compound retention time fell within 0.4 minutes of the predicted retention times from the daily calibration standard. Native compounds with labeled surrogate standards appeared within 0.1 minutes of the associated labelled surrogates.

For H&S, the analyses were performed using a capillary gas chromatography (GC) coupled with either high-resolution (HRMS) or low-resolution (LRMS).The HRMS was operated at a static mass resolution (5000) in the electron ionization (EI) mode using Voltage SIR detection. The LRMS was operated at unit mass resolution in the EI mode using multiple ion detection (MID). Two characteristic ions for each target analyte and surrogate standard were acquired. Any samples that had either the target or labeled compound did not meet the > 3:1 S/N, the ion abundance criteria specified by the method and the retention time criteria were flagged (K flag was used).

Initial Calibrations

Initial calibrations were performed for each target analyte within each batch of analysis. The frequency of analysis, percent relative standard deviations (%RSDs), percent recoveries, the correlation coefficient (r) of >0.985, the ion abundance, S/N ratios, retention times and chromatographic resolution criteria were met for most of the target compounds (Each criterion is listed in Axys' Method Summary in Appendix 1). Calibration ranges for each analyte are shown in Tables 4.2, 4.5, and 4.9. Some of the target compounds did not meet the correlation coefficient (r)

(Individual cases are demonstrated in Axys' data packages in Appendix 2). However, since those deviation were relatively small (generally greater than 0.95) and quantifications used isotope dilution techniques, none of the reported results were qualified on this basis.

Calibration Verification

Calibration verification samples were run at each batch of analysis. The frequency of analysis, the percent recoveries, retention times, chromatographic resolution, ion abundance and S/N ratio criteria were met by all calibration verification standards with a few exceptions. Some of the target and labeled compounds did not meet the criteria at the lowest or highest calibration point, in which case, those points were excluded from the calibration calculations (Refer to Appendix 1 and 2 for criteria and individual cases, respectively). None of the reported results were qualified on this basis since the final calibration curves consisted of at least 5 levels of concentrations in all instances.

Ongoing Precision and Recovery (OPR)

The OPRs for each analyte and mass labeled surrogate were provided by Axys. The OPRs were used to appropriately qualify matrix spike and surrogate recovery data. The OPR criteria specified by Draft Methods 1694 and 1698 were overridden by those from Axys' updated methods MLA-075 and MLA-068 Rev 3, respectively (For qualifiers, see Laboratory Spikes and Surrogate Recoveries sections). Recoveries for the laboratory spikes and surrogates are listed in Tables 4.3, 4.6, and 4.10. Precisions (percent relative standard deviation) are detailed in each method narrative (Appendix 1).

Laboratory Spikes

At least 5% of the samples within an analytical batch were laboratory spikes that received the method specified levels of target analytes and surrogates. Some of the laboratory spike samples showed exceeded recoveries specified by the each method's OPRs. All reportable data within a batch in which the laboratory spike did not meet the OPR were qualified with "S" flag.

Surrogate Recoveries

Some of the labeled compounds did not meet the specified OPRs. The affected target compounds were qualified accordingly ("V").

Minimum Reporting Limit (MRL)

MRLs were set for each analyte at the lowest calibration concentration that exceeded the detection limit provided by Axys. Thus the calibration ranges became narrower if the MRLs were set above the lowest point of initial calibration. Any numerical value below MRL were not reported (flagged as "< MRL").

Upper Limit of Calibration (ULC)

The ULC was set at the highest concentrations of each calibration curve. Concentrations reported by Axys that exceeded the ULCs were qualified with the flag of "E" and the ULCs were reported instead of the concentrations, e.g. > 300 E (ng/L).

Compound Identification

Most of the detected compounds met the technical acceptance criteria for identification, e.g., S/N ratios greater than 3, ion abundance ratios, retention times within established limits. Data were qualified using "K" when it didn't meet these criteria (Criteria and individual cases are reported in Appendix 1 and 2, respectively).

Analytical Sequence

All of the standards, blanks, samples, and QC samples were analyzed in accordance with the method specified analytical sequence. All instruments were checked for their performance on daily basis. All of the analytical sequences were also bracketed by the continuing calibration check standards. None of the data were qualified on this basis.

Laboratory Blanks
At least 5% of the samples within an analytical batch were laboratory blanks. None of the PFC and PPCP target analyte was found above MRL in the laboratory blanks. Cholesterol was the only analyte among the H&S group that was detected in some of the laboratory blanks at concentrations above the MRL and qualified as "B (levels)".

Duplicates
At least 5% of the samples within an analytical batch were analyzed in duplicates. Note of the duplicates showed notable difference at the level specified by each method.

Field Blanks
Three field blanks were prepared on three different field events transferring ultra pure laboratory water from pre-cleaned 4-L glass container to each sample container. The field blank samples were treated exactly the same as the other field samples. Axys were not informed of which samples were the field blanks. None of the target compounds were found in the field blanks at or above MRL except for cholesterol found in a field blank (Sample 25) at 129 ng/L in which batch cholesterol was found in the laboratory blank at 232 ng/L. Since the field blank cholesterol level was less than the laboratory blank, the data for cholesterol was not qualified on this basis.

Field Spikes
It has been shown that PFCs may be lost during long period of storage possibly due to adsorption to sample container. To quantify the loss, if any, blank ultra pure laboratory water was fortified with PFCs in two levels (20 and 200 ng/L) and shipped to Axys for analysis. The laboratory was informed only of the range of expected concentrations (10–500 ng/L). Concentrations for the field spikes were reported as Samples 26 and 27. The recovery of the field blanks should be taken into consideration when PFC data are used for any purposes.

Missing Analytes
Some analytes in some samples were not analyzed for by Axys. No data were reported for those analytes ("NA").

Overall Assessment
All of the samples were analyzed in accordance with the methods specifications. Thorough review of the entire data set resulted in some of the reported data being qualified (flagged appropriately). Any data with qualifiers should be interpreted appropriately. PFC data should also be considered qualified due to the low recoveries of field spikes.

Data Qualifiers

Flag	Description	Comment
B (BLK)	Blank for the representing batch had greater concentration than MRL.	The sample may be contaminated. Care should be taken when one performs the data interpretation.
E	The concentration exceeded the upper limit of calibration.	No data reported. The concentration can be considered to be greater than the upper limit of calibration.
K	Analyte peak did not meet the quantitation criteria: retention time, peak shape, or ion abundance ratio.	No data reported
S	Spike recovery for the representing batch was out of the range of OPR.	The reported concentration may be under or over estimated. Evaluate spike recovery for the batch of analysis to which the sample belongs.
V	Surrogate recovery for the representing sample was out of the range of OPR.	The reported concentration may be under or over estimated. Evaluate spike recovery for the batch of analysis to which the sample belongs.
NA	Not analyzed for.	No data reported

3.1.2 ORSANCO Memo

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WATER SANITATION COMMISSION
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JEFFERY A. EGER
CHAIRMAN
ALAN H. VICORY, JR., P.E., DEE
EXECUTIVE DIRECTOR
AND CHIEF ENGINEER

May 24, 2010

TO: Erich Emery, Project Manager

From: Lila Xepoleas Ziolkowski, QAO

RE: A Screening Study investigating the presence of emerging contaminants within the Ohio River Basin

An internal assessment of quality assurance and quality control procedures was performed on documents received in connection with the aforementioned study. A careful review of the materials indicates that field sampling, methodological, and analytical protocols were followed and the data generated is valid and falls within acceptable quality assurance and quality control parameters.

3.2 Laboratory Validation – See case narratives (Appendix 2).

3.3 Target Analyte Concentration

For all target analytes except for androsterone, desogestrel, androstenedione, and testosterone, the suite of labeled standards were fortified before extraction as surrogates. The concentrations for those analytes were calculated against the labeled surrogates thus were surrogate recovery corrected. Androsterone, desogestrel, androstenedione, and testosterone were quantified against labeled internal standards that were spiked just before being submitted for instrumental analysis. The concentrations for those 4 compounds were thus not recovery corrected. Data presented here were critically reviewed and appropriately flagged using qualifiers listed in Section 3.1. Minimum reporting limits (MRLs), calibration range, ongoing precision and recovery (OPR), spike recovery, and surrogate recovery data are reported in Tables 3.1 – 3.10.

3.4 QA/QC by Analyte Group

Batch-specific QA/QC results including concentration and surrogate recovery values, calibration ranges and minimum reporting limits and ongoing precision and recovery values for each analyte group are found in Tables 3.1 to 3.10.

3.4.1 Perfluorinated Compounds (PFCs)

- Table 3.1. Concentration and surrogate recovery of perfluorinated compounds (PFCs).
- Table 3.2. Calibration range and minimum reporting limit (MRL) for perfluorinated compounds (PFCs).
- Table 3.3. Ongoing precision and recovery (OPR) for perfluorinated compounds (PFCs).

3.4.2 Pharmaceuticals and Personal Care Products (PPCPs)

- Table 3.4. Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).
- Table 3.5. Calibration range and minimum reporting limit (MRL) for pharmaceuticals and personal care products (PPCPs)
- Table 3.6. Ongoing precision and recovery (OPR) for pharmaceuticals and personal care products (PPCPs)

3.4.3 Hormones and Sterols

- Table 3.7. Concentration and surrogate recovery of hormones.
- Table 3.8. Concentration and surrogate recovery of sterols.
- Table 3.9. Calibration range and minimum reporting limit (MRL) for hormones and sterols.
- Table 3.10. Ongoing precision and recovery (OPR) for hormones and sterols.

Table 3.1 Concentration and surrogate recovery of perfluorinated compounds (PFCs).

Concentration

CLIENT ID (Sample #)	1	2	3	4	5	6	7	8	9
AXYS ID	L13550-1	L13550-2	L13550-3	L13550-4	L13550-5	L13550-6	L13550-7	L13550-8	L13550-9
WORKGROUP	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317
Sample Size	0.500 L	0.502 L	0.504 L	0.513 L	0.506 L	0.507 L	0.507 L	0.509 L	0.507 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
PFBA	1.14	< MRL	1.53	1.09	< MRL	1.49	2.44	2.13	< MRL
PFPeA	1.08	1.34	1.84	1.17	< MRL	1.76	3.63	2.00	< MRL
PFHxA	< MRL	1.85	2.35	1.31	< MRL	1.56	3.95	2.49	< MRL
PFHpA	< MRL	1.11	1.57	1.24	< MRL	1.45	2.01	2.63	< MRL
PFOA	2.04	3.87	3.41	2.50	< MRL	3.01	5.39	35.2	1.48
PFNA	< MRL	< MRL	< MRL	1.04	< MRL	< MRL	1.37	< MRL	< MRL
PFDA	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	1.16	< MRL	< MRL
PFUnA	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
PFDoA	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
PFBS	2.51	< MRL	2.31	< MRL	< MRL	< MRL	< MRL	< MRL	18.4
PFHxS	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
PFOS	2.24	2.79	2.63	< MRL	< MRL	2.82	4.42	2.88	< MRL
PFOSA	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL

Surrogate recovery

CLIENT ID (Sample #)	1	2	3	4	5	6	7	8	9
AXYS ID	L13550-1	L13550-2	L13550-3	L13550-4	L13550-5	L13550-6	L13550-7	L13550-8	L13550-9
WORKGROUP	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317
Sample Size	0.500 L	0.502 L	0.504 L	0.513 L	0.506 L	0.507 L	0.507 L	0.509 L	0.507 L
UNITS	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov
13C4-PFBA	85.4	92.8	92.2	99.9	77.3	79.7	95.3	89.5	100
13C4-PFBA	85.4	92.8	92.2	99.9	77.3	79.7	95.3	89.5	100
13C2-PFHxA	89.4	94.4	90.3	93.7	97.6	83.9	91.5	92.1	98.0
13C2-PFHxA	89.4	94.4	90.3	93.7	97.6	83.9	91.5	92.1	98.0
13C2-PFOA	90.0	92.7	103	103	89.3	91.3	98.0	114	89.9
13C5-PFNA	77.3	103	99.9	75.9	117	72.7	110	91.4	81.7
13C2-PFDA	86.0	89.5	92.6	99.1	88.6	83.9	96.5	94.4	87.6
13C2-PFDA	86.0	89.5	92.6	99.1	88.6	83.9	96.5	94.4	87.6
13C2-PFDoA	60.2	64.8	57.7	63.6	89.5	55.4	65.9	71.1	74.2
13C4-PFOS(80)	81.6	106	95.6	111	106	93.1	114	108	102
13C4-PFOS(80)	81.6	106	95.6	111	106	93.1	114	108	102
13C4-PFOS(80)	81.6	106	95.6	111	106	93.1	114	108	102
13C4-PFOS(80)	81.6	106	95.6	111	106	93.1	114	108	102

Table 3.1 Concentration and surrogate recovery of perfluorinated compounds (PFCs).

Concentration

CLIENT ID (Sample #)	10	12	13	14	15	16	Lab Blank	Spiked Matrix
AXYS ID	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	WG30317-101	WG30317-102
WORKGROUP	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317
Sample Size	0.511 L	0.505 L	0.514 L	0.507 L	0.497 L	0.505 L	0.500 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
PFBA	1.37	2.21	< MRL	1.01	1.46	5.29	< MRL	110
PFPeA	1.16	1.32	< MRL	1.56	1.53	13.7	< MRL	91.1
PFHxA	1.56	2.55	< MRL	1.71	2.35	7.83	< MRL	89.5
PFHpA	1.66	1.35	< MRL	1.58	1.62	4.40	< MRL	89.2
PFOA	19.1	14.1	< MRL	13.1	14.4	14.3	< MRL	86.9
PFNA	< MRL	< MRL	< MRL	< MRL	< MRL	1.64	< MRL	91.6
PFDA	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	94.2
PFUnA	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	84.4
PFDoA	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	98.9
PFBS	< MRL	< MRL	< MRL	< MRL	< MRL	4.61	< MRL	96.5
PFHxS	< MRL	< MRL	< MRL	< MRL	< MRL	2.98	< MRL	93.3
PFOS	2.71	3.15	< MRL	2.58	4.97	7.36	< MRL	91.1
PFOSA	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	85.8

Surrogate recovery

CLIENT ID (Sample #)	10	12	13	14	15	16	Lab Blank	Spiked Matrix
AXYS ID	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	WG30317-101	WG30317-102
WORKGROUP	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317	WG30317
Sample Size	0.511 L	0.505 L	0.514 L	0.507 L	0.497 L	0.505 L	0.500 L	
UNITS	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov
13C4-PFBA	84.1	87.1	102	90.5	93.3	77.5	113	101
13C4-PFBA	84.1	87.1	102	90.5	93.3	77.5	113	101
13C2-PFHxA	93.6	103	102	102	90.9	89.4	106	114
13C2-PFHxA	93.6	103	102	102	90.9	89.4	106	114
13C2-PFOA	89.2	102	85.4	110	102	103	98.7	101
13C5-PFNA	92.8	96.8	89.9	90.0	99.2	101	113	130
13C2-PFDA	85.9	91.6	103	102	92.6	87.9	101	105
13C2-PFDA	85.9	91.6	103	102	92.6	87.9	101	105
13C2-PFDoA	65.4	63.0	88.6	71.7	60.3	59.8	85.3	92.7
13C4-PFOS(80)	96.7	93.6	109	107	103	98.3	108	123
13C4-PFOS(80)	96.7	93.6	109	107	103	98.3	108	123
13C4-PFOS(80)	96.7	93.6	109	107	103	98.3	108	123
13C4-PFOS(80)	96.7	93.6	109	107	103	98.3	108	123

Table 3.1 Concentration and surrogate recovery of perfluorinated compounds (PFCs).

Concentration

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30691-101	WG30691-102 (A)
WORKGROUP	WG30691	WG30691	WG30691	WG30691
Sample Size	0.499 L	0.503 L	0.500 L	
UNITS	ng/L	ng/L	ng/L	% Recov
PFBA	3.89	< MRL	< MRL	104
PFPeA	3.30	< MRL	< MRL	95.6
PFHxA	4.69	< MRL	< MRL	101
PFHpA	2.85	< MRL	< MRL	96.0
PFOA	6.22	< MRL	< MRL	98.8
PFNA	1.74	< MRL	< MRL	105
PFDA	1.01	< MRL	< MRL	94.1
PFUnA	< MRL	< MRL	< MRL	94.8
PFDoA	< MRL	< MRL	< MRL	102
PFBS	< MRL	< MRL	< MRL	104
PFHxS	< MRL	< MRL	< MRL	101
PFOS	5.14	< MRL	< MRL	98.3
PFOSA	< MRL	< MRL	< MRL	102

Surrogate recovery

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30691-101	WG30691-102 (A)
WORKGROUP	WG30691	WG30691	WG30691	WG30691
Sample Size	0.499 L	0.503 L	0.500 L	
UNITS	% Recov	% Recov	% Recov	% Recov
13C4-PFBA	65.0	67.6	75.5	94.5
13C4-PFBA	65.0	67.6	75.5	94.5
13C2-PFHxA	67.9	84.4	95.2	92.3
13C2-PFHxA	67.9	84.4	95.2	92.3
13C2-PFOA	99.0	98.4	101	93.0
13C5-PFNA	91.8	107	97.8	102
13C2-PFDA	80.4	79.2	82.0	96.2
13C2-PFDA	80.4	79.2	82.0	96.2
13C2-PFDoA	56.1	84.3	97.6	98.5
13C4-PFOS(80)	105	110	102	112
13C4-PFOS(80)	105	110	102	112
13C4-PFOS(80)	105	110	102	112
13C4-PFOS(80)	105	110	102	112

Table 3.1 Concentration and surrogate recovery of perfluorinated compounds (PFCs).

Concentration

CLIENT ID (Sample #)	17	18	19	20	21	22	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6	L13603-7	L13603-8	WG30329-101	WG30329-102 (A)
WORKGROUP	WG30329	WG30329	WG30329	WG30329	WG30329	WG30329	WG30329	WG30329	WG30329	WG30329
Sample Size	0.492 L	0.516 L	0.510 L	0.509 L	0.511 L	0.506 L	0.509 L	0.509 L	0.500 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
PFBA	1.83	1.97	2.69	5.75	1.4	1.52	12.5	15.7	< MRL	102
PFPeA	1.94	3.07	2.09	8.59	1.81	2.26	4.65	6.68	< MRL	109
PFHxA	2.42	2.78	2.95	10.4	2.3	1.97	9.48	14.7	< MRL	110
PFHpA	1.37	2.11	1.59	4.30	1.32	1.17	7.67	9.14	< MRL	106
PFOA	9.21	9.44	11.5	18.2	3.43	8.93	23.9	31.2	< MRL	107
PFNA	< MRL	< MRL	1.22 S	6.17 S	< MRL	< MRL	< MRL	8.08 S	< MRL	135
PFDA	< MRL	< MRL	1.65	8.04	< MRL	< MRL	< MRL	2.95	< MRL	120
PFUnA	< MRL	< MRL	3.02	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	98.9
PFDoA	< MRL	< MRL	4.75	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	104
PFBS	< MRL	< MRL	< MRL	111	< MRL	< MRL	16.5	31.8	< MRL	116
PFHxS	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	5.62	8.91	< MRL	117
PFOS	5.58	5.80	7.27	669	3.90	6.49	35.4	29.2	< MRL	115
PFOSA	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	1.60	< MRL	< MRL	105

Surrogate recovery

CLIENT ID (Sample #)	17	18	19	20	21	22	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6	L13603-7	L13603-8	WG30329-101	WG30329-102 (A)
WORKGROUP	WG30329	WG30329	WG30329	WG30329	WG30329	WG30329	WG30329	WG30329	WG30329	WG30329
Sample Size	0.492 L	0.516 L	0.510 L	0.509 L	0.511 L	0.506 L	0.509 L	0.509 L	0.500 L	
UNITS	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov
13C4-PFBA	77.3	82.1	69.9	51.1	68.8	75.6	85.4	62.5	71.7	85.0
13C4-PFBA	77.3	82.1	69.9	51.1	68.8	75.6	85.4	62.5	71.7	85.0
13C2-PFHxA	80.5	83.6	76.4	64.2	70.8	77.4	89.8	64.7	82.4	74.8
13C2-PFHxA	80.5	83.6	76.4	64.2	70.8	77.4	89.8	64.7	82.4	74.8
13C2-PFOA	82.8	90.0	79.3	88.0	84.1	83.4	87.9	82.7	79.8	90.2
13C5-PFNA	85.5	77.4	84.3	73.9	79.8	64.0	93.6	78.9	70.1	66.2
13C2-PFDA	79.3	90.2	67.6	70.3	75.1	79.1	80.6	85.4	80.3	66.2
13C2-PFDA	79.3	90.2	67.6	70.3	75.1	79.1	80.6	85.4	80.3	66.2
13C2-PFDoA	56.0	61.0	57.3	59.7	60.3	55.5	66.8	65.2	68.3	69.3
13C4-PFOS(80)	82.4	78.0	76.8	67.8	81.2	86.3	98.9	81.5	81.8	74.9
13C4-PFOS(80)	82.4	78.0	76.8	67.8	81.2	86.3	98.9	81.5	81.8	74.9
13C4-PFOS(80)	82.4	78.0	76.8	67.8	81.2	86.3	98.9	81.5	81.8	74.9
13C4-PFOS(80)	82.4	78.0	76.8	67.8	81.2	86.3	98.9	81.5	81.8	74.9

Table 3.1 Concentration and surrogate recovery of perfluorinated compounds (PFCs).

Concentration

CLIENT ID (Sample #)	26-A-7	27-A-7	Lab Blank	Spiked Matrix
AXYS ID	L13811-1	L13811-2	WG30691-101	WG30691-102 (A)
WORKGROUP	WG30691	WG30691	WG30691	WG30691
Sample Size	0.502 L	0.503 L	0.500 L	
UNITS	ng/L	ng/L	ng/L	% Recov
PFBA	15.2	215	< MRL	104
PFPeA	13.5	196	< MRL	95.6
PFHxA	13.7	199	< MRL	101
PFHpA	14.2	211	< MRL	96.0
PFOA	11.6	174	< MRL	98.8
PFNA	12.2	178	< MRL	105
PFDA	10.1	181	< MRL	94.1
PFUnA	7.96	113	< MRL	94.8
PFDoA	3.12	42.6	< MRL	102
PFBS	13.0	177	< MRL	104
PFHxS	9.67	149	< MRL	101
PFOS	4.19	79.1	< MRL	98.3
PFOSA	< MRL	< MRL	< MRL	102
	Spiked level			
	20 ng/L	200 ng/L		

Surrogate recovery

CLIENT ID (Sample #)	26-A-7	27-A-7	Lab Blank	Spiked Matrix
AXYS ID	L13811-1	L13811-2	WG30691-101	WG30691-102 (A)
WORKGROUP	WG30691	WG30691	WG30691	WG30691
Sample Size	0.502 L	0.503 L	0.500 L	
UNITS	% Recov	% Recov	% Recov	% Recov
13C4-PFBA	81.7	85.1	75.5	94.5
13C4-PFBA	81.7	85.1	75.5	94.5
13C2-PFHxA	93.0	83.8	95.2	92.3
13C2-PFHxA	93.0	83.8	95.2	92.3
13C2-PFOA	111	103	101	93.0
13C5-PFNA	108	97.8	97.8	102
13C2-PFDA	83.7	72.9	82.0	96.2
13C2-PFDA	83.7	72.9	82.0	96.2
13C2-PFDoA	82.1	81.6	97.6	98.5
13C4-PFOS(80)	114	100	102	112
13C4-PFOS(80)	114	100	102	112
13C4-PFOS(80)	114	100	102	112
13C4-PFOS(80)	114	100	102	112

Table 3.2 Calibration and minimum reporting limit (MRL) for PFCs.

	A	B	C	D	E	F	G	H
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
PFBA	**1.00**	2.50	10.0	40.0	200	400	1000	2500
PFPeA	**1.00**	2.50	10.0	40.0	200	400	1000	2500
PFHxA	**1.00**	2.50	10.0	40.0	200	400	1000	2500
PFHpA	**1.00**	2.50	10.0	40.0	200	400	1000	2500
PFOA	**1.00**	2.50	10.0	40.0	200	400	1000	2500
PFNA	**1.00**	2.50	10.0	40.0	200	400	1000	2500
PFDA	**1.00**	2.50	10.0	40.0	200	400	1000	2500
PFUnA	**1.00**	2.50	10.0	40.0	200	400	1000	2500
PFDoA	**1.00**	2.50	10.0	40.0	200	400	1000	2500
PFBS	**2.00**	5.00	20.0	80.0	400	800	2000	5000
PFHxS	**2.00**	5.00	20.0	80.0	400	800	2000	5000
PFOS	**2.00**	5.00	20.0	80.0	400	800	2000	5000
PFOSA	**1.00**	2.50	10.0	40.0	200	400	1000	2500

The MRLs are shown in bold face

Table 3.3 Ongoing precision and recovery (OPR) for PFCs.

Analyte	OPR	Class	Surrogate
PFBA	80 – 120	PFC	13C4-PFBA
PFPeA	80 – 120	PFC	13C4-PFBA
PFHxA	80 – 120	PFC	13C2-PFHxA
PFHpA	80 – 120	PFC	13C2-PFHxA
PFOA	80 – 120	PFC	13C2-PFOA
PFNA	80 – 120	PFC	13C5-PFNA
PFDA	80 – 120	PFC	13C2-PFDA
PFUnA	80 – 120	PFC	13C2-PFDA
PFDoA	80 – 120	PFC	13C2-PFDoA
PFBS	70 – 130	PFC	13C4-PFOS(80)
PFHxS	70 – 130	PFC	13C4-PFOS(80)
PFOS	70 – 130	PFC	13C4-PFOS(80)
PFOSA	70 – 130	PFC	13C4-PFOS(80)
13C4-PFBA	20 – 150	PFC-Surrogate	
13C2-PFHxA	40 – 150	PFC-Surrogate	
13C2-PFOA	40 – 150	PFC-Surrogate	
13C5-PFNA	40 – 150	PFC-Surrogate	
13C2-PFDA	40 – 150	PFC-Surrogate	
13C2-PFDoA	40 – 150	PFC-Surrogate	
13C4-PFOS(80)	40 – 150	PFC-Surrogate	

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	1	10	12	13	14	15	16	2
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	L13550-2
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.07 L	1.07 L	1.03 L	1.06 L	1.03 L	1.03 L	1.07 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Bisphenol A	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Furosemide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Gemfibrozil	7.25	< MRL	7.26	< MRL	< MRL	19.2	< MRL	13.8
Glipizide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Glyburide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Hydrochlorothiazide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
2-Hydroxy-ibuprofen	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Ibuprofen	< MRL	< MRL	< MRL	< MRL	< MRL	28.9	25.0	15.7
Naproxen	< MRL	< MRL	< MRL	< MRL	< MRL	25.5	< MRL	22.9
Triclocarban	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Triclosan	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Warfarin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL

Surrogate recovery

CLIENT ID (Sample #)	1	10	12	13	14	15	16	2
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	L13550-2
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.07 L	1.07 L	1.03 L	1.06 L	1.03 L	1.03 L	1.07 L
UNITS	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov
d6-Bisphenol A	76.6	121	95.2	115	105	99.7	93.6	109
13C-D3-Naproxen	81.9	130	76.8	103	98.2	83.1	79.5	103
d6-Gemfibrozil	96.3	131	105	120	103	104	97.6	112
d11-Glipizide	105	141	126	129	140	122	134	127
d3-Glyburide	85.3	70.0	45.0	95.5	106	107	99.8	107
13C-D3-Naproxen	81.9	130	76.8	103	98.2	83.1	79.5	103
13C3-Ibuprofen	85.6	114	91.2	129	85.1	99.0	92.3	98.5
13C3-Ibuprofen	85.6	114	91.2	129	85.1	99.0	92.3	98.5
13C-D3-Naproxen	81.9	130	76.8	103	98.2	83.1	79.5	103
13C6-Triclocarban	79.0	122	91.8	117	113	101	89.4	102
13C12-Triclosan	96.0	158	107	139	119	124	128	123
d5-Warfarin	110	153	119	164	130	125	109	130

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	3	4	5	6	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-3	L13550-4	L13550-5	L13550-6	L13550-7	L13550-8	L13550-9	WG30337-101	WG30337-102
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.06 L	1.07 L	1.08 L	1.07 L	1.08 L	1.05 L	1.01 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Bisphenol A	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	91.6
Furosemide	< MRL	< MRL	< MRL	< MRL	212	< MRL	< MRL	< MRL	94.0
Gemfibrozil	62.9	18.6	< MRL	15.8	27.6	< MRL	10.2	< MRL	109
Glipizide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	110
Glyburide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	110
Hydrochlorothiazide	< MRL	< MRL	< MRL	< MRL	105	< MRL	< MRL	< MRL	112
2-Hydroxy-ibuprofen	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	122
Ibuprofen	21.3	17.7	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	113
Naproxen	71.8	20.8	< MRL	22.0	21.4	< MRL	< MRL	< MRL	99.3
Triclocarban	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	97.2
Triclosan	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	92.7
Warfarin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	106

Surrogate recovery

CLIENT ID (Sample #)	3	4	5	6	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-3	L13550-4	L13550-5	L13550-6	L13550-7	L13550-8	L13550-9	WG30337-101	WG30337-102
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.06 L	1.07 L	1.08 L	1.07 L	1.08 L	1.05 L	1.01 L	1.00 L	
UNITS	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov
d6-Bisphenol A	92.3	103	117	90.1	85.4	97.5	90.7	113	115
13C-D3-Naproxen	87.7	95.7	104	87.0	87.0	98.6	81.0	110	113
d6-Gemfibrozil	111	110	124	103	106	109	84.5	125	110
d11-Glipizide	116	127	126	113	117	134	108	125	110
d3-Glyburide	101	101	100	94.8	90.5	98.7	84.8	101	110
13C-D3-Naproxen	87.7	95.7	104	87.0	87.0	98.6	81.0	110	113
13C3-Ibuprofen	95.0	90.5	111	91.6	97.0	106	73.5	128	95.1
13C3-Ibuprofen	95.0	90.5	111	91.6	97.0	106	73.5	128	95.1
13C-D3-Naproxen	87.7	95.7	104	87.0	87.0	98.6	81.0	110	113
13C6-Triclocarban	103	118	111	94.3	99.6	108	104	86.9	89.9
13C12-Triclosan	119	127	147	118	129	137	122	135	104
d5-Warfarin	106	127	148	115	102	125	102	144	137

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30659-101	WG30659-102
WORKGROUP	WG30659	WG30659	WG30659	WG30659
Sample Size	1.10 L	1.04 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
Bisphenol A	< MRL	< MRL	< MRL	94.7
Furosemide	< MRL	< MRL	< MRL	105
Gemfibrozil	11.3	< MRL	< MRL	107
Glipizide	< MRL	< MRL	< MRL	105
Glyburide	< MRL	< MRL	< MRL	109
Hydrochlorothiazide	< MRL	< MRL	< MRL	118
2-Hydroxy-ibuprofen	< MRL	< MRL	< MRL	118
Ibuprofen	16.4	< MRL	< MRL	101
Naproxen	29.3	< MRL	< MRL	96.0
Triclocarban	23.3	< MRL	< MRL	102
Triclosan	< MRL	< MRL	< MRL	99.1
Warfarin	< MRL	< MRL	< MRL	103

Surrogate recovery

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30659-101	WG30659-102
WORKGROUP	WG30659	WG30659	WG30659	WG30659
Sample Size	1.10 L	1.04 L	1.00 L	
UNITS	% Recov	% Recov	% Recov	% Recov
d6-Bisphenol A	91.2	97.0	97.0	111
13C-D3-Naproxen	84.6	94.6	86.0	99.2
d6-Gemfibrozil	97.0	95.2	89.0	101
d11-Glipizide	124	113	118	121
d3-Glyburide	107	112	106	115
13C-D3-Naproxen	84.6	94.6	86.0	99.2
13C3-Ibuprofen	90.2	70.3	73.0	97.0
13C3-Ibuprofen	90.2	70.3	73.0	97.0
13C-D3-Naproxen	84.6	94.6	86.0	99.2
13C6-Triclocarban	79.3	85.5	82.7	97.7
13C12-Triclosan	106	108	87.9	92.3
d5-Warfarin	115	135	135	143

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	17	18	19	20	21	22	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6	L13603-7	L13603-8	WG30338-101	WG30338-102
WORKGROUP	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338
Sample Size	1.02 L	1.06 L	1.04 L	1.05 L	1.06 L	1.05 L	1.04 L	1.06 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Bisphenol A	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	96.7
Furosemide	< MRL	< MRL	< MRL	198	< MRL	< MRL	< MRL	1570	< MRL	118
Gemfibrozil	9.52	12.8	6.53	20.7	< MRL	< MRL	< MRL	366	< MRL	110
Glipizide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	102
Glyburide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	13.0	< MRL	94.3
Hydrochlorothiazide	< MRL	< MRL	< MRL	81.4	< MRL	< MRL	< MRL	558	< MRL	70.4
2-Hydroxy-ibuprofen	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	114
Ibuprofen	< MRL	15.6	< MRL	60.1	< MRL	< MRL	< MRL	78.9	< MRL	103
Naproxen	< MRL	13.3	< MRL	59.8	< MRL	< MRL	< MRL	164	< MRL	101
Triclocarban	< MRL	< MRL	< MRL	16.6	< MRL	< MRL	< MRL	56.0	< MRL	105
Triclosan	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	94.3	< MRL	93.9
Warfarin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	3.86	< MRL	124

Surrogate recovery

CLIENT ID (Sample #)	17	18	19	20	21	22	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6	L13603-7	L13603-8	WG30338-101	WG30338-102
WORKGROUP	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338
Sample Size	1.02 L	1.06 L	1.04 L	1.05 L	1.06 L	1.05 L	1.04 L	1.06 L	1.00 L	
UNITS	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov
d6-Bisphenol A	87.7	86.5	93.0	81.9	84.7	96.2	88.4	94.9	115	106
13C-D3-Naproxen	97.5	92.8	97.7	73.0	76.6	92.1	87.2	82.8	109	98.9
d6-Gemfibrozil	90.3	102	99.8	97.5	81.7	102	89.8	110	107	108
d11-Glipizide	125	125	134	121	119	142	124	144	116	119
d3-Glyburide	92.0	87.8	99.1	94.9	97.1	102	98.1	114	103	107
13C-D3-Naproxen	97.5	92.8	97.7	73.0	76.6	92.1	87.2	82.8	109	98.9
13C3-Ibuprofen	78.4	91.9	98.9	87.9	81.7	98.9	89.7	110	101	102
13C3-Ibuprofen	78.4	91.9	98.9	87.9	81.7	98.9	89.7	110	101	102
13C-D3-Naproxen	97.5	92.8	97.7	73.0	76.6	92.1	87.2	82.8	109	98.9
13C6-Triclocarban	76.0	91.5	96.4	91.6	79.9	101	97.7	105	83.1	81.2
13C12-Triclosan	106	121	132	117	102	120	125	133	132	106
d5-Warfarin	114	115	119	87.3	109	129	111	89.3	144	135

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	11	25	Lab Blank		Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30659-101		WG30659-102
WORKGROUP	WG30659	WG30659	WG30659		WG30659
Sample Size	1.10 L	1.04 L	1.00 L		
UNITS	ng/L	ng/L	ng/L		% Recov
Acetaminophen	201	< MRL	< MRL		109
Azithromycin	140	< MRL	< MRL		63.5
Caffeine	158	< MRL	< MRL		109
Carbadox	< MRL	< MRL	< MRL		78.4
Carbamazepine	74.0	< MRL	< MRL		110
Cefotaxime	< MRL	< MRL	< MRL		105
Ciprofloxacin	27.8	< MRL	< MRL		134
Clarithromycin	48.6	< MRL	< MRL		87.7
Clinafloxacin	< MRL	< MRL	< MRL		116
Cloxacillin	< MRL	< MRL	< MRL		65.2
Dehydronifedipine	2.13	< MRL	< MRL		103
1,7-Dimethylxanthine	< MRL	< MRL	< MRL		179
Diphenhydramine	31.4	< MRL	< MRL		83.1
Diltiazem	16.0	< MRL	< MRL		83.3
Digoxin	< MRL	< MRL	< MRL		121
Digoxigenin	< MRL	< MRL	< MRL		99.7
Enrofloxacin	< MRL	< MRL	3.14	B (3.14)	136
Erythromycin-H2O	24.1	< MRL	< MRL		103
Flumequine	< MRL	< MRL	< MRL		109
Fluoxetine	7.28	< MRL	< MRL		124
Lincomycin	< MRL	< MRL	< MRL		42.6
Lomefloxacin	< MRL	< MRL	< MRL		156

Surrogate recovery

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30659-101	WG30659-102
WORKGROUP	WG30659	WG30659	WG30659	WG30659
Sample Size	1.10 L	1.04 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
13C2-15N-Acetaminophen	136	108	107	105
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C3-Caffeine	71.4	88.1	104	84.7
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C3-N15-Ciprofloxacin	51.5	75.5	61.7	71.1
13C6-Sulfamethazine	68.2	124	93.6	102
13C3-N15-Ciprofloxacin	51.5	75.5	61.7	71.1
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C3-Trimethoprim	71.4	88.1	104	98.3
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C3-N15-Ciprofloxacin	74.1	99.2	90.9	71.1
13C2-Erythromycin-H2O	51.5	75.5	61.7	74.0
13C3-Trimethoprim	72.5	79.8	73.1	98.3
d5-Fluoxetine	74.1	99.2	90.9	81.3
13C3-Trimethoprim	85.6	89.9	77.7	98.3
13C3-N15-Ciprofloxacin	74.1	99.2	90.9	71.1
13C3-Trimethoprim	51.5	75.5	61.7	98.3

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	1	10	12	13	14	15
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.07 L	1.07 L	1.03 L	1.06 L	1.03 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Acetaminophen	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Azithromycin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Caffeine	79.6	< MRL	72.2	< MRL	< MRL	198
Carbadox	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Carbamazepine	9.33	11.7	22.7	< MRL	15.2	17.9
Cefotaxime	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Ciprofloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Clarithromycin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Clinafloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Cloxacillin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Dehydronifedipine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
1,7-Dimethylxanthine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Diphenhydramine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Diltiazem	< MRL	< MRL	< MRL	< MRL	< MRL	1.70
Digoxin	NA	NA	NA	NA	NA	NA
Digoxigenin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Enrofloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Erythromycin-H2O	1.80	2.41	2.64	< MRL	1.31	1.76
Flumequine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Fluoxetine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Lincomycin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Lomefloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL

Surrogate recovery

CLIENT ID (Sample #)	1	10	12	13	14	15
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.07 L	1.07 L	1.03 L	1.06 L	1.03 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
13C2-15N-Acetaminophen	180	173	171	128	167	155
13C3-Trimethoprim	111	98.7	78.2	94.2	80.0	82.5
13C3-Caffeine	95.9	175	85.6	132	98.1	104
13C3-Trimethoprim	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	111	98.7	78.2	94.2	80.0	82.5
13C3-N15-Ciprofloxacin	49.4	52.4	57.5	60.1	68.4	69.3
13C6-Sulfamethazine	92.2	113	110	110	95.8	101
13C3-N15-Ciprofloxacin	49.4	52.4	57.5	60.1	68.4	69.3
13C3-Trimethoprim	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	95.9	175	85.6	132	98.1	104
13C3-Trimethoprim	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	NA	NA	NA	NA	NA	NA
13C3-N15-Ciprofloxacin	111	98.7	78.2	94.2	80.0	82.5
13C2-Erythromycin-H2O	49.4	52.4	57.5	60.1	68.4	69.3
13C3-Trimethoprim	81.4	61.5	58.6	73.1	72.1	68.2
d5-Fluoxetine	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	78.0	68.4	78.3	50.7	90.0	73.6
13C3-N15-Ciprofloxacin	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	49.4	52.4	57.5	60.1	68.4	69.3

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	16	2	3	4	5	6
AXYS ID	L13550-15	L13550-2	L13550-3	L13550-4	L13550-5	L13550-6
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.03 g	1.07 L	1.06 L	1.07 L	1.08 L	1.07 L
UNITS	ng/g	ng/L	ng/L	ng/L	ng/L	ng/L
Acetaminophen	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Azithromycin	< MRL	< MRL	67.1	< MRL	< MRL	< MRL
Caffeine	< MRL	72.2 V	66.8	114	< MRL	129
Carbadox	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Carbamazepine	56.6	18.4	44.1	20.0	< MRL	19.9
Cefotaxime	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Ciprofloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Clarithromycin	< MRL	1.93	11.2	2.07	< MRL	< MRL
Clinafloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Cloxacillin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Dehydronifedipine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
1,7-Dimethylxanthine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Diphenhydramine	< MRL	< MRL	34.5	4.44	< MRL	< MRL
Diltiazem	4.44	2.86	20.5	4.09	< MRL	1.82
Digoxin	NA	NA	NA	NA	NA	NA
Digoxigenin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Enrofloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Erythromycin-H2O	12.3	3.28	14.9	7.31	< MRL	3.77
Flumequine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Fluoxetine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Lincomycin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Lomefloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL

Surrogate recovery

CLIENT ID (Sample #)	16	2	3	4	5	6
AXYS ID	L13550-15	L13550-2	L13550-3	L13550-4	L13550-5	L13550-6
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.03 g	1.07 L	1.06 L	1.07 L	1.08 L	1.07 L
UNITS	ng/g	ng/L	ng/L	ng/L	ng/L	ng/L
13C2-15N-Acetaminophen	185	167	158	158	126	159
13C3-Trimethoprim	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Caffeine	150	142	133	113	121	113
13C3-Trimethoprim	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	96.6	90.6	84.0	97.1	93.5	84.6
13C3-N15-Ciprofloxacin	108	45.5	62.4	60.8	32.4	47.3
13C6-Sulfamethazine	139	97.1	93.3	104	110	83.8
13C3-N15-Ciprofloxacin	108	45.5	62.4	60.8	32.4	47.3
13C3-Trimethoprim	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	150	142	133	113	121	113
13C3-Trimethoprim	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	NA	NA	NA	NA	NA	NA
13C3-N15-Ciprofloxacin	96.6	90.6	84.0	97.1	93.5	84.6
13C2-Erythromycin-H2O	108	45.5	62.4	60.8	32.4	47.3
13C3-Trimethoprim	59.3	72.8	73.3	61.5	73.7	68.8
d5-Fluoxetine	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	71.7	99.1	86.1	77.6	87.6	91.5
13C3-N15-Ciprofloxacin	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	108	45.5	62.4	60.8	32.4	47.3

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-7	L13550-8	L13550-9	WG30337-101	WG30337-102
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.05 L	1.01 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	% Recov
Acetaminophen	< MRL	< MRL	< MRL	< MRL	86.7
Azithromycin	147	< MRL	< MRL	< MRL	43.9
Caffeine	57.8	66.2	< MRL	< MRL	100
Carbadox	< MRL	< MRL	< MRL	< MRL	100
Carbamazepine	136	19.1	10.5	< MRL	106
Cefotaxime	< MRL	< MRL	< MRL	< MRL	99.5
Ciprofloxacin	28.5	< MRL	< MRL	< MRL	149
Clarithromycin	16.7	< MRL	< MRL	< MRL	116
Clinafloxacin	< MRL	< MRL	< MRL	< MRL	139
Cloxacillin	< MRL	< MRL	< MRL	< MRL	64.9
Dehydronifedipine	2.01	< MRL	< MRL	< MRL	68.6
1,7-Dimethylxanthine	< MRL	< MRL	< MRL	< MRL	104
Diphenhydramine	42.2	< MRL	< MRL	< MRL	81.9
Diltiazem	31.8	< MRL	< MRL	< MRL	70.0
Digoxin	NA	NA	NA	NA	NA
Digoxigenin	< MRL	< MRL	< MRL	< MRL	127
Enrofloxacin	< MRL	< MRL	< MRL	< MRL	200
Erythromycin-H2O	17.9	2.18	1.45	< MRL	103
Flumequine	< MRL	< MRL	< MRL	< MRL	112
Fluoxetine	1.78	< MRL	< MRL	< MRL	80.8
Lincomycin	< MRL	< MRL	< MRL	< MRL	43.0
Lomefloxacin	< MRL	< MRL	< MRL	< MRL	179

Surrogate recovery

CLIENT ID (Sample #)	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-7	L13550-8	L13550-9	WG30337-101	WG30337-102
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.05 L	1.01 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	% Recov
13C2-15N-Acetaminophen	158	179	154	109	123
13C3-Trimethoprim	81.9	84.6	90.1	94.3	105
13C3-Caffeine	93.7	114	138	122	119
13C3-Trimethoprim	81.9	84.6	90.1	94.3	105
13C3-Trimethoprim	81.9	84.6	90.1	94.3	105
13C3-Trimethoprim	81.9	84.6	90.1	94.3	105
13C3-N15-Ciprofloxacin	65.9	63.1	73.6	42.7	58.3
13C6-Sulfamethazine	110	96.7	115	92.8	93.1
13C3-N15-Ciprofloxacin	65.9	63.1	73.6	42.7	58.3
13C3-Trimethoprim	81.9	84.6	90.1	94.3	105
13C3-Trimethoprim	81.9	84.6	90.1	94.3	105
13C3-Trimethoprim	93.7	114	138	122	105
13C3-Trimethoprim	81.9	84.6	90.1	94.3	105
13C3-Trimethoprim	81.9	84.6	90.1	94.3	105
13C3-Trimethoprim	NA	NA	NA	NA	105
13C3-N15-Ciprofloxacin	81.9	84.6	90.1	94.3	58.3
13C2-Erythromycin-H2O	65.9	63.1	73.6	42.7	79.7
13C3-Trimethoprim	68.8	73.0	69.7	64.5	105
d5-Fluoxetine	81.9	84.6	90.1	94.3	109
13C3-Trimethoprim	85.4	74.9	93.1	78.7	105
13C3-N15-Ciprofloxacin	81.9	84.6	90.1	94.3	58.3
13C3-Trimethoprim	65.9	63.1	73.6	42.7	105

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	17	18	19	20	21	22
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6
WORKGROUP	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338
Sample Size	1.02 L	1.06 L	1.04 L	1.05 L	1.06 L	1.05 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Acetaminophen	< MRL	< MRL	< MRL	506	< MRL	< MRL
Azithromycin	< MRL	< MRL	< MRL	41.2	< MRL	< MRL
Caffeine	105	167	73.1	243	< MRL	< MRL
Carbadox	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Carbamazepine	11.1	14.5	13.0	107.0	14.0	10.9
Cefotaxime	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Ciprofloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Clarithromycin	< MRL	< MRL	< MRL	9.94	< MRL	< MRL
Clinafloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Cloxacillin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Dehydronifedipine	< MRL	< MRL	< MRL	2.03	< MRL	< MRL
1,7-Dimethylxanthine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Diphenhydramine	< MRL	< MRL	< MRL	3.29	< MRL	< MRL
Diltiazem	< MRL	< MRL	< MRL	1.35	< MRL	< MRL
Digoxin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Digoxigenin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Enrofloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Erythromycin-H2O	1.99	1.76	1.72	33.5	1.07	< MRL
Flumequine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Fluoxetine	< MRL	< MRL	< MRL	3.92	< MRL	< MRL
Lincomycin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Lomefloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL

Surrogate recovery

CLIENT ID (Sample #)	17	18	19	20	21	22
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6
WORKGROUP	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338
Sample Size	1.02 L	1.06 L	1.04 L	1.05 L	1.06 L	1.05 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
13C2-15N-Acetaminophen	112	131	127	136	122	158
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C3-Caffeine	83.0	106	105	114	79.4	164
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C3-N15-Ciprofloxacin	95.2	95.1	72.0	88.1	97.3	142
13C6-Sulfamethazine	46.9	49.5	60.4	61.7	49.8	79.7
13C3-N15-Ciprofloxacin	95.2	95.1	72.0	88.1	97.3	142
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	83.0	106	105	114	79.4	164
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C3-N15-Ciprofloxacin	84.9	86.8	83.1	89.1	70.0	117
13C2-Erythromycin-H2O	95.2	95.1	72.0	88.1	97.3	142
13C3-Trimethoprim	59.2	71.4	66.2	66.4	60.1	91.9
d5-Fluoxetine	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	58	100	81.2	76.6	83.0	122
13C3-N15-Ciprofloxacin	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	95.2	95.1	72.0	88.1	97.3	142

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-7	L13603-8	WG30338-101	WG30338-102
WORKGROUP	WG30338	WG30338	WG30338	WG30338
Sample Size	1.05 L	1.06 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
Acetaminophen	< MRL	< MRL	< MRL	96.0
Azithromycin	< MRL	834	< MRL	67.8
Caffeine	< MRL	< MRL	< MRL	55.8
Carbadox	< MRL	< MRL	< MRL	81.2
Carbamazepine	< MRL	159.0	< MRL	94.6
Cefotaxime	< MRL	< MRL	< MRL	102
Ciprofloxacin	< MRL	162	< MRL	108
Clarithromycin	< MRL	530	< MRL	96.6
Clinafloxacin	< MRL	< MRL	< MRL	92.9
Cloxacillin	< MRL	< MRL	< MRL	62.0
Dehydronifedipine	< MRL	5.40	< MRL	72.2
1,7-Dimethylxanthine	< MRL	< MRL	< MRL	105
Diphenhydramine	< MRL	>400 E	< MRL	93.2
Diltiazem	< MRL	172	< MRL	90.1
Digoxin	< MRL	< MRL	< MRL	101
Digoxigenin	< MRL	< MRL	< MRL	90.4
Enrofloxacin	< MRL	< MRL	< MRL	99.8
Erythromycin-H2O	< MRL	167	< MRL	98.1
Flumequine	< MRL	< MRL	< MRL	109
Fluoxetine	< MRL	25.0	< MRL	115
Lincomycin	< MRL	< MRL	< MRL	35.7
Lomefloxacin	< MRL	< MRL	< MRL	101

Surrogate recovery

CLIENT ID (Sample #)	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-7	L13603-8	WG30338-101	WG30338-102
WORKGROUP	WG30338	WG30338	WG30338	WG30338
Sample Size	1.05 L	1.06 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
13C2-15N-Acetaminophen	116	136	98.2	105
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C3-Caffeine	53.4	111	112	120
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C3-N15-Ciprofloxacin	91.2	77.6	112	108
13C6-Sulfamethazine	54.8	38.6	70.1	62.6
13C3-N15-Ciprofloxacin	91.2	77.6	112	108
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C3-Trimethoprim	53.4	111	112	98.2
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C3-N15-Ciprofloxacin	80.2	86.9	86.5	108
13C2-Erythromycin-H2O	91.2	77.6	112	62.5
13C3-Trimethoprim	71.3	58.3	69.2	98.2
d5-Fluoxetine	80.2	86.9	86.5	72.6
13C3-Trimethoprim	86.8	74.3	98.9	98.2
13C3-N15-Ciprofloxacin	80.2	86.9	86.5	108
13C3-Trimethoprim	91.2	77.6	112	98.2

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30659-101	WG30659-102
WORKGROUP	WG30659	WG30659	WG30659	WG30659
Sample Size	1.10 L	1.04 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
Miconazole	< MRL	< MRL	< MRL	81.1
Norfloxacin	< MRL	< MRL	< MRL	132
Norgestimate	< MRL	< MRL	< MRL	79.3
Ofloxacin	27.7	< MRL	< MRL	168
Ormetoprim	< MRL	< MRL	< MRL	99.4
Oxacillin	< MRL	< MRL	< MRL	40.1
Oxolinic Acid	< MRL	< MRL	< MRL	148
Penicillin G	< MRL	< MRL	< MRL	54.8
Penicillin V	< MRL	< MRL	< MRL	77.4
Roxithromycin	< MRL	< MRL	< MRL	65.3
Sarafloxacin	< MRL	< MRL	< MRL	121
Sulfachloropyridazine	< MRL	< MRL	< MRL	103
Sulfadiazine	< MRL	< MRL	< MRL	97.7
Sulfadimethoxine	< MRL	< MRL	< MRL	90.2
Sulfamerazine	< MRL	< MRL	< MRL	102
Sulfamethazine	< MRL	< MRL	< MRL	95.5
Sulfamethizole	< MRL	< MRL	< MRL	93.2
Sulfamethoxazole	279	< MRL	< MRL	96.9
Sulfanilamide	< MRL	< MRL	< MRL	41.9
Sulfathiazole	< MRL	< MRL	< MRL	91.6
Thiabendazole	10.7	< MRL	< MRL	89.9
Trimethoprim	76.8	< MRL	< MRL	100
Tylosin	< MRL	< MRL	< MRL	81.0
Virginiamycin	< MRL	< MRL	< MRL	95.6

Surrogate recovery

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30659-101	WG30659-102
WORKGROUP	WG30659	WG30659	WG30659	WG30659
Sample Size	1.10 L	1.04 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
13C3-N15-Ciprofloxacin	74.1	99.2	90.9	71.1
13C3-Trimethoprim	51.5	75.5	61.7	98.3
13C3-N15-Ciprofloxacin	74.1	99.2	90.9	71.1
13C3-Trimethoprim	51.5	75.5	61.7	98.3
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C3-Trimethoprim	74.1	99.2	90.9	98.3
13C6-Sulfamethazine	74.1	99.2	90.9	102
13C3-N15-Ciprofloxacin	68.2	124	93.6	71.1
13C6-Sulfamethazine	51.5	75.5	61.7	102
13C6-Sulfamethazine	68.2	124	93.6	102
13C6-Sulfamethoxazole	68.2	124	93.6	98.9
13C6-Sulfamethazine	82.3	112	96.3	102
13C6-Sulfamethazine	68.2	124	93.6	102
13C6-Sulfamethoxazole	68.2	124	93.6	98.9
13C6-Sulfamethoxazole	82.3	112	96.3	98.9
13C6-Sulfamethazine	82.3	112	96.3	102
13C6-Sulfamethoxazole	68.2	124	93.6	98.9
d6-Thiabendazole	82.3	112	96.3	120
13C3-Trimethoprim	55.2	122	110	98.3
13C6-Sulfamethazine	74.1	99.2	90.9	102
13C3-Trimethoprim	68.2	124	93.6	98.3
13C3-Caffeine	74.1	99.2	90.9	84.7

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	1	10	12	13	14	15
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.07 L	1.07 L	1.03 L	1.06 L	1.03 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Miconazole	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Norfloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Norgestimate	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Ofloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Ormetoprim	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Oxacillin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Oxolinic Acid	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Penicillin G	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Penicillin V	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Roxithromycin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sarafloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfachloropyridazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfadiazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfadimethoxine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfamerazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfamethazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfamethizole	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfamethoxazole	12.4	21.3	35.5	< MRL	20.4	31.6
Sulfanilamide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfathiazole	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Thiabendazole	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Trimethoprim	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Tylosin	NA	NA	NA	NA	NA	NA
Virginiamycin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL

Surrogate recovery

CLIENT ID (Sample #)	1	10	12	13	14	15
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.07 L	1.07 L	1.03 L	1.06 L	1.03 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
13C3-N15-Ciprofloxacin	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	49.4	52.4	57.5	60.1	68.4	69.3
13C3-N15-Ciprofloxacin	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	49.4	52.4	57.5	60.1	68.4	69.3
13C3-Trimethoprim	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	111	98.7	78.2	94.2	80.0	82.5
13C3-Trimethoprim	111	98.7	78.2	94.2	80.0	82.5
13C6-Sulfamethazine	111	98.7	78.2	94.2	80.0	82.5
13C3-N15-Ciprofloxacin	73.2	84.6	82.9	109	77.4	81.7
13C6-Sulfamethazine	49.4	52.4	57.5	60.1	68.4	69.3
13C6-Sulfamethazine	73.2	84.6	82.9	109	77.4	81.7
13C6-Sulfamethoxazole	73.2	84.6	82.9	109	77.4	81.7
13C6-Sulfamethazine	92.2	113	110	110	95.8	101
13C6-Sulfamethazine	73.2	84.6	82.9	109	77.4	81.7
13C6-Sulfamethoxazole	73.2	84.6	82.9	109	77.4	81.7
13C6-Sulfamethoxazole	92.2	113	110	110	95.8	101
13C6-Sulfamethazine	92.2	113	110	110	95.8	101
13C6-Sulfamethoxazole	73.2	84.6	82.9	109	77.4	81.7
d6-Thiabendazole	92.2	113	110	110	95.8	101
13C3-Trimethoprim	65.3	73.3	65.9	111	63.6	68.5
13C6-Sulfamethazine	111	98.7	78.2	94.2	80	82.5
13C3-Trimethoprim	NA	NA	NA	NA	NA	NA
13C3-Caffeine	111	98.7	78.2	94.2	80	82.5

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	16	2	3	4	5	6
AXYS ID	L13550-15	L13550-2	L13550-3	L13550-4	L13550-5	L13550-6
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.03 g	1.07 L	1.06 L	1.07 L	1.08 L	1.07 L
UNITS	ng/g	ng/L	ng/L	ng/L	ng/L	ng/L
Miconazole	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Norfloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Norgestimate	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Ofloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Ormetoprim	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Oxacillin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Oxolinic Acid	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Penicillin G	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Penicillin V	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Roxithromycin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sarafloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfachloropyridazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfadiazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfadimethoxine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfamerazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfamethazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfamethizole	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfamethoxazole	188	24.2	57.2	32.9	< MRL	21.7
Sulfanilamide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfathiazole	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Thiabendazole	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Trimethoprim	< MRL	< MRL	50.0	< MRL	< MRL	< MRL
Tylosin	NA	NA	NA	NA	NA	NA
Virginiamycin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL

Surrogate recovery

CLIENT ID (Sample #)	16	2	3	4	5	6
AXYS ID	L13550-15	L13550-2	L13550-3	L13550-4	L13550-5	L13550-6
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.03 g	1.07 L	1.06 L	1.07 L	1.08 L	1.07 L
UNITS	ng/g	ng/L	ng/L	ng/L	ng/L	ng/L
13C3-N15-Ciprofloxacin	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	108	45.5	62.4	60.8	32.4	47.3
13C3-N15-Ciprofloxacin	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	108	45.5	62.4	60.8	32.4	47.3
13C3-Trimethoprim	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	96.6	90.6	84.0	97.1	93.5	84.6
13C3-Trimethoprim	96.6	90.6	84.0	97.1	93.5	84.6
13C6-Sulfamethazine	96.6	90.6	84.0	97.1	93.5	84.6
13C3-N15-Ciprofloxacin	113	66.8	72.4	82.9	123	67.3
13C6-Sulfamethazine	108	45.5	62.4	60.8	32.4	47.3
13C6-Sulfamethazine	113	66.8	72.4	82.9	123	67.3
13C6-Sulfamethoxazole	113	66.8	72.4	82.9	123	67.3
13C6-Sulfamethazine	139	97.1	93.3	104	110	83.8
13C6-Sulfamethazine	113	66.8	72.4	82.9	123	67.3
13C6-Sulfamethoxazole	113	66.8	72.4	82.9	123	67.3
13C6-Sulfamethoxazole	139	97.1	93.3	104	110	83.8
13C6-Sulfamethazine	139	97.1	93.3	104	110	83.8
13C6-Sulfamethoxazole	113	66.8	72.4	82.9	123	67.3
d6-Thiabendazole	139	97.1	93.3	104	110	83.8
13C3-Trimethoprim	67.3	67.4	62.7	72.0	113	60.3
13C6-Sulfamethazine	96.6	90.6	84	97.1	93.5	84.6
13C3-Trimethoprim	NA	NA	NA	NA	NA	NA
13C3-Caffeine	96.6	90.6	84.0	97.1	93.5	84.6

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-7	L13550-8	L13550-9	WG30337-101	WG30337-102
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.05 L	1.01 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	% Recov
Miconazole	< MRL	< MRL	< MRL	< MRL	93.3
Norfloxacin	< MRL	< MRL	< MRL	< MRL	177
Norgestimate	< MRL	< MRL	< MRL	< MRL	88.1
Ofloxacin	< MRL	< MRL	< MRL	< MRL	196
Ormetoprim	< MRL	< MRL	< MRL	< MRL	110
Oxacillin	< MRL	< MRL	< MRL	< MRL	43.9
Oxolinic Acid	< MRL	< MRL	< MRL	< MRL	145
Penicillin G	< MRL	< MRL	< MRL	< MRL	49.1
Penicillin V	< MRL	< MRL	< MRL	< MRL	71.5
Roxithromycin	< MRL	< MRL	< MRL	< MRL	102
Sarafloxacin	< MRL	< MRL	< MRL	< MRL	156
Sulfachloropyridazine	< MRL	< MRL	< MRL	< MRL	117
Sulfadiazine	< MRL	< MRL	< MRL	< MRL	112
Sulfadimethoxine	< MRL	< MRL	< MRL	< MRL	99.1
Sulfamerazine	< MRL	< MRL	< MRL	< MRL	99.6
Sulfamethazine	< MRL	< MRL	< MRL	< MRL	101
Sulfamethizole	< MRL	< MRL	< MRL	< MRL	80.3
Sulfamethoxazole	213	35.9	16.5	< MRL	88.3
Sulfanilamide	< MRL	< MRL	< MRL	< MRL	48.8
Sulfathiazole	< MRL	< MRL	< MRL	< MRL	94.6
Thiabendazole	< MRL	< MRL	< MRL	< MRL	89.9
Trimethoprim	108	< MRL	< MRL	< MRL	110
Tylosin	NA	NA	NA	NA	NA
Virginiamycin	< MRL	< MRL	< MRL	< MRL	124

Surrogate recovery

CLIENT ID (Sample #)	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-7	L13550-8	L13550-9	WG30337-101	WG30337-102
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.05 L	1.01 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	% Recov
13C3-N15-Ciprofloxacin	81.9	84.6	90.1	94.3	58.3
13C3-Trimethoprim	65.9	63.1	73.6	42.7	105
13C3-N15-Ciprofloxacin	81.9	84.6	90.1	94.3	58.3
13C3-Trimethoprim	65.9	63.1	73.6	42.7	105
13C3-Trimethoprim	81.9	84.6	90.1	94.3	105
13C3-Trimethoprim	81.9	84.6	90.1	94.3	105
13C3-Trimethoprim	81.9	84.6	90.1	94.3	105
13C3-Trimethoprim	81.9	84.6	90.1	94.3	105
13C6-Sulfamethazine	81.9	84.6	90.1	94.3	93.1
13C3-N15-Ciprofloxacin	82.2	75.6	92.7	80.6	58.3
13C6-Sulfamethazine	65.9	63.1	73.6	42.7	93.1
13C6-Sulfamethazine	82.2	75.6	92.7	80.6	93.1
13C6-Sulfamethoxazole	82.2	75.6	92.7	80.6	100
13C6-Sulfamethazine	110	96.7	115	92.8	93.1
13C6-Sulfamethazine	82.2	75.6	92.7	80.6	93.1
13C6-Sulfamethoxazole	82.2	75.6	92.7	80.6	100
13C6-Sulfamethoxazole	110	96.7	115	92.8	100
13C6-Sulfamethazine	110	96.7	115	92.8	93.1
13C6-Sulfamethoxazole	82.2	75.6	92.7	80.6	100
d6-Thiabendazole	110	96.7	115	92.8	107
13C3-Trimethoprim	59.1	56.5	66.1	112	105
13C6-Sulfamethazine	81.9	84.6	90.1	94.3	93.1
13C3-Trimethoprim	NA	NA	NA	NA	105
13C3-Caffeine	81.9	84.6	90.1	94.3	119

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	17	18	19	20	21	22
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6
WORKGROUP	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338
Sample Size	1.02 L	1.06 L	1.04 L	1.05 L	1.06 L	1.05 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Miconazole	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Norfloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Norgestimate	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Ofloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Ormetoprim	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Oxacillin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Oxolinic Acid	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Penicillin G	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Penicillin V	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Roxithromycin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sarafloxacin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfachloropyridazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfadiazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfadimethoxine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfamerazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfamethazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfamethizole	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfamethoxazole	18.9	28.5	32.2	38.6	30.3	26.0
Sulfanilamide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sulfathiazole	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Thiabendazole	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Trimethoprim	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Tylosin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Virginiamycin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL

Surrogate recovery

CLIENT ID (Sample #)	17	18	19	20	21	22
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6
WORKGROUP	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338
Sample Size	1.02 L	1.06 L	1.04 L	1.05 L	1.06 L	1.05 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
13C3-N15-Ciprofloxacin	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	95.2	95.1	72.0	88.1	97.3	142
13C3-N15-Ciprofloxacin	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	95.2	95.1	72.0	88.1	97.3	142
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	84.9	86.8	83.1	89.1	70.0	117
13C6-Sulfamethazine	84.9	86.8	83.1	89.1	70.0	117
13C3-N15-Ciprofloxacin	46.9	49.5	60.4	61.7	49.8	79.7
13C6-Sulfamethazine	95.2	95.1	72.0	88.1	97.3	142
13C6-Sulfamethazine	46.9	49.5	60.4	61.7	49.8	79.7
13C6-Sulfamethoxazole	46.9	49.5	60.4	61.7	49.8	79.7
13C6-Sulfamethazine	61.5	63.5	83.7	82.1	65.6	108
13C6-Sulfamethazine	46.9	49.5	60.4	61.7	49.8	79.7
13C6-Sulfamethoxazole	46.9	49.5	60.4	61.7	49.8	79.7
13C6-Sulfamethoxazole	61.5	63.5	83.7	82.1	65.6	108
13C6-Sulfamethazine	61.5	63.5	83.7	82.1	65.6	108
13C6-Sulfamethoxazole	46.9	49.5	60.4	61.7	49.8	79.7
d6-Thiabendazole	61.5	63.5	83.7	82.1	65.6	108
13C3-Trimethoprim	77.6	73.5	67.4	70.7	66.5	100
13C6-Sulfamethazine	84.9	86.8	83.1	89.1	70.0	117
13C3-Trimethoprim	46.9	49.5	60.4	61.7	49.8	79.7
13C3-Caffeine	84.9	86.8	83.1	89.1	70.0	117

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-7	L13603-8	WG30338-101	WG30338-102
WORKGROUP	WG30338	WG30338	WG30338	WG30338
Sample Size	1.05 L	1.06 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
Miconazole	< MRL	< MRL	< MRL	101
Norfloxacin	< MRL	< MRL	< MRL	121
Norgestimate	< MRL	< MRL	< MRL	79.1
Ofloxacin	< MRL	504	< MRL	122
Ormetoprim	< MRL	< MRL	< MRL	94.1
Oxacillin	< MRL	< MRL	< MRL	37.5
Oxolinic Acid	< MRL	< MRL	< MRL	111
Penicillin G	< MRL	< MRL	< MRL	33.0
Penicillin V	< MRL	< MRL	< MRL	65.0
Roxithromycin	< MRL	< MRL	< MRL	95.6
Sarafloxacin	< MRL	< MRL	< MRL	97.9
Sulfachloropyridazine	< MRL	< MRL	< MRL	113
Sulfadiazine	< MRL	7.45	< MRL	86.0
Sulfadimethoxine	< MRL	< MRL	< MRL	104
Sulfamerazine	< MRL	< MRL	< MRL	86.1
Sulfamethazine	< MRL	< MRL	< MRL	113
Sulfamethizole	< MRL	< MRL	< MRL	89.9
Sulfamethoxazole	6.77	481	< MRL	94.7
Sulfanilamide	< MRL	< MRL	< MRL	41.4
Sulfathiazole	2.70	< MRL	< MRL	91.5
Thiabendazole	< MRL	10.8	< MRL	97.0
Trimethoprim	< MRL	187	< MRL	90.5
Tylosin	< MRL	< MRL	< MRL	91.5
Virginiamycin	< MRL	< MRL	< MRL	118

Surrogate recovery

CLIENT ID (Sample #)	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-7	L13603-8	WG30338-101	WG30338-102
WORKGROUP	WG30338	WG30338	WG30338	WG30338
Sample Size	1.05 L	1.06 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
13C3-N15-Ciprofloxacin	80.2	86.9	86.5	108
13C3-Trimethoprim	91.2	77.6	112	98.2
13C3-N15-Ciprofloxacin	80.2	86.9	86.5	108
13C3-Trimethoprim	91.2	77.6	112	98.2
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C3-Trimethoprim	80.2	86.9	86.5	98.2
13C6-Sulfamethazine	80.2	86.9	86.5	62.6
13C3-N15-Ciprofloxacin	54.8	38.6	70.1	108
13C6-Sulfamethazine	91.2	77.6	112	62.6
13C6-Sulfamethazine	54.8	38.6	70.1	62.6
13C6-Sulfamethoxazole	54.8	38.6	70.1	64.2
13C6-Sulfamethazine	63.4	35.8	73.5	62.6
13C6-Sulfamethazine	54.8	38.6	70.1	62.6
13C6-Sulfamethoxazole	54.8	38.6	70.1	64.2
13C6-Sulfamethoxazole	63.4	35.8	73.5	64.2
13C6-Sulfamethazine	63.4	35.8	73.5	62.6
13C6-Sulfamethoxazole	54.8	38.6	70.1	64.2
d6-Thiabendazole	63.4	35.8	73.5	104
13C3-Trimethoprim	71.4	53.2	103	98.2
13C6-Sulfamethazine	80.2	86.9	86.5	62.6
13C3-Trimethoprim	54.8	38.6	70.1	98.2
13C3-Caffeine	80.2	86.9	86.5	120

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	1	10	12	13	14	15	16	2	3
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	L13550-2	L13550-3
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.07 L	1.07 L	1.03 L	1.06 L	1.03 L	1.03 L	1.07 L	1.06 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Alprazolam	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Amitriptyline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	1.97
10-Hydroxy-amitriptyline	< MRL	< MRL	< MRL	< MRL	< MRL	0.329	0.304	0.202	0.414
Amlodipine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Benzoylecgonine	1.09	< MRL	1.70	< MRL	1.83	4.94	5.49	6.72	3.24
Benztropine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Betamethasone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Cocaine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	0.827	0.341	0.172
DEET	17.5	16.4	24.6	< MRL	19.6	19.3	36.4	30.9	26.5
Desmethyldiltiazem	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	1.19	0.729	8.24
Diazepam	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Fluocinonide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Fluticasone propionate	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Hydrocortisone	NA	NA	NA	NA	NA	NA	NA	NA	NA
Meprobamate	< MRL	13.7	26.3	< MRL	15.0	24.1	43.2	< MRL	27.3
Methylprednisolone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL

Surrogate recovery

CLIENT ID (Sample #)	1	10	12	13	14	15	16	2	3
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	L13550-2	L13550-3
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.07 L	1.07 L	1.03 L	1.06 L	1.03 L	1.03 L	1.07 L	1.06 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
d5-Alprazolam	88.6	91.4	95.5	80.8	84.1	92.4	94.6	90.1	96.9
d6-Amitriptyline	61.7	96.6	100	67.7	87.4	85.9	94.7	77.3	103
d7-Propranolol	115	116	106	97.1	95.4	105	107	121	116
d5-Norfluoxetine	90.2	132	140	62.3	111	119	136	94.0	116
d8-Benzoylecgonine	91.5	87.0	80.8	94.9	79.9	76.1	74.0	83.6	96.3
d3-Benztropine	89.4	94.4	88.1	73.6	80.5	85.9	83.8	68.5	103
d6-Amitriptyline	61.7	96.6	100	67.7	87.4	85.9	94.7	77.3	103
d3-Cocaine	84.6	86.5	86.4	62.4	74.4	76.4	76.6	85.2	96.2
d7-DEET	95.4	101	104	88.5	88.2	95.8	97.0	90.9	95.2
d4-Promethazine	88.3	87.0	95.1	89.0	76.0	85.7	82.3	90.1	87.0
d5-Diazepam	106	99.2	107	94.5	89.3	99.5	80.7	101	95.0
d5-Alprazolam	88.6	91.4	95.5	80.8	84.1	92.4	94.6	90.1	96.9
d7-Metoprolol	124	102	104	95.6	98.5	98.2	110	119	118
d4-Hydrocortisone	144	69.1	234	47.4	82.7	76.9	100	82.7	99.4
d7-Metoprolol	124	102	104	95.6	98.5	98.2	110	119	118
d2-Methylprednisolone	40.8	108	147	115	131	157	157	49.7	49.5

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	4	5	6	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-4	L13550-5	L13550-6	L13550-7	L13550-8	L13550-9	WG30337-101	WG30337-102
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.07 L	1.08 L	1.07 L	1.08 L	1.05 L	1.01 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Alprazolam	< MRL	< MRL	< MRL	1.49	< MRL	< MRL	< MRL	114
Amitriptyline	< MRL	< MRL	< MRL	4.92	< MRL	< MRL	< MRL	110
10-Hydroxy-amitriptyline	< MRL	< MRL	0.163	0.415	< MRL	< MRL	< MRL	96.7
Amlodipine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	108
Benzoylecgonine	3.10	< MRL	4.05	5.16	1.35	< MRL	< MRL	120
Benztropine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	101
Betamethasone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	127
Cocaine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	111
DEET	18.6	< MRL	20.7	41.2	23.8	11.2	< MRL	121
Desmethyldiltiazem	1.25	< MRL	< MRL	9.22	< MRL	< MRL	< MRL	63.7
Diazepam	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	113
Fluocinonide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	101
Fluticasone propionate	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	93.6
Hydrocortisone	NA	NA	NA	NA	NA	NA	NA	NA
Meprobamate	< MRL	< MRL	17.0	130	16.9	< MRL	< MRL	97.6
Methylprednisolone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	168

Surrogate recovery

CLIENT ID (Sample #)	4	5	6	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-4	L13550-5	L13550-6	L13550-7	L13550-8	L13550-9	WG30337-101	WG30337-102
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.07 L	1.08 L	1.07 L	1.08 L	1.05 L	1.01 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
d5-Alprazolam	89.6	70.2	84.6	109	98.7	88.2	88.6	77.7
d6-Amitriptyline	96.3	87.4	88.3	107	105	92.2	61.7	81.4
d7-Propranolol	126	97.3	106	116	119	105	115	101
d5-Norfluoxetine	121	51.5	112	147	139	125	90.2	40.8
d8-Benzoylecgonine	91.0	99.8	86.8	94.1	96.0	86.1	91.5	115
d3-Benztropine	97.8	79.9	81.1	106	92.6	90.4	89.4	94.0
d6-Amitriptyline	96.3	87.4	88.3	107	105	92.2	61.7	81.4
d3-Cocaine	86.2	72.5	82.3	95.5	89.0	79.5	84.6	72.9
d7-DEET	105	94.5	86.8	104	99.9	91.7	95.4	69.6
d4-Promethazine	92.4	82.6	78.9	106	92.6	84.7	88.3	62.0
d5-Diazepam	95.9	109	101	91.0	111	101	121	98.7
d5-Alprazolam	89.6	70.2	84.6	109	98.7	88.2	88.6	77.7
d7-Metoprolol	118	92.0	107	123	112	109	124	115
d4-Hydrocortisone	65.6	64.8	132	94.6	99.5	92.8	159	66.7
d7-Metoprolol	118	92.0	107	123	112	109	124	115
d2-Methylprednisolone	80.8	44.3	93.0	178	134	126	40.8	24.1

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	17	18	19	20	21	22	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6	L13603-7	L13603-8	WG30338-101	WG30338-102
WORKGROUP	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338
Sample Size	1.02 L	1.06 L	1.04 L	1.05 L	1.06 L	1.05 L	1.05 L	1.06 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Alprazolam	< MRL	< MRL	< MRL	2.43	< MRL	< MRL	< MRL	7.69	< MRL	105
Amitriptyline	< MRL	< MRL	< MRL	3.57	1.05	< MRL	1.09	77.8	< MRL	101
10-Hydroxy-amitriptyline	< MRL	< MRL	< MRL	0.292	< MRL	< MRL	< MRL	7.04	< MRL	101
Amlodipine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	3.48	< MRL	80.7
Benzoylecgonine	2.05	4.46	3.23	6.47	< MRL	1.26	< MRL	56.5	< MRL	101
Benztropine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	98.7
Betamethasone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	88.4
Cocaine	< MRL	0.434	< MRL	0.794	< MRL	< MRL	< MRL	7.91	< MRL	104
DEET	15.9	28.8	28.0	62.1	8.37	15.9	9.39	>100 E	< MRL	97.8
Desmethyldiltiazem	< MRL	< MRL	< MRL	1.00	< MRL	< MRL	< MRL	79.6	< MRL	172
Diazepam	< MRL	< MRL	< MRL	1.01	< MRL	< MRL	< MRL	4.12	< MRL	102
Fluocinonide	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	117
Fluticasone propionate	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	72.3
Hydrocortisone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	104
Meprobamate	< MRL	14.7	13.9	127	< MRL	13.6	< MRL	646	< MRL	88.3
Methylprednisolone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	127

Surrogate recovery

CLIENT ID (Sample #)	17	18	19	20	21	22	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6	L13603-7	L13603-8	WG30338-101	WG30338-102
WORKGROUP	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338
Sample Size	1.02 L	1.06 L	1.04 L	1.05 L	1.06 L	1.05 L	1.05 L	1.06 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
d5-Alprazolam	93.3	91.1	87.6	89.1	99.2	86.2	86.8	88.8	95.6	95.6
d6-Amitriptyline	95.0	92.2	89.3	87.0	98.5	84.9	86.8	79.1	99.9	99.9
d7-Propranolol	111	112	101	99.5	109	103	101	87.9	110	110
d5-Norfluoxetine	96.0	90.3	93.4	128	122	110	108	124	94.3	94.3
d8-Benzoylecgonine	98.7	88.8	82.6	79.2	89.7	85.8	81.0	67.0	110	110
d3-Benztropine	90.6	84.7	84.2	83.1	90.8	78.7	80.7	78.5	93.8	93.8
d6-Amitriptyline	95.0	92.2	89.3	87.0	98.5	84.9	86.8	79.1	99.9	99.9
d3-Cocaine	91.1	87.8	81.9	83.7	93.7	81.4	81.8	81.8	97.5	97.5
d7-DEET	95.9	99.5	99.0	99.7	98.8	92.2	95.1	99.9	95.5	95.5
d4-Promethazine	71.6	64.1	67.8	65.3	78.8	68.1	67.7	64.8	67.0	67.0
d5-Diazepam	98.2	96.8	97.4	98.8	103	92.9	93.8	94.3	108	108
d5-Alprazolam	93.3	91.1	87.6	89.1	99.2	86.2	86.8	88.8	95.6	95.6
d7-Metoprolol	114	108	97.1	93.6	107	97.3	92.5	82.7	106	106
d4-Hydrocortisone	99.3	78.3	78.1	109	149	159	83.7	134	111	111
d7-Metoprolol	114	108	97.1	93.6	107	97.3	92.5	82.7	106	106
d2-Methylprednisolone	90.9	76.1	80.4	132	129	127	117	132	91.7	91.7

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30659-101	WG30659-102
WORKGROUP	WG30659	WG30659	WG30659	WG30659
Sample Size	1.10 L	1.04 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
Alprazolam	< MRL	< MRL	< MRL	112
Amitriptyline	4.79	< MRL	< MRL	99.3
10-Hydroxy-amitriptyline	1.25	< MRL	< MRL	99.4
Amlodipine	< MRL	< MRL	< MRL	94.6
Benzoylecgonine	6.07	< MRL	< MRL	102
Benztropine	< MRL	< MRL	< MRL	112
Betamethasone	< MRL	< MRL	< MRL	103
Cocaine	0.784	< MRL	< MRL	110
DEET	26.0	< MRL	< MRL	78.2
Desmethyldiltiazem	7.60	< MRL	< MRL	109
Diazepam	< MRL	< MRL	< MRL	99.1
Fluocinonide	< MRL	< MRL	< MRL	139
Fluticasone propionate	2.02	< MRL	< MRL	87.3
Hydrocortisone	< MRL	< MRL	< MRL	107
Meprobamate	111	< MRL	< MRL	95.4
Methylprednisolone	< MRL	< MRL	< MRL	97.6

Surrogate recovery

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30659-101	WG30659-102
WORKGROUP	WG30659	WG30659	WG30659	WG30659
Sample Size	1.10 L	1.04 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
d5-Alprazolam	83.7	69.3	75.5	75.5
d6-Amitriptyline	85.1	86.7	78.6	78.6
d7-Propranolol	106	91.3	95.6	95.6
d5-Norfluoxetine	104	85.0	51.2	51.2
d8-Benzoylecgonine	73.4	93.2	95.1	95.1
d3-Benztropine	78.0	80.4	73.9	73.9
d6-Amitriptyline	85.1	86.7	78.6	78.6
d3-Cocaine	90.1	85.7	51.3	51.3
d7-DEET	90.1	76.2	86.4	86.4
d4-Promethazine	78.6	77.8	71.1	71.1
d5-Diazepam	87.9	103	96.6	96.6
d5-Alprazolam	83.7	69.3	75.5	75.5
d7-Metoprolol	104	88.1	96.2	96.2
d4-Hydrocortisone	112	112	128	128
d7-Metoprolol	104	88.1	96.2	96.2
d2-Methylprednisolone	90.5	101	80.5	80.5

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	1	10	12	13	14	15	16	2	3
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	L13550-2	L13550-3
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.07 L	1.07 L	1.03 L	1.06 L	1.03 L	1.03 L	1.07 L	1.06 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Metoprolol	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	18.8	19.2	42.3
Norfluoxetine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Norverapamil	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Paroxetine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Prednisolone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Prednisone	NA	NA	NA	NA	NA	NA	NA	NA	NA
Promethazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Propoxyphene	1.15	< MRL	< MRL	< MRL	< MRL	< MRL	1.35	< MRL	3.94
Propranolol	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Sertraline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	3.39
Simvastatin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Theophylline	< MRL	< MRL	< MRL	< MRL	< MRL	204	< MRL	< MRL	< MRL
Trenbolone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Trenbolone acetate	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Valsartan	27.2 S	< MRL	17.6 S	< MRL	< MRL	40.4 S	18.1 S	31.2 S	87.5 S
Verapamil	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	2.86

Surrogate recovery

CLIENT ID (Sample #)	1	10	12	13	14	15	16	2	3
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	L13550-2	L13550-3
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.07 L	1.07 L	1.03 L	1.06 L	1.03 L	1.03 L	1.07 L	1.06 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
d7-Metoprolol	124	102	104	95.6	98.5	98.2	110	119	118
d5-Norfluoxetine	90.2	132	140	62.3	111	119	136	94.0	116
d7-Propranolol	115	116	106	97.1	95.4	105	107	121	116
d6-Paroxetine	103	89.7	97.9	71.5	87.3	87.7	90.6	93.1	97.1
d7-Propranolol	115	116	106	97.1	95.4	105	107	121	116
d7-Propranolol	115	116	106	97.1	95.4	105	107	121	116
d4-Promethazine	88.3	87	95.1	89.0	76.0	85.7	82.3	90.1	87.0
d5-Propoxyphene	67.2	127	122	112	112	125	119	108	100
d7-Propranolol	115	116	106	97.1	95.4	105	107	121	116
d7-Propranolol	115	116	106	97.1	95.4	105	107	121	116
d5-Propoxyphene	67.2	127	122	112	112	125	119	108	100
13C1-15N2-Theophylline	86.2	92.5	75.9	103	76.6	54.2	86.0	83.5	66.5
d5-Alprazolam	88.6	91.4	95.5	80.8	84.1	92.4	94.6	90.1	96.9
d5-Alprazolam	88.6	91.4	95.5	80.8	84.1	92.4	94.6	90.1	96.9
d5-Propoxyphene	67.2	127	122	112	112	125	119	108	100
d6-Amitriptyline	61.7	96.6	100	67.7	87.4	85.9	94.7	77.3	103

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	4	5	6	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-4	L13550-5	L13550-6	L13550-7	L13550-8	L13550-9	WG30337-101	WG30337-102
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.07 L	1.08 L	1.07 L	1.08 L	1.05 L	1.01 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Metoprolol	17.8	< MRL	< MRL	94.5	< MRL	< MRL	< MRL	112
Norfluoxetine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	104
Norverapamil	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	122
Paroxetine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	107
Prednisolone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	197
Prednisone	NA	NA	NA	NA	NA	NA	NA	NA
Promethazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	90.8
Propoxyphene	< MRL	< MRL	1.73	13.5	< MRL	< MRL	< MRL	90.5
Propranolol	< MRL	< MRL	< MRL	10.1	< MRL	< MRL	< MRL	102
Sertraline	< MRL	< MRL	< MRL	6.02	< MRL	< MRL	< MRL	105
Simvastatin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	20.7
Theophylline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	485
Trenbolone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	124
Trenbolone acetate	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	124
Valsartan	40.9 S	< MRL	22.0 S	18.9 S	< MRL	< MRL	< MRL	134
Verapamil	< MRL	< MRL	< MRL	3.05	< MRL	< MRL	< MRL	94.9

Surrogate recovery

CLIENT ID (Sample #)	4	5	6	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-4	L13550-5	L13550-6	L13550-7	L13550-8	L13550-9	WG30337-101	WG30337-102
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.07 L	1.08 L	1.07 L	1.08 L	1.05 L	1.01 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
d7-Metoprolol	118	92.0	107	123	112	109	124	115
d5-Norfluoxetine	121	51.5	112	147	139	125	90.2	40.8
d7-Propranolol	126	97.3	106	116	119	105	115	101
d6-Paroxetine	93.3	75.8	88.5	111	101	83.5	118	68.2
d7-Propranolol	126	97.3	106	116	119	105	115	101
d7-Propranolol	126	97.3	106	116	119	105	115	101
d4-Promethazine	92.4	82.6	78.9	106	92.6	84.7	88.3	62.0
d5-Propoxyphene	120	106	85.9	118	126	115	67.2	63.7
d7-Propranolol	126	97.3	106	116	119	105	115	101
d7-Propranolol	126	97.3	106	116	119	105	115	101
d5-Propoxyphene	120	106	85.9	118	126	115	67.2	63.7
13C1-15N2-Theophylline	78.2	110	66.9	85.3	86.3	61.1	101.2	110
d5-Alprazolam	89.6	70.2	84.6	109	98.7	88.2	88.6	77.7
d5-Alprazolam	89.6	70.2	84.6	109	98.7	88.2	88.6	77.7
d5-Propoxyphene	120	106	85.9	118	126	115	67.2	63.7
d6-Amitriptyline	96.3	87.4	88.3	107	105	92.2	61.7	81.4

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	17	18	19	20	21	22	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6	L13603-7	L13603-8	WG30338-101	WG30338-102
WORKGROUP	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338
Sample Size	1.02 L	1.06 L	1.04 L	1.05 L	1.06 L	1.05 L	1.05 L	1.06 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Metoprolol	< MRL	< MRL	< MRL	83.5	< MRL	< MRL	< MRL	701	< MRL	98.6
Norfluoxetine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	2.22	< MRL	97.0
Norverapamil	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	3.45	< MRL	89.4
Paroxetine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	5.54	< MRL	103
Prednisolone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	100
Prednisone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	79.5
Promethazine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	129
Propoxyphene	< MRL	< MRL	< MRL	14.7	< MRL	< MRL	< MRL	123	< MRL	101
Propranolol	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	96.0	< MRL	109
Sertraline	< MRL	< MRL	< MRL	4.27	< MRL	< MRL	< MRL	64.6	< MRL	91.6
Simvastatin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	41.7
Theophylline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	304	< MRL	439
Trenbolone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	131
Trenbolone acetate	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	94.3
Valsartan	17.9	22.3	< MRL	41.1	< MRL	< MRL	< MRL	625	< MRL	90.6
Verapamil	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	64.3 S	< MRL	98.2

Surrogate recovery

CLIENT ID (Sample #)	17	18	19	20	21	22	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6	L13603-7	L13603-8	WG30338-101	WG30338-102
WORKGROUP	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338
Sample Size	1.02 L	1.06 L	1.04 L	1.05 L	1.06 L	1.05 L	1.05 L	1.06 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
d7-Metoprolol	114	108	97.1	93.6	107	97.3	92.5	82.7	106	106
d5-Norfluoxetine	96.0	90.3	93.4	128	122	110	108	124	94.3	94.3
d7-Propranolol	111	112	101	99.5	109	103	101	87.9	110	110
d6-Paroxetine	97.8	91.5	90.2	91.6	97.6	83.8	82.5	77.2	101	101
d7-Propranolol	111	112	101	99.5	109	103	101	87.9	110	110
d7-Propranolol	111	112	101	99.5	109	103	101	87.9	110	110
d4-Promethazine	71.6	64.1	67.8	65.3	78.8	68.1	67.7	64.8	67.0	67.0
d5-Propoxyphene	82.2	70.9	76.0	89.6	107	88.0	89.8	79.9	96.0	96.0
d7-Propranolol	111	112	101	99.5	109	103	101	87.9	110	110
d7-Propranolol	111	112	101	99.5	109	103	101	87.9	100	110
d5-Propoxyphene	82.2	70.9	76.0	89.6	107	88.0	89.8	79.9	96.0	96.0
13C1-15N2-Theophylline	83.2	61.1	52.0	53.0	50.6	64.1	76.4	51.9	106	106
d5-Alprazolam	93.3	91.1	87.6	89.1	99.2	86.2	86.8	88.8	95.6	95.6
d5-Alprazolam	93.3	91.1	87.6	89.1	99.2	86.2	86.8	88.8	95.6	95.6
d5-Propoxyphene	82.2	70.9	76.0	89.6	107	88.0	89.8	79.9	96.0	96.0
d6-Amitriptyline	95.0	92.2	89.3	87.0	98.5	84.9	86.8	79.1	99.9	99.9

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30659-101	WG30659-102
WORKGROUP	WG30659	WG30659	WG30659	WG30659
Sample Size	1.10 L	1.04 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
Metoprolol	97.5	< MRL	< MRL	105
Norfluoxetine	< MRL	< MRL	< MRL	104
Norverapamil	0.153	< MRL	< MRL	85.1
Paroxetine	< MRL	< MRL	< MRL	110
Prednisolone	< MRL	< MRL	< MRL	100
Prednisone	< MRL	< MRL	< MRL	105
Promethazine	< MRL	< MRL	< MRL	97.1
Propoxyphene	7.77	< MRL	< MRL	117
Propranolol	10.7	< MRL	< MRL	109
Sertraline	3.08	< MRL	< MRL	78.8
Simvastatin	< MRL	< MRL	< MRL	61.9
Theophylline	< MRL	< MRL	< MRL	364
Trenbolone	< MRL	< MRL	< MRL	160
Trenbolone acetate	< MRL	< MRL	< MRL	117
Valsartan	79.2	< MRL	< MRL	102
Verapamil	2.44 S	< MRL	< MRL	102

Surrogate recovery

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30659-101	WG30659-102
WORKGROUP	WG30659	WG30659	WG30659	WG30659
Sample Size	1.10 L	1.04 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
d7-Metoprolol	104	88.1	96.2	96.2
d5-Norfluoxetine	104	85.0	51.2	51.2
d7-Propranolol	106	91.3	95.6	95.6
d6-Paroxetine	98.4	77.6	78.0	78.0
d7-Propranolol	106	91.3	95.6	95.6
d7-Propranolol	106	91.3	95.6	95.6
d4-Promethazine	78.6	77.8	71.1	71.1
d5-Propoxyphene	79.1	104	85.2	85.2
d7-Propranolol	106	91.3	95.6	95.6
d7-Propranolol	106	91.3	95.6	95.6
d5-Propoxyphene	79.1	104	85.2	85.2
13C1-15N2-Theophylline	70.1	95.5	112	112
d5-Alprazolam	83.7	69.3	75.5	75.5
d5-Alprazolam	83.7	69.3	75.5	75.5
d5-Propoxyphene	79.1	104	85.2	85.2
d6-Amitriptyline	85.1	86.7	78.6	78.6

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30661-101	WG30661-102
WORKGROUP	WG30661	WG30661	WG30661	WG30661
Sample Size	1.10 L	1.05 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
Albuterol	1.39	< MRL	< MRL	93.8
Amphetamine	< MRL	< MRL	< MRL	87.0
Atenolol	152	< MRL	< MRL	100
Atorvastatin	< MRL	< MRL	< MRL	78.6
Cimetidine	2.37	< MRL	< MRL	103
Clonidine	< MRL	< MRL	< MRL	126
Codeine	11.8	< MRL	< MRL	125
Cotinine	26.8	< MRL	< MRL	99.0
Enalapril	< MRL	< MRL	< MRL	95.4
Hydrocodone	7.96	< MRL	< MRL	116
Metformin	1460	< MRL	< MRL	119
Oxycodone	20.7	< MRL	< MRL	104
Ranitidine	4.17	< MRL	< MRL	53.1
Triamterene	32.4	< MRL	< MRL	109

Surrogate recovery

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30661-101	WG30661-102
WORKGROUP	WG30661	WG30661	WG30661	WG30661
Sample Size	1.10 L	1.05 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
d3-Albuterol	120	106	94.5	98.5
d5-Amphetamine	43.5	62.0	51.1	50.1
d7-Atenolol	130	113	95.1	106
d5-Enalapril	97.2	91.9	95.7	91.9
d3-Cimetidine	95.7	71.2	71.4	52.2
d4-Clonidine	129	99.2	102	94.3
d6-Codeine	110	106	97.8	84.6
d3-Cotinine	82.1	104	102	105
d5-Enalapril	97.2	91.9	95.7	91.9
d3-Hydrocodone	100	103	80.9	80.7
d6-Metformin	10.2	41.0	40.7	31.0
d6-Oxycodone	104	107	101	89.9
d3-Albuterol	120	106	94.5	98.5
d4-Clonidine	129	99.2	102	94.3

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	17	18	19	20	21	22	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6	L13603-7	L13603-8	WG30313-101	WG30313-102
WORKGROUP	WG30313	WG30313	WG30313	WG30313	WG30313	WG30313	WG30313	WG30313	WG30313	WG30313
Sample Size	1.05 L	1.02 L	1.05 L	1.04 L	1.05 L	1.06 L	1.05 L	1.04 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Albuterol	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	17.4	< MRL	98.1
Amphetamine	< MRL	< MRL	< MRL	5.36	< MRL	< MRL	< MRL	91.9 V	< MRL	99.0
Atenolol	6.61	8.28	6.49	137	< MRL	2.81	< MRL	502 V	< MRL	106
Atorvastatin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	78.5
Cimetidine	< MRL	< MRL	< MRL	1.57	< MRL	< MRL	< MRL	49.3	< MRL	102
Clonidine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	105
Codeine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	88.2	< MRL	101
Cotinine	14.8	15.5	13.4 V	31.5 V	7.94	10.4	7.76	51.3	< MRL	97.4
Enalapril	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	98.9
Hydrocodone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	159	< MRL	127
Metformin	597	736	870	1490	476	395	< MRL	15300	< MRL	116
Oxycodone	< MRL	< MRL	< MRL	12.5	< MRL	< MRL	< MRL	66.1	< MRL	103
Ranitidine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	203	< MRL	60.3
Triamterene	3.11	2.41	1.66	30.7	2.59	1.79	< MRL	200 V	< MRL	85.6

Surrogate recovery

CLIENT ID (Sample #)	17	18	19	20	21	22	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6	L13603-7	L13603-8	WG30313-101	WG30313-102
WORKGROUP	WG30313	WG30313	WG30313	WG30313	WG30313	WG30313	WG30313	WG30313	WG30313	WG30313
Sample Size	1.05 L	1.02 L	1.05 L	1.04 L	1.05 L	1.06 L	1.05 L	1.04 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
d3-Albuterol	86.4	99.2	75.8	87.6	78.9	77.4	81.5	107	75.7	81.1
d5-Amphetamine	47.6	55.6	46.5	34.1	39.1	32.5	45.5	14.2	54.2	44.6
d7-Atenolol	99.0	108	89.3	105	101	87.6	84.1	149	94.8	91.8
d5-Enalapril	85.3	96.2	65.7	77.2	40.9	65.1	65.3	116	88.6	86.7
d3-Cimetidine	72.9	79.8	59.4	63.7	66.6	63.3	61.9	120	58.7	48.8
d4-Clonidine	89.5	93.1	76.5	88.3	84.2	89.7	90.6	137	89.1	89.1
d6-Codeine	108	114	88.6	108	105	97.4	95.2	130	93.5	96.3
d3-Cotinine	75.4	86.0	68.0	60.5	71.1	73.8	76.4	107	99.0	99.2
d5-Enalapril	85.3	96.2	65.7	77.2	40.9	65.1	65.3	116	88.6	86.7
d3-Hydrocodone	97.1	109	88.3	93.6	91.5	100	91.7	126	77.5	69.9
d6-Metformin	13.5	14.7	10.2	12.3	10.1	24.2	13.4	12.2	56.0	38.1
d6-Oxycodone	89.9	107	84.9	81.7	79.8	86.0	95.5	85.8	103	69.5
d3-Albuterol	86.4	99.2	75.8	87.6	78.9	77.4	81.5	107	75.7	81.1
d4-Clonidine	89.5	93.1	76.5	88.3	84.2	89.7	90.6	137	89.1	89.1

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	1	10	12	13	14	15	16	2	3	4	5
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	L13550-2	L13550-3	L13550-4	L13550-5
WORKGROUP	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278
Sample Size	1.03 L	1.05 L	1.03 L	1.02 L	1.04 L	1.05 L	1.02 L	1.05 L	1.05 L	1.05 L	1.02 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Albuterol	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	1.20	< MRL	< MRL
Amphetamine	< MRL	< MRL	< MRL	< MRL	< MRL	13.0	5.10	< MRL	< MRL	< MRL	< MRL
Atenolol	8.53	4.24	9.46	< MRL	8.05	16.5	29.0	28.5 V	99.5	34.1 V	< MRL
Atorvastatin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Cimetidine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	2.96	< MRL	< MRL	< MRL	< MRL
Clonidine	< MRL	< MRL	< MRL	2.73	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Codeine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Cotinine	8.83	10.5	14.3	< MRL	13.9	19.9	21.6	12.3	21.1	12.6	< MRL
Enalapril	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Hydrocodone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	5.19	< MRL	6.34	< MRL	< MRL
Metformin	324	325	604	< MRL	410	651	467	948	3760	1240	< MRL
Oxycodone	< MRL	< MRL	< MRL	< MRL	< MRL	3.28	5.27	3.87	13.3	3.39	< MRL
Ranitidine	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Triamterene	1.54	1.89	4.31	< MRL	2.49	4.84	18.3	3.64 V	15.0	4.39 V	< MRL

Surrogate recovery

CLIENT ID (Sample #)	1	10	12	13	14	15	16	2	3	4	5
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	L13550-2	L13550-3	L13550-4	L13550-5
WORKGROUP	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278
Sample Size	1.03 L	1.05 L	1.03 L	1.02 L	1.04 L	1.05 L	1.02 L	1.05 L	1.05 L	1.05 L	1.02 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
d3-Albuterol	91.7	96.5	89.5	102	108	89.2	103	140	95.1	138	97.0
d5-Amphetamine	56.2	50.8	48.9	49.3	60.6	48.4	57.4	88.3	49.9	86.5	73.1
d7-Atenolol	103	123	101	109	124	107	128	168	112	159	105
d5-Enalapril	84.5	98.3	86.8	113	110	96.9	103	140	94.4	140	109
d3-Cimetidine	68.4	77.3	69.2	62.6	80.3	76.9	90.3	102	80.2	109	58.8
d4-Clonidine	92.6	97.6	91.5	103	110	101	117	152	90.5	142	92.6
d6-Codeine	107	116	102	113	117	100	117	169	115	161	106
d3-Cotinine	77.0	81.7	71.4	104	88.1	72.1	71.6	123	76.8	113	104
d5-Enalapril	84.5	98.3	86.8	113	110	96.9	103	140	94.4	140	109
d3-Hydrocodone	102	114	100	103	123	101	113	169	104	150	96.2
d6-Metformin	13.1	14.7	10.5	91.6	13.6	11.8	6.05	12.5	11.3	18.6	112
d6-Oxycodone	87.6	89.8	91.3	118	107	90.0	108	150	91.4	123	87.2
d3-Albuterol	91.7	96.5	89.5	102	108	89.2	103	140	95.1	138	97.0
d4-Clonidine	92.6	97.6	91.5	103	110	101	117	152	90.5	142	92.6

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	6	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-6	L13550-7	L13550-8	L13550-9	WG30278-101	WG30278-102
WORKGROUP	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278
Sample Size	1.09 L	1.06 L	1.05 L	1.06 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Albuterol	< MRL	5.16	< MRL	< MRL	< MRL	97.7
Amphetamine	< MRL	7.23	< MRL	< MRL	< MRL	99.5
Atenolol	24.1	269	8.62	6.78	< MRL	87.4
Atorvastatin	< MRL	< MRL	< MRL	< MRL	< MRL	90.8
Cimetidine	< MRL	21.5	< MRL	< MRL	< MRL	99.8
Clonidine	< MRL	< MRL	< MRL	< MRL	< MRL	113
Codeine	< MRL	< MRL	< MRL	< MRL	< MRL	95.9
Cotinine	12.3	17.6	12.5	7.56	< MRL	96.7
Enalapril	< MRL	< MRL	< MRL	< MRL	< MRL	95.8
Hydrocodone	< MRL	18.3	< MRL	< MRL	< MRL	101
Metformin	585	1550	489	< MRL	< MRL	104
Oxycodone	2.64	26.3	< MRL	< MRL	< MRL	117
Ranitidine	< MRL	22.8	< MRL	< MRL	< MRL	42.1
Triamterene	3.83	23.0	2.53	1.97	< MRL	97.6

Surrogate recovery

CLIENT ID (Sample #)	6	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-6	L13550-7	L13550-8	L13550-9	WG30278-101	WG30278-102
WORKGROUP	WG30278	WG30278	WG30278	WG30278	WG30278	WG30278
Sample Size	1.09 L	1.06 L	1.05 L	1.06 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
d3-Albuterol	98.6	103	97.1	86.6	105	95.2
d5-Amphetamine	54.5	51.4	54.4	50.4	71.0	60.5
d7-Atenolol	119	121	105	92.2	116	102
d5-Enalapril	95.2	107	99.0	81.4	103	103
d3-Cimetidine	65.8	87.7	69.8	64.9	48.2	48.0
d4-Clonidine	93.9	109	99.0	88.5	103	98.3
d6-Codeine	115	129	112	98.2	108	102
d3-Cotinine	74.2	83.1	75.1	70.1	122	108
d5-Enalapril	95.2	107	99.0	81.4	103	103
d3-Hydrocodone	109	113	112	103	82.6	83.4
d6-Metformin	12.6	13.1	11.8	13.8	121	94.7
d6-Oxycodone	99.5	99.0	91.6	84.8	92.9	77.6
d3-Albuterol	98.6	103	97.1	86.6	105	95.2
d4-Clonidine	93.9	109	99.0	88.5	103	98.3

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30659-101	WG30659-102
WORKGROUP	WG30659	WG30659	WG30659	WG30659
Sample Size	1.10 L	1.04 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
Anhydrochlortetracycline [ACTC]	< MRL	< MRL	< MRL	65.8
Anhydrotetracycline [ATC]	< MRL	< MRL	< MRL	60.8
Chlortetracycline [CTC]	< MRL	< MRL	< MRL	61.6
Demeclocycline	< MRL	< MRL	< MRL	94.9
Doxycycline	< MRL	< MRL	< MRL	114
4-Epianhydrochlortetracycline [EACTC]	< MRL	< MRL	< MRL	49.5
4-Epianhydrotetracycline [EATC]	< MRL	< MRL	< MRL	135
4-Epichlortetracycline [ECTC]	< MRL	< MRL	< MRL	87.8
4-Epioxytetracycline [EOTC]	< MRL	< MRL	< MRL	90.4
4-Epitetracycline [ETC]	< MRL	< MRL	< MRL	98.2
Isochlortetracycline [ICTC]	< MRL	< MRL	< MRL	105
Minocycline	< MRL	< MRL	< MRL	78.1
Oxytetracyclin [OTC]	< MRL	< MRL	< MRL	89.8
Tetracycline [TC]	< MRL	< MRL	< MRL	98.7

Surrogate recovery

CLIENT ID (Sample #)	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13813-1	L13813-2	WG30659-101	WG30659-102
WORKGROUP	WG30659	WG30659	WG30659	WG30659
Sample Size	1.10 L	1.04 L	1.00 L	
UNITS	% Recov	% Recov	% Recov	% Recov
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6
d6-Thiabendazole	70.2	82.4	89.0	92.6

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	1	10	12	13	14	15	16	2	3
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	L13550-2	L13550-3
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.07 L	1.07 L	1.03 L	1.06 L	1.03 L	1.03 L	1.07 L	1.06 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Anhydrochlortetracycline [ACTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Anhydrotetracycline [ATC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Chlortetracycline [CTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Demeclocycline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Doxycycline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
4-Epianhydrochlortetracycline [EACTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
4-Epianhydrotetracycline [EATC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
4-Epichlortetracycline [ECTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
4-Epioxytetracycline [EOTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
4-Epitetracycline [ETC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Isochlortetracycline [ICTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Minocycline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Oxytetracyclin [OTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Tetracycline [TC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL

Surrogate recovery

CLIENT ID (Sample #)	1	10	12	13	14	15	16	2	3
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	L13550-2	L13550-3
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.08 L	1.07 L	1.07 L	1.03 L	1.06 L	1.03 L	1.03 L	1.07 L	1.06 L
UNITS	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121
d6-Thiabendazole	93.7	84.5	88.1	84.7	98.0	98.3	113	90.2	121

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	4	5	6	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-4	L13550-5	L13550-6	L13550-7	L13550-8	L13550-9	WG30337-101	WG30337-102
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.07 L	1.08 L	1.07 L	1.08 L	1.05 L	1.01 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Anhydrochlortetracycline [ACTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	91.1
Anhydrotetracycline [ATC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	136
Chlortetracycline [CTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	74.6
Demeclocycline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	105
Doxycycline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	104
4-Epianhydrochlortetracycline [EACTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	73.8
4-Epianhydrotetracycline [EATC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	198
4-Epichlortetracycline [ECTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	81.3
4-Epioxytetracycline [EOTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	97.5
4-Epitetracycline [ETC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	98.3
Isochlortetracycline [ICTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	139
Minocycline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	92.2
Oxytetracyclin [OTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	116
Tetracycline [TC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	141

Surrogate recovery

CLIENT ID (Sample #)	4	5	6	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-4	L13550-5	L13550-6	L13550-7	L13550-8	L13550-9	WG30337-101	WG30337-102
WORKGROUP	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337	WG30337
Sample Size	1.07 L	1.08 L	1.07 L	1.08 L	1.05 L	1.01 L	1.00 L	
UNITS	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107
d6-Thiabendazole	87.6	92.2	83.3	86.2	84.7	87.1	113	107

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	17	18	19	20	21	22
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6
WORKGROUP	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338
Sample Size	1.02 L	1.06 L	1.04 L	1.05 L	1.06 L	1.05 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Anhydrochlortetracycline [ACTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Anhydrotetracycline [ATC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Chlortetracycline [CTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Demeclocycline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Doxycycline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
4-Epianhydrochlortetracycline [EACTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
4-Epianhydrotetracycline [EATC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
4-Epichlortetracycline [ECTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
4-Epioxytetracycline [EOTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
4-Epitetracycline [ETC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Isochlortetracycline [ICTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Minocycline	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Oxytetracyclin [OTC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Tetracycline [TC]	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL

Surrogate recovery

CLIENT ID (Sample #)	17	18	19	20	21	22
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-5	L13603-6
WORKGROUP	WG30338	WG30338	WG30338	WG30338	WG30338	WG30338
Sample Size	1.02 L	1.06 L	1.04 L	1.05 L	1.06 L	1.05 L
UNITS	% Recov	% Recov	% Recov	% Recov	% Recov	% Recov
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8
d6-Thiabendazole	117	97.4	87.4	88.4	82.8	92.8

Table 3.4 Concentration and surrogate recovery of pharmaceuticals and personal care products (PPCPs).

Concentration

CLIENT ID (Sample #)	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-7	L13603-8	WG30338-101	WG30338-102
WORKGROUP	WG30338	WG30338	WG30338	WG30338
Sample Size	1.04 L	1.06 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
Anhydrochlortetracycline [ACTC]	< MRL	< MRL	< MRL	151
Anhydrotetracycline [ATC]	< MRL	< MRL	< MRL	77.0
Chlortetracycline [CTC]	< MRL	< MRL	< MRL	95.1
Demeclocycline	< MRL	< MRL	< MRL	81.0
Doxycycline	< MRL	< MRL	< MRL	100
4-Epianhydrochlortetracycline [EACTC]	< MRL	< MRL	< MRL	53.4
4-Epianhydrotetracycline [EATC]	< MRL	< MRL	< MRL	97.6
4-Epichlortetracycline [ECTC]	< MRL	< MRL	< MRL	89.5
4-Epioxytetracycline [EOTC]	< MRL	< MRL	< MRL	118
4-Epitetracycline [ETC]	< MRL	< MRL	< MRL	97.8
Isochlortetracycline [ICTC]	< MRL	< MRL	< MRL	75.1
Minocycline	< MRL	< MRL	< MRL	54.6
Oxytetracyclin [OTC]	< MRL	< MRL	< MRL	116
Tetracycline [TC]	< MRL	< MRL	< MRL	97.0

Surrogate recovery

CLIENT ID (Sample #)	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-7	L13603-8	WG30338-101	WG30338-102
WORKGROUP	WG30338	WG30338	WG30338	WG30338
Sample Size	1.04 L	1.06 L	1.00 L	
UNITS	% Recov	% Recov	% Recov	% Recov
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102
d6-Thiabendazole	97.7	103	127	102

Table 3.5 Calibration range and minimum reporting limit (MRL) for PPCPs.

	A ng/L	B ng/L	C ng/L	D ng/L	E ng/L	F ng/L	G ng/L
Acetaminophen		**200**	600	3000	10000	40000	200000
Albuterol		**1.00**	3.00	15.0	50.0	200	1000
Alprazolam		**1.00**	3.00	15.0	50.0	200	600
Amitriptyline		**1.00**	3.00	15.0	50.0	200	600
10-Hydroxy-amitriptyline	**0.150**	0.500	1.50	7.50	25.0	100	300
Amlodipine	**1.50**	5.00	15.0	75.0	250	1000	3000
Amphetamine		**5.00**	15.0	75.0	250	1000	5000
Atenolol		**2.00**	6.00	30.0	100	400	2000
Atorvastatin			**15.0**	75.0	250	1000	5000
Azithromycin	**1.50**	5.00	15.0	75.0	250	1000	5000
Benzoylecgonine		**1.00**	3.00	15.0	50.0	200	600
Benztropine	**0.300**	1.00	3.00	15.0	50.0	200	600
Betamethasone	**1.50**	5.00	15.0	75.0	250	1000	3000
Bisphenol A	**2500**	5000	10000	20000	40000	80000	160000
Caffeine		**50.0**	150	750	2500	10000	50000
Carbadox	**1.50**	5.00	15.0	75.0	250	1000	5000
Carbamazepine		**5.00**	15.0	75.0	250	1000	5000
Cefotaxime	**6.00**	20.0	60.0	300	1000	4000	20000
Cimetidine		**1.00**	3.00	15.0	50.0	200	1000
Ciprofloxacin		**20.0**	60.0	300	1000	4000	20000
Clarithromycin	**1.50**	5.00	15.0	75.0	250	1000	5000
Clinafloxacin	**6.00**	20.0	60.0	300	1000	4000	20000
Clonidine	**1.50**	5.00	15.0	75.0	250	1000	5000
Cloxacillin	**1.20**	4.00	12.0	60.0	200	800	4000
Cocaine	**0.150**	0.500	1.50	7.50	25.0	100	300
Codeine		**10.0**	30.0	150	500	2000	10000
Cotinine		**5.00**	15.0	75.0	250	1000	5000
DEET			**1.50**	7.50	25.0	100	300
Dehydronifedipine		**2.00**	6.00	30.0	100	400	2000
Desmethyldiltiazem		**0.500**	1.50	7.50	25.0	100	300
Diazepam		**1.00**	3.00	15.0	50.0	200	600
Digoxigenin	**6.00**	20.0	60.0	300	1000	4000	20000

Table 3.5 Calibration range and minimum reporting limit (MRL) for PPCPs.

	A	B	C	D	E	F	G
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Digoxin	**15.0**	50.0	150	750	2500	10000	50000
Diltiazem		**1.00**	3.00	15.0	50.0	200	1000
1,7-Dimethylxanthine	**150**	500	1500	7500	25000	100000	500000
Diphenhydramine		**2.00**	6.00	30.0	100	400	2000
Enalapril		**1.00**	3.00	15.0	50	200	1000
Enrofloxacin	**3.00**	10.0	30.0	150	500	2000	10000
Erythromycin-H2O		**1.00**	3.00	15.0	50.0	200	1000
Flumequine	**1.50**	5.00	15.0	75.0	250	1000	5000
Fluocinonide	**6.00**	20.0	60.0	300	1000	4000	12000
Fluoxetine	**1.50**	5.00	15.0	75.0	250	1000	5000
Fluticasone propionate	**2.00**	6.67	20.0	100	334	1330	4000
Furosemide		**133**	400	2000	6650	26600	79800
Gemfibrozil		**5.14**	15.4	77.0	257	1030	3080
Glipizide	**6.00**	20.0	60.0	300	1000	4000	12000
Glyburide	**3.00**	10.0	30.0	150	500	2000	6000
Hydrochlorothiazide		**66.7**	200	1000	3340	13300	40020
Hydrocodone		**5.00**	15.0	75.0	250	1000	5000
Hydrocortisone			**600**	3000	10000	40000	120000
Ibuprofen	**15.0**	50.0	150	750	2500	10000	30000
2-Hydroxy-ibuprofen			**800**	4000	13400	53400	160000
Lincomycin	**7.02**	23.4	70.2	351	1170	4680	23400
Lomefloxacin	**3.00**	10.0	30.0	150	500	2000	10000
Meprobamate		**13.3**	40.0	200	667	2670	8000
Metformin			**300**	1500	5000	20000	100000
Methylprednisolone			**40.0**	200	667	2670	8000
Metoprolol			**15.0**	75.0	250	1000	3000
Miconazole	**1.50**	5.00	15.0	75.0	250	1000	5000
Naproxen		**10.0**	30.0	150	500	2000	6000
Norfloxacin	**15.0**	50.0	150	750	2500	10000	50000
Norfluoxetine	**1.50**	5.00	15.0	75.0	250	1000	3000
Norgestimate	**3.00**	10.0	30.0	150	500	2000	10000
Norverapamil	**0.150**	0.500	1.50	7.50	25.0	100	300

Table 3.5 Calibration range and minimum reporting limit (MRL) for PPCPs.

	A	B	C	D	E	F	G
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Ofloxacin	**15.0**	50.0	150	750	2500	10000	50000
Ormetoprim	**0.600**	2.00	6.00	30.0	100	400	2000
Oxacillin	**3.00**	10.0	30.0	150	500	2000	10000
Oxolinic Acid	**0.600**	2.00	6.00	30.0	100	400	2000
Oxycodone		**2.00**	6.00	30.0	100	400	2000
Paroxetine	**4.00**	13.3	40.0	200	667	2670	8000
Penicillin G	**1.20**	4.00	12.0	60.0	200	800	4000
Penicillin V	**3.00**	10.0	30.0	150	500	2000	10000
Prednisolone	**6.00**	20.0	60.0	300	1000	4000	12000
Prednisone			**200**	1000	3330	13300	40000
Promethazine	**0.399**	1.33	3.99	20.0	66.5	266	798
Propoxyphene		**1.00**	3.00	15.0	50.0	200	600
Propranolol		**6.67**	20.0	100	334	1330	4000
Ranitidine		**2.00**	6.00	30.0	100	400	2000
Roxithromycin	**0.300**	1.00	3.00	15.0	50.0	200	1000
Sarafloxacin		**50.0**	150	750	2500	10000	50000
Sertraline		**1.33**	3.99	20.0	66.5	266	798
Simvastatin	**20.0**	66.7	200	1000	3330	13300	40000
Sulfachloropyridazine	**1.50**	5.00	15.0	75.0	250	1000	5000
Sulfadiazine		**5.00**	15.0	75.0	250	1000	5000
Sulfadimethoxine		**1.00**	3.00	15.0	50	200	1000
Sulfamerazine	**0.600**	2.00	6.00	30.0	100	400	2000
Sulfamethazine	**0.600**	2.00	6.00	30.0	100	400	2000
Sulfamethizole	**0.600**	2.00	6.00	30.0	100	400	2000
Sulfamethoxazole		**2.00**	6.00	30.0	100	400	2000
Sulfanilamide	**15.0**	50.0	150	750	2500	10000	50000
Sulfathiazole	**1.50**	5.00	15.0	75.0	250	1000	5000
Anhydrochlortetracycline [ACTC]	**15.0**	50.0	125	250	500	1500	5000
Anhydrotetracycline [ATC]	**15.0**	50.0	125	250	500	1500	5000
Chlortetracycline [CTC]	**6.00**	20.0	50.0	100	200	600	2000
Demeclocycline	**15.0**	50.0	125	250	500	1500	5000
Doxycycline		**20.0**	50.0	100	200	600	2000

Table 3.5 Calibration range and minimum reporting limit (MRL) for PPCPs.

	A	B	C	D	E	F	G
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
4-Epianhydrochlortetracycline [EACTC]	**60.0**	200	500	1000	2000	6000	20000
4-Epianhydrotetracycline [EATC]	**15.0**	50.0	125	250	500	1500	5000
4-Epichlortetracycline [ECTC]	**15.0**	50.0	125	250	500	1500	5000
4-Epioxytetracycline [EOTC]	**6.00**	20.0	50.0	100	200	600	2000
4-Epitetracycline [ETC]	**6.00**	20.0	50.0	100	200	600	2000
Isochlortetracycline [ICTC]	**6.00**	20.0	50.0	100	200	600	2000
Minocycline	**60.0**	200	500	1000	2000	6000	20000
Oxytetracyclin [OTC]	**6.00**	20.0	50.0	100	200	600	2000
Tetracycline [TC]		**20.0**	50.0	100	200	600	2000
Theophylline		**200**	600	3000	10000	40000	120000
Thiabendazole		**5.0**	15.0	75.0	250	1000	5000
Trenbolone	**4.00**	13.3	40.0	200	667	2670	8000
Trenbolone acetate	**0.300**	1.00	3.00	15.0	50.0	200	600
Triamterene		**1.00**	3.00	15.0	50.0	200	1000
Triclocarban		**10.0**	30.0	150	500	2000	6000
Triclosan	**60.0**	200	600	3000	10000	40000	120000
Trimethoprim			**15.0**	75.0	250	1000	5000
Tylosin	**6.00**	20.0	60.0	300	1000	4000	20000
Valsartan		**13.3**	40.0	200	667	2670	8000
Verapamil			**1.50**	7.50	25.0	100	300
Virginiamycin	**3.00**	10.0	30.0	150	500	2000	10000
Warfarin	**1.50**	5.00	15.0	75.0	250	1000	3000

The MRLs are shown in bold face

Table 3.6 Ongoing precision and recovery (OPR) for PPCPs.

Analyte	OPR	Class	Surrogate
Acetaminophen	70 – 140	PPCP	13C2-15N-Acetaminophen
Albuterol	50 – 160	PPCP	d3-Albuterol
Alprazolam	70 – 130	PPCP	d5-Alprazolam
Amitriptyline	70 – 130	PPCP	d6-Amitriptyline
10-Hydroxy-amitriptyline	70 – 130	PPCP	d7-Propranolol
Amlodipine	45 – 130	PPCP	d5-Norfluoxetine
Amphetamine	50 – 160	PPCP	d5-Amphetamine
Atenolol	70 – 130	PPCP	d7-Atenolol
Atorvastatin	20 – 130	PPCP	d5-Enalapril
Azithromycin	10 – 130	PPCP	13C3-Trimethoprim
Benzoylecgonine	70 – 130	PPCP	d8-Benzoylecgonine
Benztropine	70 – 130	PPCP	d3-Benztropine
Betamethasone	20 – 240	PPCP	d6-Amitriptyline
Bisphenol A	70 – 130	PPCP	d6-Bisphenol A
Caffeine	25 – 160	PPCP	13C3-Caffeine
Carbacox	25 – 180	PPCP	13C3-Trimethoprim
Carbamazepine	25 – 200	PPCP	13C3-Trimethoprim
Cefotaxime	10 – 300	PPCP	13C3-Trimethoprim
Cimetidine	15 – 130	PPCP	d3-Cimetidine
Ciprofloxacin	25 – 180	PPCP	13C3-N15-Ciprofloxacin
Clarithromycin	50 – 160	PPCP	13C6-Sulfamethazine
Clinafloxacin	25 – 300	PPCP	13C3-N15-Ciprofloxacin
Clonidine	70 – 130	PPCP	d4-Clonidine
Cloxacillin	35 – 160	PPCP	13C3-Trimethoprim
Cocaine	70 – 130	PPCP	d3-Cocaine
Codeine	70 – 130	PPCP	d6-Codeine
Cotinine	70 – 130	PPCP	d3-Cotinine
DEET	70 – 130	PPCP	d7-DEET
Dehydronifedipine	35 – 160	PPCP	13C3-Trimethoprim
Desmethyldiltiazem	3 – 350	PPCP	d4-Promethazine
Diazepam	70 – 130	PPCP	d5-Diazepam
Digoxigenin	50 – 150	PPCP	13C3-Trimethoprim
Digoxin	10 – 300	PPCP	13C3-Trimethoprim
Diltiazem	20 – 160	PPCP	13C3-Trimethoprim
1,7-Dimethylxanthine	30 – 300	PPCP	13C3-Trimethoprim
Diphenhydramine	30 – 200	PPCP	13C3-N15-Ciprofloxacin
Enalapril	70 – 130	PPCP	d5-Enalapril
Enrofloxacin	30 – 220	PPCP	13C2-Erythromycin-H2O
Erythromycin - H2O	70 – 130	PPCP	13C3-Trimethoprim
Flumequine	40 – 160	PPCP	d5-Fluoxetine
Fluocinonide	7 – 230	PPCP	d5-Alprazolam
Fluoxetine	60 – 150	PPCP	13C3-Trimethoprim
Fluticasone propionate	20 – 160	PPCP	d7-Metoprolol
Furosemide	65 – 130	PPCP	13C-D3-Naproxen
Gemfibrozil	60 – 140	PPCP	d6-Gemfibrozil
Glipizide	55 – 170	PPCP	d11-Glipizide

Table 3.6 Ongoing precision and recovery (OPR) for PPCPs.

Analyte	OPR	Class	Surrogate
Glyburide	50 – 180	PPCP	d3-Glyburide
Hydrochlorothiazide	70 – 200	PPCP	13C-D3-Naproxen
Hydrocodone	70 – 130	PPCP	d3-Hydrocodone
Hydrocortisone	15 – 220	PPCP	d4-Hydrocortisone
Ibuprofen	70 – 130	PPCP	13C3-Ibuprofen
2-Hydroxy-ibuprofen	70 – 130	PPCP	13C3-Ibuprofen
Lincomycin	10 – 300	PPCP	13C3-N15-Ciprofloxacin
Lomefloxacin	50 – 250	PPCP	13C3-Trimethoprim
Meprobamate	65 – 150	PPCP	d7-Metoprolol
Metformin	70 – 160	PPCP	d6-Metformin
Methylprednisolone	35 – 240	PPCP	d2-Methylprednisolone
Metoprolol	70 – 130	PPCP	d7-Metoprolol
Miconazole	35 – 130	PPCP	13C3-N15-Ciprofloxacin
Naproxen	50 – 150	PPCP	13C-D3-Naproxen
Norfloxacin	10 – 250	PPCP	13C3-Trimethoprim
Norfluoxetine	70 – 130	PPCP	d5-Norfluoxetine
Norgestimate	35 – 130	PPCP	13C3-N15-Ciprofloxacin
Norverapamil	55 – 130	PPCP	d7-Propranolol
Ofloxacin	60 – 250	PPCP	13C3-Trimethoprim
Ormetoprim	70 – 150	PPCP	13C3-Trimethoprim
Oxacillin	20 – 130	PPCP	13C3-Trimethoprim
Oxolinic Acid	60 – 150	PPCP	13C3-Trimethoprim
Oxycodone	65 – 130	PPCP	d6-Oxycodone
Paroxetine	70 – 130	PPCP	d6-Paroxetine
Penicillin G	10 – 130	PPCP	13C3-Trimethoprim
Penicillin V	40 – 140	PPCP	13C6-Sulfamethazine
Prednisolone	35 – 240	PPCP	d7-Propranolol
Prednisone	50 – 180	PPCP	d7-Propranolol
Promethazine	70 – 130	PPCP	d4-Promethazine
Propoxyphene	70 – 130	PPCP	d5-Propoxyphene
Propranolol	70 – 150	PPCP	d7-Propranolol
Ranitidine	25 – 140	PPCP	d3-Albuterol
Roxithromycin	50 – 140	PPCP	13C3-N15-Ciprofloxacin
Sarafloxacin	50 – 200	PPCP	13C6-Sulfamethazine
Sertraline	50 – 130	PPCP	d7-Propranolol
Simvastatin	1 – 150	PPCP	d5-Propoxyphene
Sulfachloropyridazine	60 – 160	PPCP	13C6-Sulfamethazine
Sulfadiazine	70 – 130	PPCP	13C6-Sulfamethoxazole
Sulfadimethoxine	35 – 160	PPCP	13C6-Sulfamethazine
Sulfamerazine	60 – 140	PPCP	13C6-Sulfamethazine
Sulfamethazine	70 – 130	PPCP	13C6-Sulfamethoxazole
Sulfamethizole	30 – 140	PPCP	13C6-Sulfamethoxazole
Sulfamethoxazole	70 – 130	PPCP	13C6-Sulfamethazine
Sulfanilamide	2 – 160	PPCP	13C6-Sulfamethoxazole
Sulfathiazole	30 – 180	PPCP	d6-Thiabendazole
Anhydrochlortetracycline [ACTC]	15 – 200	PPCP	d6-Thiabendazole

Table 3.6 Ongoing precision and recovery (OPR) for PPCPs.

Analyte	OPR	Class	Surrogate
Anhydrotetracycline [ATC]	20 – 160	PPCP	d6-Thiabendazole
Chlortetracycline [CTC]	30 – 250	PPCP	d6-Thiabendazole
Demeclocycline	35 – 180	PPCP	d6-Thiabendazole
Doxycycline	35 – 180	PPCP	d6-Thiabendazole
4-Epianhydrochlortetracycline [EACTC]	6 – 130	PPCP	d6-Thiabendazole
4-Epianhydrotetracycline [EATC]	15 – 200	PPCP	d6-Thiabendazole
4-Epichlortetracycline [ECTC]	25 – 180	PPCP	d6-Thiabendazole
4-Epioxytetracycline [EOTC]	25 – 180	PPCP	d6-Thiabendazole
4-Epitetracycline [ETC]	35 – 200	PPCP	d6-Thiabendazole
Isochlortetracycline [ICTC]	25 – 180	PPCP	d6-Thiabendazole
Minocycline	1 – 250	PPCP	d6-Thiabendazole
Oxytetracyclin [OTC]	20 – 200	PPCP	d6-Thiabendazole
Tetracycline [TC]	20 – 200	PPCP	d6-Thiabendazole
Theophylline	10 – 1000	PPCP	13C1-15N2-Theophylline
Thiabendazole	60 – 150	PPCP	13C3-Trimethoprim
Trenbolone	70 – 140	PPCP	d5-Alprazolam
Trenbolone acetate	55 – 130	PPCP	d5-Alprazolam
Triamterene	70 – 140	PPCP	d4-Clonidine
Triclocarban	60 – 140	PPCP	13C6-Triclocarban
Triclosan	70 – 130	PPCP	13C12-Triclosan
Trimethoprim	50 – 150	PPCP	13C6-Sulfamethazine
Tylosin	10 – 180	PPCP	13C3-Trimethoprim
Valsartan	70 – 130	PPCP	d5-Propoxyphene
Verapamil	70 – 145	PPCP	d6-Amitriptyline
Virginiamycin	15 – 300	PPCP	13C3-Caffeine
Warfarin	70 – 140	PPCP	d5-Warfarin
13C2, 15N-Acetaminophen	30 – 160	PPCP-Surrogate	
d3-Albuterol	20 – 140	PPCP-Surrogate	
d5-Alprazolam	45 – 130	PPCP-Surrogate	
d6-Amitriptyline	10 – 130	PPCP-Surrogate	
d5-Amphetamine	20 – 130	PPCP-Surrogate	
d7-Atenolol	70 – 130	PPCP-Surrogate	
d8-Benzoylecgonine	10 – 170	PPCP-Surrogate	
d3-Benztropine	20 – 140	PPCP-Surrogate	
d6-Bisphenol A	50 – 170	PPCP-Surrogate	
13C3-Caffeine	40 – 140	PPCP-Surrogate	
d3-Cimetidine	15 – 130	PPCP-Surrogate	
13C3, 15N-Ciprofloxacin	7 – 150	PPCP-Surrogate	
d4-Clonidine	70 – 130	PPCP-Surrogate	
d3-Cocaine	25 – 140	PPCP-Surrogate	
d6-Codeine	70 – 130	PPCP-Surrogate	
d3-Cotinine	70 – 140	PPCP-Surrogate	
d7-DEET	15 – 160	PPCP-Surrogate	
d5-Diazepam	15 – 160	PPCP-Surrogate	
d5-Enalapril	65 – 130	PPCP-Surrogate	
13C2-Erythromycin - H2O	35 – 130	PPCP-Surrogate	

Table 3.6 Ongoing precision and recovery (OPR) for PPCPs.

Analyte	OPR	Class	Surrogate
d5-Fluoxetine	40 – 130	PPCP-Surrogate	
d6-Gemfibrozil	50 – 150	PPCP-Surrogate	
d11-Glipizide	30 – 180	PPCP-Surrogate	
d3-Glyburide	20 – 160	PPCP-Surrogate	
d3-Hydrocodone	70 – 130	PPCP-Surrogate	
d4-Hydrocortisone	40 – 240	PPCP-Surrogate	
13C3-Ibuprofen	50 – 140	PPCP-Surrogate	
d6-Metformin	3 – 130	PPCP-Surrogate	
d2-Methylprednisolone	15 – 160	PPCP-Surrogate	
d7-Metoprolol	25 – 140	PPCP-Surrogate	
13C-D3-Naproxen	30 – 150	PPCP-Surrogate	
d5-Norfluoxetine	20 – 130	PPCP-Surrogate	
d6-Oxycodone	50 – 150	PPCP-Surrogate	
d6-Paroxetine	7 – 150	PPCP-Surrogate	
d4-Promethazine	3 – 140	PPCP-Surrogate	
d5-Propoxyphene	30 – 130	PPCP-Surrogate	
d7-Propranolol	25 – 140	PPCP-Surrogate	
13C6-Sulfamethazine	30 – 160	PPCP-Surrogate	
13C6-Sulfamethoxazole	30 – 140	PPCP-Surrogate	
d6-Thiabendazole	25 – 140	PPCP-Surrogate	
13C1-15N2-Theophylline	20 – 200	PPCP-Surrogate	
d6-Thiabendazole	25 – 180	PPCP-Surrogate	
13C6-Triclocarban	20 – 160	PPCP-Surrogate	
13C12-Triclosan	20 – 160	PPCP-Surrogate	
13C3-Trimethoprim	30 – 140	PPCP-Surrogate	
d5-Warfarin	35 – 250	PPCP-Surrogate	

Table 3.7 Concentration and surrogate recovery of hormones.

Concentration

CLIENT ID (Sample #)	1	10	12	13	14	15	16	2
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	L13550-2
WORKGROUP	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228
Sample Size	0.978 L	0.971 L	0.979 L	0.937 L	0.976 L	0.956 L	0.956 L	0.968 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Androsterone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Desogestrel	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
17 alpha-Estradiol	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Estrone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	K
Equilin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Androstenedione	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
17 alpha-Dihydroequilin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
17 beta-Estradiol	< MRL	K	K	K	< MRL	K	K	K
Testosterone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Equilenin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Mestranol	< MRL	K	< MRL	K	< MRL	K	< MRL	K
Norethindrone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
17 alpha-Ethinyl-Estradiol	< MRL	K	K	K	< MRL	K	< MRL	K
Progesterone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Norgestrel	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
Estriol	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
beta-Estradiol 3-benzoate	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL

Surrogate recovery

CLIENT ID (Sample #)	1	10	12	13	14	15	16	2
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14	L13550-15	L13550-2
WORKGROUP	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228
Sample Size	0.978 L	0.971 L	0.979 L	0.937 L	0.976 L	0.956 L	0.956 L	0.968 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
D4-17 beta-Estradiol	107	105	98.8	81.1	106	96.1	77.9	94.1
D4-17 beta-Estradiol	107	105	98.8	81.1	106	96.1	77.9	94.1
D4-17 beta-Estradiol	107	105	98.8	81.1	106	96.1	77.9	94.1
D4-17 beta-Estradiol	107	105	98.8	81.1	106	96.1	77.9	94.1
D4-17 beta-Estradiol	107	105	98.8	81.1	106	96.1	77.9	94.1
D4-17 beta-Estradiol	107	105	98.8	81.1	106	96.1	77.9	94.1
D6-Norethindrone	167	168	157	104	170	153	106	147
D4-17 beta-Estradiol	107	105	98.8	81.1	106	96.1	77.9	94.1
D4-17 beta-Estradiol	107	105	98.8	81.1	106	96.1	77.9	94.1
D4-17 beta-Estradiol	107	105	98.8	81.1	106	96.1	77.9	94.1
D4-Mestranol	111	112	104	84.7	114	104	73.4	100
D6-Norethindrone	167	168	157	104	170	153	106	147
D4-17 alpha-Ethinyl-Estradiol	113	115	105	84.1	113	102	71.5	97.9
D9-Progesterone	172	174	165	104	177	157	96.5	157
D6-Norgestrel	158	148	140	92.1	148	139	81.1	127
D6-Norgestrel	158	148	140	92.1	148	139	81.1	127
D6-Norgestrel	158	148	140	92.1	148	139	81.1	127

Table 3.7 Concentration and surrogate recovery of hormones.

Concentration

CLIENT ID (Sample #)	3	4	5	6	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-3	L13550-4	L13550-5	L13550-6	L13550-7	L13550-8	L13550-9	WG30228-101	WG30228-102
WORKGROUP	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228
Sample Size	0.973 L	0.963 L	0.915 L	0.976 L	0.982 L	0.971 L	0.969 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Androsterone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	K	96.4
Desogestrel	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	33.7
17 alpha-Estradiol	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	114
Estrone	< MRL	< MRL	< MRL	< MRL	K	< MRL	< MRL	< MRL	121
Equilin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	111
Androstenedione	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	K	94.4
17 alpha-Dihydroequilin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	96.6
17 beta-Estradiol	K	K	K	K	K	< MRL	K	K	96.5
Testosterone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	88.8
Equilenin	< MRL	< MRL	K	< MRL	< MRL	< MRL	< MRL	K	100
Mestranol	< MRL	K	K	< MRL	< MRL	< MRL	< MRL	K	97.8
Norethindrone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	104
17 alpha-Ethinyl-Estradiol	K	K	K	< MRL	< MRL	< MRL	K	K	99.6
Progesterone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	106
Norgestrel	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	102
Estriol	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	106
beta-Estradiol 3-benzoate	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	33.3

Surrogate recovery

CLIENT ID (Sample #)	3	4	5	6	7	8	9	Lab Blank	Spiked Matrix
AXYS ID	L13550-3	L13550-4	L13550-5	L13550-6	L13550-7	L13550-8	L13550-9	WG30228-101	WG30228-102
WORKGROUP	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228	WG30228
Sample Size	0.973 L	0.963 L	0.915 L	0.976 L	0.982 L	0.971 L	0.969 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
D4-17 beta-Estradiol	102	101	97.6	98	101	100	84.7	50.4	71.5
D4-17 beta-Estradiol	102	101	97.6	98	101	100	84.7	50.4	71.5
D4-17 beta-Estradiol	102	101	97.6	98	101	100	84.7	50.4	71.5
D4-17 beta-Estradiol	102	101	97.6	98	101	100	84.7	50.4	71.5
D4-17 beta-Estradiol	102	101	97.6	98	101	100	84.7	50.4	71.5
D4-17 beta-Estradiol	102	101	97.6	98	101	100	84.7	50.4	71.5
D6-Norethindrone	156	164	125	155	156	165	133	47.4	67.8
D4-17 beta-Estradiol	102	101	97.6	98	101	100	84.7	50.4	71.5
D4-17 beta-Estradiol	102	101	97.6	98	101	100	84.7	50.4	71.5
D4-17 beta-Estradiol	102	101	97.6	98	101	100	84.7	50.4	71.5
D4-Mestranol	108	109	99.7	104	105	109	92.9	45.1	63.3
D6-Norethindrone	156	164	125	155	156	165	133	47.4	67.8
D4-17 alpha-Ethinyl-Estradiol	108	107	99.6	103	106	108	90.8	39.9	57.5
D9-Progesterone	157	176	131	162	155	167	138	45	64
D6-Norgestrel	141	143	111	139	142	145	117	34	51.7
D6-Norgestrel	141	143	111	139	142	145	117	34	51.7
D6-Norgestrel	141	143	111	139	142	145	117	34	51.7

Table 3.7 Concentration and surrogate recovery of hormones.

Concentration

CLIENT ID (Sample #)	17	18	19	20	22	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-6	WG30292-101	WG30292-102
WORKGROUP	WG30292	WG30292	WG30292	WG30292	WG30292	WG30292	WG30292
Sample Size	0.572 L	0.973 L	0.965 L	0.976 L	0.959 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Androsterone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	100
Desogestrel	< MRL	< MRL	< MRL	< MRL	< MRL	K	48.6
17 alpha-Estradiol	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	109
Estrone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	124
Equilin	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	115
Androstenedione	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	118
17 alpha-Dihydroequilin	< MRL	< MRL	< MRL	9.08 V	< MRL	< MRL	101
17 beta-Estradiol	K	K	K	< MRL	< MRL	K	93.1
Testosterone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	114
Equilenin	< MRL	< MRL	< MRL	< MRL	< MRL	K	109
Mestranol	< MRL	< MRL	< MRL	< MRL	< MRL	K	93
Norethindrone	< MRL	< MRL	< MRL	< MRL	< MRL	K	96
17 alpha-Ethinyl-Estradiol	K	K	K	< MRL	< MRL	K	93.2
Progesterone	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	97.7
Norgestrel	< MRL	< MRL	< MRL	< MRL	< MRL	K	95.3
Estriol	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	81.4
beta-Estradiol 3-benzoate	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	62.7

Surrogate recovery

CLIENT ID (Sample #)	17	18	19	20	22	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-6	WG30292-101	WG30292-102
WORKGROUP	WG30292	WG30292	WG30292	WG30292	WG30292	WG30292	WG30292
Sample Size	0.572 L	0.973 L	0.965 L	0.976 L	0.959 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
D4-17 beta-Estradiol	60.6	97.3	83.5	113	85.9	84.7	79.5
D4-17 beta-Estradiol	60.6	97.3	83.5	113	85.9	84.7	79.5
D4-17 beta-Estradiol	60.6	97.3	83.5	113	85.9	84.7	79.5
D4-17 beta-Estradiol	60.6	97.3	83.5	113	85.9	84.7	79.5
D4-17 beta-Estradiol	60.6	97.3	83.5	113	85.9	84.7	79.5
D4-17 beta-Estradiol	60.6	97.3	83.5	113	85.9	84.7	79.5
D6-Norethindrone	103	161	155	192	185	97.5	108
D4-17 beta-Estradiol	60.6	97.3	83.5	113	85.9	84.7	79.5
D4-17 beta-Estradiol	60.6	97.3	83.5	113	85.9	84.7	79.5
D4-17 beta-Estradiol	60.6	97.3	83.5	113	85.9	84.7	79.5
D4-Mestranol	75.1	109	106	121	113	88.1	82.5
D6-Norethindrone	103	161	155	192	185	97.5	108
D4-17 alpha-Ethinyl-Estradiol	64.9	105	95.1	120	102	85.2	77.6
D9-Progesterone	103	163	156	203	202	94.4	103
D6-Norgestrel	94.8	149	144	179	179	95.8	100
D6-Norgestrel	94.8	149	144	179	179	95.8	100
D6-Norgestrel	94.8	149	144	179	179	95.8	100

Table 3.7 Concentration and surrogate recovery of hormones.

Concentration

CLIENT ID (Sample #)	21	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13603-5	L13813-1	L13813-2	WG30695-101	WG30695-102
WORKGROUP	WG30695	WG30695	WG30695	WG30695	WG30695
Sample Size	0.908 L	0.971 L	0.899 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	% Recov
Androsterone	< MRL	< MRL	< MRL	< MRL	67.5
Desogestrel	< MRL	< MRL	< MRL	< MRL	36.7
17 alpha-Estradiol	< MRL	< MRL	< MRL	< MRL	112
Estrone	< MRL	< MRL	< MRL	< MRL	117
Equilin	< MRL	< MRL	< MRL	< MRL	103
Androstenedione	< MRL	< MRL	< MRL	< MRL	78.9
17 alpha-Dihydroequilin	< MRL	< MRL	< MRL	< MRL	85.2
17 beta-Estradiol	< MRL	< MRL	K	K	96.4
Testosterone	< MRL	< MRL	< MRL	< MRL	75.1
Equilenin	< MRL	< MRL	K	< MRL	120
Mestranol	< MRL	< MRL	< MRL	< MRL	89.9
Norethindrone	< MRL	< MRL	< MRL	< MRL	129
17 alpha-Ethinyl-Estradiol	< MRL	K	K	K	97.6
Progesterone	< MRL	< MRL	< MRL	< MRL	114
Norgestrel	< MRL	< MRL	< MRL	< MRL	106
Estriol	< MRL	< MRL	< MRL	< MRL	103
beta-Estradiol 3-benzoate	< MRL	< MRL	< MRL	< MRL	100

Surrogate recovery

CLIENT ID (Sample #)	21	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13603-5	L13813-1	L13813-2	WG30695-101	WG30695-102
WORKGROUP	WG30695	WG30695	WG30695	WG30695	WG30695
Sample Size	0.908 L	0.971 L	0.899 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	% Recov
D4-17 beta-Estradiol	50.4	62.7	89.9	81.2	101
D4-17 beta-Estradiol	50.4	62.7	89.9	81.2	101
D4-17 beta-Estradiol	50.4	62.7	89.9	81.2	101
D4-17 beta-Estradiol	50.4	62.7	89.9	81.2	101
D4-17 beta-Estradiol	50.4	62.7	89.9	81.2	101
D4-17 beta-Estradiol	50.4	62.7	89.9	81.2	101
D6-Norethindrone	84.9	87.7	124	83.2	121
D4-17 beta-Estradiol	50.4	62.7	89.9	81.2	101
D4-17 beta-Estradiol	50.4	62.7	89.9	81.2	101
D4-17 beta-Estradiol	50.4	62.7	89.9	81.2	101
D4-Mestranol	65.7	107	91.2	104	130
D6-Norethindrone	84.9	87.7	124	83.2	121
D4-17 alpha-Ethinyl-Estradiol	41.8	110	90.8	74.7	94.6
D9-Progesterone	107	89.8	153	89.1	123
D6-Norgestrel	73.8	73.2	98.7	69.5	94.5
D6-Norgestrel	73.8	73.2	98.7	69.5	94.5
D6-Norgestrel	73.8	73.2	98.7	69.5	94.5

Table 3.7 Concentration and surrogate recovery of hormones.

Concentration

CLIENT ID (Sample #)	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-7	L13603-8	WG30417-101	WG30417-102
WORKGROUP	WG30417	WG30417	WG30417	WG30417
Sample Size	0.974 L	0.965 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
Androsterone	< MRL	< MRL	< MRL	110
Desogestrel	K	< MRL	K	44.9
17 alpha-Estradiol	< MRL	< MRL	< MRL	108
Estrone	< MRL	K	< MRL	104
Equilin	< MRL	< MRL	< MRL	94.1
Androstenedione	< MRL	< MRL	< MRL	108
17 alpha-Dihydroequilin	< MRL	100	< MRL	97.4
17 beta-Estradiol	< MRL	< MRL	K	97.8
Testosterone	< MRL	< MRL	< MRL	102
Equilenin	< MRL	< MRL	K	86
Mestranol	< MRL	< MRL	K	92.8
Norethindrone	< MRL	< MRL	< MRL	108
17 alpha-Ethinyl-Estradiol	< MRL	< MRL	K	94.4
Progesterone	< MRL	< MRL	< MRL	97.8
Norgestrel	< MRL	< MRL	< MRL	96.3
Estriol	< MRL	< MRL	< MRL	166
beta-Estradiol 3-benzoate	< MRL	< MRL	< MRL	112

Surrogate recovery

CLIENT ID (Sample #)	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-7	L13603-8	WG30417-101	WG30417-102
WORKGROUP	WG30417	WG30417	WG30417	WG30417
Sample Size	0.974 L	0.965 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
D4-17 beta-Estradiol	47.2	106	84.5	98.4
D4-17 beta-Estradiol	47.2	106	84.5	98.4
D4-17 beta-Estradiol	47.2	106	84.5	98.4
D4-17 beta-Estradiol	47.2	106	84.5	98.4
D4-17 beta-Estradiol	47.2	106	84.5	98.4
D4-17 beta-Estradiol	47.2	106	84.5	98.4
D6-Norethindrone	120	110	64.3	69.9
D4-17 beta-Estradiol	47.2	106	84.5	98.4
D4-17 beta-Estradiol	47.2	106	84.5	98.4
D4-17 beta-Estradiol	47.2	106	84.5	98.4
D4-Mestranol	88	75.2	69.4	76.9
D6-Norethindrone	120	110	64.3	69.9
D4-17 alpha-Ethinyl-Estradiol	43.1	106	70.2	76.1
D9-Progesterone	119	144	87.1	98.9
D6-Norgestrel	83.6	133	55.3	58.9
D6-Norgestrel	83.6	133	55.3	58.9
D6-Norgestrel	83.6	133	55.3	58.9

Table 3.8 Concentration and surrogate recovery of sterols.

Concentration

CLIENT ID (Sample #)	17	18	19	20	22	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-6	WG30292-101	WG30292-102
WORKGROUP	WG30292	WG30292	WG30292	WG30292	WG30292	WG30292	WG30292
Sample Size	0.572 L	0.973 L	0.965 L	0.976 L	0.959 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Coprostanol	< MRL	624	< MRL	1830	< MRL	< MRL	108
Epicoprostanol	< MRL	< MRL	< MRL	120	< MRL	< MRL	109
Cholesterol	1120	1400	1160	>1890 E	>1890 E	< MRL	114
Cholestanol	91.3	153	97.5	562	164	< MRL	108
Desmosterol	K	K	K	K	275	< MRL	80.4
Ergosterol	< MRL	< MRL	< MRL	K	K	< MRL	25.6
Campesterol	223	197	165	353	507	< MRL	105
Stigmasterol	662	475	365	>810 E	750	< MRL	109
beta-Sitosterol	543	579	397	896	649	< MRL	96.4
beta Stigmastanol	97	137	< MRL	185	< MRL	< MRL	104

Surrogate recovery

CLIENT ID (Sample #)	17	18	19	20	22	Lab Blank	Spiked Matrix
AXYS ID	L13603-1	L13603-2	L13603-3	L13603-4	L13603-6	WG30292-101	WG30292-102
WORKGROUP	WG30292	WG30292	WG30292	WG30292	WG30292	WG30292	WG30292
Sample Size	0.572 L	0.973 L	0.965 L	0.976 L	0.959 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Cholesterol-d7	34.8	55.7	56.6	54.7	54.4	40.2	45.8
Cholesterol-d7	34.8	55.7	56.6	54.7	54.4	40.2	45.8
Cholesterol-d7	34.8	55.7	56.6	54.7	54.4	40.2	45.8
Cholesterol-d7	34.8	55.7	56.6	54.7	54.4	40.2	45.8
Cholesterol-d7	34.8	55.7	56.6	54.7	54.4	40.2	45.8
Cholesterol-d7	34.8	55.7	56.6	54.7	54.4	40.2	45.8
Cholesterol-d7	34.8	55.7	56.6	54.7	54.4	40.2	45.8
Cholesterol-d7	34.8	55.7	56.6	54.7	54.4	40.2	45.8
Cholesterol-d7	34.8	55.7	56.6	54.7	54.4	40.2	45.8
Cholesterol-d7	34.8	55.7	56.6	54.7	54.4	40.2	45.8

Table 3.8 Concentration and surrogate recovery of sterols.

Concentration

CLIENT ID (Sample #)	23		24		Lab Blank		Spiked Matrix
AXYS ID	L13603-7		L13603-8		WG30417-101		WG30417-102
WORKGROUP	WG30417		WG30417		WG30417		WG30417
Sample Size	0.974 L		0.965 L		1.00 L		
UNITS	ng/L		ng/L		ng/L		% Recov
Coprostanol	< MRL		1750		< MRL		109
Epicoprostanol	< MRL		121		< MRL		108
Cholesterol	1050	B (128)	>1890	E	128	B (128)	103
Cholestanol	111		507		< MRL		112
Desmosterol		K		K	< MRL		95.3
Ergosterol		K	< MRL		< MRL		40
Campesterol	256		>800	E	< MRL		106
Stigmasterol	779	S	>810	E	< MRL		153
beta-Sitosterol	481		>1890	E	< MRL		117
beta Stigmastanol	< MRL		135		< MRL		104

Surrogate recovery

CLIENT ID (Sample #)	23	24	Lab Blank	Spiked Matrix
AXYS ID	L13603-7	L13603-8	WG30417-101	WG30417-102
WORKGROUP	WG30417	WG30417	WG30417	WG30417
Sample Size	0.974 L	0.965 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	% Recov
Cholesterol-d7	48.4	56.9	42.5	44.9
Cholesterol-d7	48.4	56.9	42.5	44.9
Cholesterol-d7	48.4	56.9	42.5	44.9
Cholesterol-d7	48.4	56.9	42.5	44.9
Cholesterol-d7	48.4	56.9	42.5	44.9
Cholesterol-d7	48.4	56.9	42.5	44.9
Cholesterol-d7	48.4	56.9	42.5	44.9
Cholesterol-d7	48.4	56.9	42.5	44.9
Cholesterol-d7	48.4	56.9	42.5	44.9
Cholesterol-d7	48.4	56.9	42.5	44.9

Table 3.8 Concentration and surrogate recovery of sterols.

Concentration

CLIENT ID (Sample #)	1		10		12		13		14		15	
AXYS ID	L13550-1		L13550-10		L13550-11		L13550-12		L13550-13		L13550-14	
WORKGROUP	WG30695		WG30695		WG30695		WG30695		WG30695		WG30695	
Sample Size	0.973 L		0.972 L		0.973 L		0.927 L		0.963 L		0.950 L	
UNITS	ng/L		ng/L		ng/L		ng/L		ng/L		ng/L	
Coprostanol	< MRL		< MRL		< MRL		< MRL		< MRL		< MRL	
Epicoprostanol	< MRL		< MRL		< MRL		< MRL		< MRL		< MRL	
Cholesterol	195	B (232)	741	B (232)	911	B (232)	< MRL		834	B (232)	955	B (232)
Cholestanol	78.9		< MRL		204		< MRL		118		125	
Desmosterol	< MRL			K		K	< MRL			K	219	
Ergosterol		K		K		K	< MRL		< MRL			K
Campesterol	< MRL		67.9		587		< MRL		189		117	
Stigmasterol	90.9		159		>810	E		K	226		255	
beta-Sitosterol	98		144		1420			K	193		260	
beta Stigmastanol	< MRL		< MRL		213		< MRL			K	< MRL	

Surrogate recovery

CLIENT ID (Sample #)	1	10	12	13	14	15
AXYS ID	L13550-1	L13550-10	L13550-11	L13550-12	L13550-13	L13550-14
WORKGROUP	WG30695	WG30695	WG30695	WG30695	WG30695	WG30695
Sample Size	0.973 L	0.972 L	0.973 L	0.927 L	0.963 L	0.950 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Cholesterol-d7	32.9	52.5	50	50.6	62.1	47
Cholesterol-d7	32.9	52.5	50	50.6	62.1	47
Cholesterol-d7	32.9	52.5	50	50.6	62.1	47
Cholesterol-d7	32.9	52.5	50	50.6	62.1	47
Cholesterol-d7	32.9	52.5	50	50.6	62.1	47
Cholesterol-d7	32.9	52.5	50	50.6	62.1	47
Cholesterol-d7	32.9	52.5	50	50.6	62.1	47
Cholesterol-d7	32.9	52.5	50	50.6	62.1	47
Cholesterol-d7	32.9	52.5	50	50.6	62.1	47
Cholesterol-d7	32.9	52.5	50	50.6	62.1	47

Table 3.8 Concentration and surrogate recovery of sterols.

Concentration

CLIENT ID (Sample #)	16		2		3		4		5		6		7	
AXYS ID	L13550-15		L13550-2		L13550-3		L13550-4		L13550-5		L13550-6		L13550-7	
WORKGROUP	WG30695		WG30695		WG30695		WG30695		WG30695		WG30695		WG30695	
Sample Size	0.948 L		0.968 L		0.955 L		0.966 L		0.928 L		0.961 L		0.955 L	
UNITS	ng/L		ng/L		ng/L		ng/L		ng/L		ng/L		ng/L	
Coprostanol	462		< MRL		1060		< MRL		< MRL		< MRL		818	
Epicoprostanol	< MRL		< MRL		< MRL			K	< MRL		< MRL		< MRL	
Cholesterol	1470	B (232)	516	B (232)	1710	B (232)	676	B (232)	< MRL		278	B (232)	1570	B (232)
Cholestanol	308		60.7		522		65.6			K	< MRL		277	
Desmosterol		K	< MRL			K	< MRL		< MRL		82.5		199	
Ergosterol	< MRL			K	< MRL		< MRL		< MRL		< MRL		< MRL	
Campesterol	>800	E	62.6		130		< MRL		< MRL		63.4		186	
Stigmasterol	>810	E	213		472		107		< MRL		265		>810	E
beta-Sitosterol	794		176		415		128		< MRL		948		629	
beta Stigmastanol	111		< MRL		< MRL			K		K	< MRL		< MRL	

Surrogate recovery

CLIENT ID (Sample #)	16	2	3	4	5	6	7
AXYS ID	L13550-15	L13550-2	L13550-3	L13550-4	L13550-5	L13550-6	L13550-7
WORKGROUP	WG30695	WG30695	WG30695	WG30695	WG30695	WG30695	WG30695
Sample Size	0.948 L	0.968 L	0.955 L	0.966 L	0.928 L	0.961 L	0.955 L
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
Cholesterol-d7	61.7	43.5	52.8	44.1	56.9	47.9	35.7
Cholesterol-d7	61.7	43.5	52.8	44.1	56.9	47.9	35.7
Cholesterol-d7	61.7	43.5	52.8	44.1	56.9	47.9	35.7
Cholesterol-d7	61.7	43.5	52.8	44.1	56.9	47.9	35.7
Cholesterol-d7	61.7	43.5	52.8	44.1	56.9	47.9	35.7
Cholesterol-d7	61.7	43.5	52.8	44.1	56.9	47.9	35.7
Cholesterol-d7	61.7	43.5	52.8	44.1	56.9	47.9	35.7
Cholesterol-d7	61.7	43.5	52.8	44.1	56.9	47.9	35.7
Cholesterol-d7	61.7	43.5	52.8	44.1	56.9	47.9	35.7
Cholesterol-d7	61.7	43.5	52.8	44.1	56.9	47.9	35.7

Table 3.8 Concentration and surrogate recovery of sterols.

Concentration

CLIENT ID (Sample #)	8		9		21		11		25		Lab Blank		Spiked Matrix
AXYS ID	L13550-8		L13550-9		L13603-5		L13813-1		L13813-2		WG30695-101		WG30695-102
WORKGROUP	WG30695		WG30695		WG30695		WG30695		WG30695		WG30695		WG30695
Sample Size	0.954 L		0.958 L		0.908 L		0.971 L		0.899 L		1.00 L		
UNITS	ng/L		ng/L		ng/L		ng/L		ng/L		ng/L		% Recov
Coprostanol	< MRL		< MRL		< MRL		602		< MRL		< MRL		131
Epicoprostanol	< MRL		< MRL		< MRL		< MRL		< MRL		< MRL		126
Cholesterol	449	B (232)	503	B (232)	1680	B (232)	1830	B (232)	129	B (232)	232	B (232)	154
Cholestanol	52.2		69.3		259		214		< MRL		< MRL		134
Desmosterol	112			K	867			K	< MRL		< MRL		120
Ergosterol		K		K	< MRL		< MRL		< MRL		< MRL		42.6
Campesterol	52.3		140		>800	E	200		< MRL		< MRL		122
Stigmasterol	194		374		>810	E	651		< MRL		< MRL		133
beta-Sitosterol	231		196		1640		1440		< MRL		< MRL		146
beta Stigmastanol		K	< MRL		102		< MRL			K	< MRL		115

Surrogate recovery

CLIENT ID (Sample #)	8	9	21	11	25	Lab Blank	Spiked Matrix
AXYS ID	L13550-8	L13550-9	L13603-5	L13813-1	L13813-2	WG30695-101	WG30695-102
WORKGROUP	WG30695	WG30695	WG30695	WG30695	WG30695	WG30695	WG30695
Sample Size	0.954 L	0.958 L	0.908 L	0.971 L	0.899 L	1.00 L	
UNITS	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	% Recov
Cholesterol-d7	30	52.2	30.9	41.3	58.1	38.7	59.1
Cholesterol-d7	30	52.2	30.9	41.3	58.1	38.7	59.1
Cholesterol-d7	30	52.2	30.9	41.3	58.1	38.7	59.1
Cholesterol-d7	30	52.2	30.9	41.3	58.1	38.7	59.1
Cholesterol-d7	30	52.2	30.9	41.3	58.1	38.7	59.1
Cholesterol-d7	30	52.2	30.9	41.3	58.1	38.7	59.1
Cholesterol-d7	30	52.2	30.9	41.3	58.1	38.7	59.1
Cholesterol-d7	30	52.2	30.9	41.3	58.1	38.7	59.1
Cholesterol-d7	30	52.2	30.9	41.3	58.1	38.7	59.1
Cholesterol-d7	30	52.2	30.9	41.3	58.1	38.7	59.1

Table 3.9 Calibration range and minimum reporting limit (MRL) for hormones and sterols.

	A ng/L	B ng/L	C ng/L	D ng/L	E ng/L
Hormones					
Androstenedione	**20.6**	103	411	617	822
Androsterone	**8.32**	41.6	166	250	333
Desogestrel	**8.56**	42.8	171	257	342
17 alpha-Dihydroequilin	**8.08**	40.4	162	242	323
Equilenin	**8.31**	41.6	166	249	332
Equilin	**7.85**	39.2	157	235	314
17 alpha-Estradiol	**7.92**	39.6	158	238	317
17 beta-Estradiol	**8.16**	40.8	163	245	326
17 alpha-Ethinyl-Estradiol	**8.56**	42.8	171	257	342
beta-Estradiol 3-benzoate	**8.64**	43.2	173	259	346
Estriol	**7.92**	39.6	158	238	317
Estrone	**9.28**	46.4	186	278	371
Mestranol	**8.16**	40.8	163	245	326
Norethindrone	**8.59**	43.0	172	258	344
Norgestrel	**8.16**	40.8	163	245	326
Progesterone	**20.5**	102	410	614	819
Testosterone	**8.56**	42.8	171	257	342
Sterols					
Campesterol	**40.0**	160	240	320	800
Cholestanol	**40.0**	160	240	320	800
Cholesterol	**94.4**	378	567	755	1890
Coprostanol	**96.9**	388	581	775	1940
Desmosterol	**57.5**	230	345	460	1150
Epicoprostanol	**95.9**	383	575	767	1920
Ergosterol	**96.3**	385	578	771	1930
beta-Sitosterol	**94.5**	378	567	756	1890
Stigmasterol	**40.5**	162	243	324	810
beta-Stigmastanol	**96.8**	387	581	774	1940

The MRLs are shown in bold face

Table 3.10 Ongoing precision and recovery (OPR) for hormones and sterols.

Analyte	OPR	Class	Surrogate
Androsterone	50 – 150	Hormones	d4-17 beta-Estradiol
Desogestrel	29 – 150	Hormones	d4-17 beta-Estradiol
17 alpha-Estradiol	50 – 150	Hormones	d4-17 beta-Estradiol
Estrone	50 – 150	Hormones	d4-17 beta-Estradiol
Equilin	50 – 150	Hormones	d4-17 beta-Estradiol
Androstenedione	50 – 193	Hormones	d4-17 beta-Estradiol
17 alpha-Dihydroequilin	50 – 150	Hormones	d6-Norethindrone
17 beta-Estradiol	70 – 130	Hormones	d4-17 beta-Estradiol
Testosterone	50 – 150	Hormones	d4-17 beta-Estradiol
Equilenin	50 – 150	Hormones	d4-17 beta-Estradiol
Mestranol	50 – 150	Hormones	d4-Mestranol
Norethindrone	70 – 130	Hormones	d6-Norethindrone
17 alpha-Ethinyl-Estradiol	70 – 130	Hormones	d4-17 alpha-Ethinyl-Estradiol
Progesterone	70 – 130	Hormones	d9-Progesterone
Norgestrel	70 – 130	Hormones	d6-Norgestrel
Estriol	6 – 169	Hormones	d6-Norgestrel
beta-Estradiol 3-benzoate	5 – 189	Hormones	d6-Norgestrel
d4-17 beta-Estradiol	30 – 150	Hormones-Surrogate	
d4-Mestranol	30 – 150	Hormones-Surrogate	
d6-Norethindrone	30 – 150	Hormones-Surrogate	
d4-17 alpha-Ethinyl-Estradiol	30 – 150	Hormones-Surrogate	
d9-Progesterone	30 – 200	Hormones-Surrogate	
d6-Norgestrel	30 – 150	Hormones-Surrogate	
Coprostanol	50 – 150	Sterols	d7-Cholesterol
Epicoprostanol	50 – 150	Sterols	d7-Cholesterol
Cholesterol	50 – 237	Sterols	d7-Cholesterol
Cholestanol	50 – 150	Sterols	d7-Cholesterol
Desmosterol	47 – 150	Sterols	d7-Cholesterol
Ergosterol	NA	Sterols	d7-Cholesterol
Campesterol	50 – 156	Sterols	d7-Cholesterol
β-sitosterol	5 – 200	Sterols	d7-Cholesterol
Stigmasterol	46 – 152	Sterols	d7-Cholesterol
β-stigmastanol	50 – 150	Sterols	d7-Cholesterol
d7-Cholesterol	13 – 150	Sterols-Surrogate	

4.0 Results

Results from this screening study are found in Table 4.

Table 4 Concentrations of perfluoroalkyl compounds (PFCs), pharmaceuticals and personal care products (PPCPs), and hormones/sterols in the Ohio River and its tributaries.

Sample number	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA		PFDA	PFUnA	PFDoA	PFBS	PFHxS	PFOS	PFOSA
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L		ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
1	1.14	1.08	< MRL	< MRL	2.04	< MRL		< MRL	< MRL	< MRL	2.51	< MRL	2.24	< MRL
2	< MRL	1.34	1.85	1.11	3.87	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	2.79	< MRL
3	1.53	1.84	2.35	1.57	3.41	< MRL		< MRL	< MRL	< MRL	2.31	< MRL	2.63	< MRL
4	1.09	1.17	1.31	1.24	2.50	1.04		< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
5	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
6	1.49	1.76	1.56	1.45	3.01	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	2.82	< MRL
7	2.44	3.63	3.95	2.01	5.39	1.37		1.16	< MRL	< MRL	< MRL	< MRL	4.42	< MRL
8	2.13	2.00	2.49	2.63	35.2	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	2.88	< MRL
9	< MRL	< MRL	< MRL	< MRL	1.48	< MRL		< MRL	< MRL	< MRL	18.4	< MRL	< MRL	< MRL
10	1.37	1.16	1.56	1.66	19.1	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	2.71	< MRL
11	3.89	3.30	4.69	2.85	6.22	1.74		1.01	< MRL	< MRL	< MRL	< MRL	5.14	< MRL
12	2.21	1.32	2.55	1.35	14.1	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	3.15	< MRL
13	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
14	1.01	1.56	1.71	1.58	13.1	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	2.58	< MRL
15	1.46	1.53	2.35	1.62	14.4	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	4.97	< MRL
16	5.29	13.7	7.83	4.4	14.3	1.64		< MRL	< MRL	< MRL	4.61	2.98	7.36	< MRL
17	1.83	1.94	2.42	1.37	9.21	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	5.58	< MRL
18	1.97	3.07	2.78	2.11	9.44	0.993	S	< MRL	< MRL	< MRL	< MRL	< MRL	5.80	< MRL
19	2.69	2.09	2.95	1.59	11.5	1.22	S	1.65	3.02	4.75	< MRL	< MRL	7.27	< MRL
20	5.75	8.59	10.4	4.30	18.2	6.17	S	8.04	< MRL	< MRL	111	< MRL	669	< MRL
21	1.40	1.81	2.30	1.32	3.43	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	3.90	< MRL
22	1.52	2.26	1.97	1.17	8.93	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	6.49	< MRL
23	12.5	4.65	9.48	7.67	23.9	< MRL		< MRL	< MRL	< MRL	16.5	5.62	35.4	1.60
24	15.7	6.68	14.7	9.14	31.2	8.08	S	2.95	< MRL	< MRL	31.8	8.91	29.2	< MRL
25	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL		< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
26 (FS)	15.2	13.5	13.7	14.2	11.6	12.2		10.1	7.96	3.12	13.0	9.67	4.19	< MRL
26 (SL)	20.0	20.0	20.0	20.0	20.0	20.0		20.0	20.0	20.0	20.0	20.0	20.0	0
27 (FS)	215	196	199	211	174	178		181	113	42.6	177	149	79.1	< MRL
27 (SL)	200	200	200	200	200	200		200	200	200	200	200	200	0
MRL	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00	2.00	2.00	2.00	1.00

FS - Field Spike (result)

SL - Spiked Level (expected)

Table 4 Continued...

Sample number	Acetaminophen	Albuterol	Alprazolam	Amitriptyline	10-Hydroxy-amitriptyline	Amlodipine	Amphetamine	Atenolol	Atorvastatin	Azithromycin	Benzoylecgonine	Benztropine	Betamethasone	Bisphenol A
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
1	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	8.53	<MRL	<MRL	1.09	<MRL	<MRL	<MRL
2	<MRL	<MRL	<MRL	<MRL	0.202	<MRL	<MRL	28.5 V	<MRL	<MRL	6.72	<MRL	<MRL	<MRL
3	<MRL	1.20	<MRL	1.97	0.414	<MRL	<MRL	99.5	<MRL	67.1	3.24	<MRL	<MRL	<MRL
4	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	34.1 V	<MRL	<MRL	3.10	<MRL	<MRL	<MRL
5	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
6	<MRL	<MRL	<MRL	<MRL	0.163	<MRL	<MRL	24.1	<MRL	<MRL	4.05	<MRL	<MRL	<MRL
7	<MRL	5.16	1.49	4.92	0.415	<MRL	7.23	269	<MRL	147	5.16	<MRL	<MRL	<MRL
8	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	8.62	<MRL	<MRL	1.35	<MRL	<MRL	<MRL
9	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	6.78	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
10	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	4.24	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
11	201	1.39	<MRL	4.79	1.25	<MRL	<MRL	152	<MRL	140	6.07	<MRL	<MRL	<MRL
12	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	9.46	<MRL	<MRL	1.70	<MRL	<MRL	<MRL
13	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
14	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	8.05	<MRL	<MRL	1.83	<MRL	<MRL	<MRL
15	<MRL	<MRL	<MRL	<MRL	0.329	<MRL	13.0	16.5	<MRL	<MRL	4.94	<MRL	<MRL	<MRL
16	<MRL	<MRL	<MRL	<MRL	0.304	<MRL	5.10	29.0	<MRL	<MRL	5.49	<MRL	<MRL	<MRL
17	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	6.61	<MRL	<MRL	2.05	<MRL	<MRL	<MRL
18	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	8.28	<MRL	<MRL	4.46	<MRL	<MRL	<MRL
19	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	6.49	<MRL	<MRL	3.23	<MRL	<MRL	<MRL
20	506	<MRL	2.43	3.57	0.292	<MRL	5.36	137	<MRL	41.2	6.47	<MRL	<MRL	<MRL
21	<MRL	<MRL	<MRL	1.05	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
22	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	2.81	<MRL	<MRL	1.26	<MRL	<MRL	<MRL
23	<MRL	<MRL	<MRL	1.09	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
24	<MRL	17.4	7.69	77.8	7.04	3.48	<MRL	502 V	<MRL	834	56.5	<MRL	<MRL	<MRL
25	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
MRL	200	1.00	1.00	1.00	0.150	1.50	5.00	2.00	15.0	1.50	1.00	0.300	1.50	2500

Table 4 Continued...

Sample number	Caffeine	Carbadox	Carbamazepine	Cefotaxime	Cimetidine	Ciprofloxacin	Clarithromycin	Clinafloxacin	Clonidine	Cloxacillin	Cocaine	Codeine	Cotinine	DEET
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
1	79.6	<MRL	9.33	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	8.83	17.5
2	72.2	<MRL	18.4	<MRL	<MRL	<MRL	1.93	<MRL	<MRL	<MRL	0.341	<MRL	12.3	30.9
3	66.8	<MRL	44.1	<MRL	<MRL	<MRL	11.2	<MRL	<MRL	<MRL	0.172	<MRL	21.1	26.5
4	114	<MRL	20.0	<MRL	<MRL	<MRL	2.07	<MRL	<MRL	<MRL	<MRL	<MRL	12.6	18.6
5	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
6	129	<MRL	19.9	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	12.3	20.7
7	57.8	<MRL	136	<MRL	21.5	28.5	16.7	<MRL	<MRL	<MRL	<MRL	<MRL	17.6	41.2
8	66.2	<MRL	19.1	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	12.5	23.8
9	<MRL	<MRL	10.5	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	7.56	11.2
10	<MRL	<MRL	11.7	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	10.5	16.4
11	158	<MRL	74.0	<MRL	2.37	27.8	48.6	<MRL	<MRL	<MRL	0.784	11.8	26.8	26.0
12	72.2	<MRL	22.7	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	14.3	24.6
13	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	2.73	<MRL	<MRL	<MRL	<MRL	<MRL
14	<MRL	<MRL	15.2	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	13.9	19.6
15	198	<MRL	17.9	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	19.9	19.3
16	<MRL	<MRL	56.6	<MRL	2.96	<MRL	<MRL	<MRL	<MRL	<MRL	0.827	<MRL	21.6	36.4
17	105	<MRL	11.1	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	14.8	15.9
18	167	<MRL	14.5	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	0.434	<MRL	15.5	28.8
19	73.1	<MRL	13.0	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	13.4 V	28.0
20	243	<MRL	107	<MRL	1.57	<MRL	9.94	<MRL	<MRL	<MRL	0.794	<MRL	31.5 V	62.1
21	<MRL	<MRL	14.0	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	7.94	8.37
22	<MRL	<MRL	10.9	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	10.4	15.9
23	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	7.76	9.39
24	<MRL	<MRL	159	<MRL	49.3	162	530	<MRL	<MRL	<MRL	7.91	88.2	51.3	231
25	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
MRL	50.0	1.50	5.00	6.00	1.00	20.0	1.50	6.00	1.50	1.20	0.150	10.0	5.00	1.50

Table 4 Continued...

Sample number	Dehydronifedipine	Desmethyldiltiazem	Diazepam	Digoxigenin	Digoxin	Diltiazem	1,7-Dimethylxanthine	Diphenhydramine	Enalapril	Enrofloxacin	Erythromycin-H2O	Flumequine	Fluocinonide
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
1	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	1.80	<MRL	<MRL
2	<MRL	0.729	<MRL	<MRL	NA	2.86	<MRL	<MRL	<MRL	<MRL	3.28	<MRL	<MRL
3	<MRL	8.24	<MRL	<MRL	NA	20.5	<MRL	34.5	<MRL	<MRL	14.9	<MRL	<MRL
4	<MRL	1.25	<MRL	<MRL	NA	4.09	<MRL	4.44	<MRL	<MRL	7.31	<MRL	<MRL
5	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
6	<MRL	<MRL	<MRL	<MRL	NA	1.82	<MRL	<MRL	<MRL	<MRL	3.77	<MRL	<MRL
7	2.01	9.22	<MRL	<MRL	NA	31.8	<MRL	42.2	<MRL	<MRL	17.9	<MRL	<MRL
8	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	2.18	<MRL	<MRL
9	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	1.45	<MRL	<MRL
10	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	2.41	<MRL	<MRL
11	2.13	7.60	<MRL	<MRL	<MRL	16.0	<MRL	31.4	<MRL	<MRL	24.1	<MRL	<MRL
12	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	2.64	<MRL	<MRL
13	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
14	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	1.31	<MRL	<MRL
15	<MRL	<MRL	<MRL	<MRL	NA	1.70	<MRL	<MRL	<MRL	<MRL	1.76	<MRL	<MRL
16	<MRL	1.19	<MRL	<MRL	NA	4.44	<MRL	<MRL	<MRL	<MRL	12.3	<MRL	<MRL
17	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	1.99	<MRL	<MRL
18	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	1.76	<MRL	<MRL
19	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	1.72	<MRL	<MRL
20	2.03	1.00	1.01	<MRL	<MRL	1.35	<MRL	3.29	<MRL	<MRL	33.5	<MRL	<MRL
21	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	1.07	<MRL	<MRL
22	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
23	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
24	5.40	79.6	4.12	<MRL	<MRL	172	<MRL	> 300 E	<MRL	<MRL	167	<MRL	<MRL
25	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
MRL	2.00	0.50	1.00	6.00	15.0	1.00	150	2.00	1.00	3.00	1.00	1.50	6.00

Table 4 Continued...

Sample number	Fluoxetine	Fluticasone propionate	Furosemide	Gemfibrozil	Glipizide	Glyburide	Hydrochlorothiazide	Hydrocodone	Hydrocortisone	Ibuprofen	2-Hydroxy-ibuprofen	Lincomycin	Lomefloxacin	Meprobamate
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
1	<MRL	<MRL	<MRL	7.25	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL
2	<MRL	<MRL	<MRL	13.8	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL
3	<MRL	<MRL	<MRL	62.9	<MRL	<MRL	<MRL	6.34	NA	<MRL	<MRL	<MRL	<MRL	27.3
4	<MRL	<MRL	<MRL	18.6	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL
5	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL
6	<MRL	<MRL	<MRL	15.8	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	17.0
7	1.78	<MRL	212	27.6	<MRL	<MRL	105	18.3	NA	<MRL	<MRL	<MRL	<MRL	130
8	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	16.9
9	<MRL	<MRL	<MRL	10.2	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL
10	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	13.7
11	7.28	2.02	<MRL	11.3	<MRL	<MRL	<MRL	7.96	<MRL	<MRL	<MRL	<MRL	<MRL	111
12	<MRL	<MRL	<MRL	7.26	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	26.3
13	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL
14	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	15.0
15	<MRL	<MRL	<MRL	19.2	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	24.1
16	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	5.19	NA	<MRL	<MRL	<MRL	<MRL	43.2
17	<MRL	<MRL	<MRL	9.52	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
18	<MRL	<MRL	<MRL	12.8	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	14.7
19	<MRL	<MRL	<MRL	6.53	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	13.9
20	3.92	<MRL	198	20.7	<MRL	<MRL	81.4	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	127
21	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
22	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	13.6
23	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
24	25.0	<MRL	1570	366	<MRL	13.0 V	558	159	<MRL	<MRL	<MRL	<MRL	<MRL	646
25	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
MRL	1.50	2.00	133	5.14	6.00	3.00	66.7	5.00	600	15.0	800	7.02	3.00	13.3

Table 4 Continued...

Sample number	Metformin	Methylprednisolone	Metoprolol	Miconazole	Naproxen	Norfloxacin	Norfluoxetine	Norgestimate	Norverapamil	Ofloxacin	Ormetoprim	Oxacillin	Oxolinic Acid	Oxycodone
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
1	324	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
2	948	<MRL	19.2	<MRL	22.9	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	3.87
3	3760	<MRL	42.3	<MRL	71.8	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	13.3
4	1240	<MRL	17.8	<MRL	20.8	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	3.39
5	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
6	585	<MRL	<MRL	<MRL	22.0	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	2.64
7	1550	<MRL	94.5	<MRL	21.4	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	26.3
8	489	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
9	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
10	325	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
11	1460	<MRL	97.5	<MRL	29.3	<MRL	<MRL	<MRL	0.153	27.7	<MRL	<MRL	<MRL	20.7
12	604	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
13	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
14	410	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
15	651	<MRL	<MRL	<MRL	25.5	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	3.28
16	467	<MRL	18.8	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	5.27
17	597	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
18	736	<MRL	<MRL	<MRL	13.3	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
19	870	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
20	1490	<MRL	83.5	<MRL	59.8	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	12.5
21	476	<MRL	<MRL	<MRL	<MRL	14.3	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
22	395	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
23	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
24	15300	<MRL	701	<MRL	164	<MRL	2.22	<MRL	3.45	504	<MRL	<MRL	<MRL	66.1
25	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
MRL	300	40.0	15.0	1.50	10.0	15.0	1.50	3.00	0.150	15.0	0.600	3.00	0.600	2.00

Table 4 Continued...

Sample number	Paroxetine	Penicillin G	Penicillin V	Prednisolone	Prednisone	Promethazine	Propoxyphene	Propranolol	Ranitidine	Roxithromycin	Sarafloxacin	Sertraline	Simvastatin	Sulfachloropyridazine	Sulfadiazine
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
1	<MRL	<MRL	<MRL	<MRL	NA	<MRL	1.15	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
2	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
3	<MRL	<MRL	<MRL	<MRL	NA	<MRL	3.94	<MRL	<MRL	<MRL	<MRL	3.39	<MRL	<MRL	<MRL
4	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
5	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
6	<MRL	<MRL	<MRL	<MRL	NA	<MRL	1.73	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
7	<MRL	<MRL	<MRL	<MRL	NA	<MRL	13.5	10.1	22.8	<MRL	<MRL	6.02	<MRL	<MRL	<MRL
8	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
9	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
10	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
11	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	7.77	10.7	4.17	<MRL	<MRL	3.08	<MRL	<MRL	<MRL
12	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
13	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
14	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
15	<MRL	<MRL	<MRL	<MRL	NA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
16	<MRL	<MRL	<MRL	<MRL	NA	<MRL	1.35	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
17	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
18	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
19	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
20	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	14.7	<MRL	<MRL	<MRL	<MRL	4.27	<MRL	<MRL	<MRL
21	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
22	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
23	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
24	5.54	<MRL	<MRL	<MRL	<MRL	<MRL	123	96	203	<MRL	<MRL	64.6	<MRL	<MRL	7.45
25	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
MRL	4.00	1.20	3.00	6.00	200	0.399	1.00	6.67	2.00	0.300	50.0	1.33	20.0	1.50	5.00

Table 4 Continued...

Sample number	Sulfadimethoxine	Sulfamerazine	Sulfamethazine	Sulfamethizole	Sulfamethoxazole	Sulfanilamide	Sulfathiazole	Anhydrochlortetracycline [ACTC]	Anhydrotetracycline [ATC]	Chlortetracycline [CTC]	Demeclocycline	Doxycycline	4-Epianhydrochlortetracycline [EACTC]	4-Epianhydrotetracycline [EATC]	4-Epichlortetracycline [ECTC]
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
1	<MRL	<MRL	<MRL	<MRL	12.4	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
2	<MRL	<MRL	<MRL	<MRL	24.2	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
3	<MRL	<MRL	<MRL	<MRL	57.2	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
4	<MRL	<MRL	<MRL	<MRL	32.9	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
5	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
6	<MRL	<MRL	<MRL	<MRL	21.7	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
7	<MRL	<MRL	<MRL	<MRL	213	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
8	<MRL	<MRL	<MRL	<MRL	35.9	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
9	<MRL	<MRL	<MRL	<MRL	16.5	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
10	<MRL	<MRL	<MRL	<MRL	21.3	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
11	<MRL	<MRL	<MRL	<MRL	279	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
12	<MRL	<MRL	<MRL	<MRL	35.5	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
13	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
14	<MRL	<MRL	<MRL	<MRL	20.4	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
15	<MRL	<MRL	<MRL	<MRL	31.6	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
16	<MRL	<MRL	<MRL	<MRL	188	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
17	<MRL	<MRL	<MRL	<MRL	18.9	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
18	<MRL	<MRL	<MRL	<MRL	28.5	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
19	<MRL	<MRL	<MRL	<MRL	32.2	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
20	<MRL	<MRL	<MRL	<MRL	38.6	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
21	<MRL	<MRL	<MRL	<MRL	30.3	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
22	<MRL	<MRL	<MRL	<MRL	26.0	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
23	<MRL	<MRL	<MRL	<MRL	6.77	<MRL	2.70	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
24	<MRL	<MRL	<MRL	<MRL	481	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
25	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
MRL	1.00	0.600	0.600	0.600	2.00	15.0	1.50	15.0	15.0	6.00	15.0	20.0	60.0	15.0	15.0

Table 4 Continued...

Sample number	4-Epioxytetracycline [EOTC]	4-Epitetracycline [ETC]	Isochlortetracycline [ICTC]	Minocycline	Oxytetracyclin [OTC]	Tetracycline [TC]	Theophylline	Thiabendazole	Trenbolone	Trenbolone acetate	Triamterene	Triclocarban	Triclosan	Trimethoprim
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
1	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	1.54	<MRL	<MRL	<MRL
2	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	3.64 V	<MRL	<MRL	<MRL
3	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	15.0	<MRL	<MRL	50.0
4	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	4.39 V	<MRL	<MRL	<MRL
5	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
6	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	3.83	<MRL	<MRL	<MRL
7	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	23.0	<MRL	<MRL	108
8	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	2.53	<MRL	<MRL	<MRL
9	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	1.97	<MRL	<MRL	<MRL
10	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	1.89	<MRL	<MRL	<MRL
11	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	10.7	<MRL	<MRL	32.4	23.3	<MRL	76.8
12	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	4.31	<MRL	<MRL	<MRL
13	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
14	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	2.49	<MRL	<MRL	<MRL
15	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	204	<MRL	<MRL	<MRL	4.84	<MRL	<MRL	<MRL
16	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	18.3	<MRL	<MRL	<MRL
17	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	3.11	<MRL	<MRL	<MRL
18	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	2.41	<MRL	<MRL	<MRL
19	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	1.66	<MRL	<MRL	<MRL
20	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	30.7	16.6	<MRL	<MRL
21	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	2.59	<MRL	<MRL	<MRL
22	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	1.79	<MRL	<MRL	<MRL
23	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
24	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	304	10.8	<MRL	<MRL	200 V	56.0	94.3	187
25	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
MRL	6.00	6.00	6.00	60.0	6.00	20.0	200	5.00	4.00	0.300	1.00	10.0	60.0	15.0

Table 4 Continued...

Sample number	Tylosin	Valsartan		Verapamil		Virginiamycin	Warfarin
	ng/L	ng/L		ng/L		ng/L	ng/L
1	NA	27.2	S	<MRL		<MRL	<MRL
2	NA	31.2	S	<MRL		<MRL	<MRL
3	NA	87.5	S	2.86		<MRL	<MRL
4	NA	40.9	S	<MRL		<MRL	<MRL
5	NA	<MRL		<MRL		<MRL	<MRL
6	NA	22.0	S	<MRL		<MRL	<MRL
7	NA	18.9	S	3.05		<MRL	<MRL
8	NA	<MRL		<MRL		<MRL	<MRL
9	NA	<MRL		<MRL		<MRL	<MRL
10	NA	<MRL		<MRL		<MRL	<MRL
11	<MRL	79.2		2.44	S	<MRL	<MRL
12	NA	17.6	S	<MRL		<MRL	<MRL
13	NA	<MRL		<MRL		<MRL	<MRL
14	NA	<MRL		<MRL		<MRL	<MRL
15	NA	40.4	S	<MRL		<MRL	<MRL
16	NA	18.1	S	<MRL		<MRL	<MRL
17	<MRL	17.9		<MRL		<MRL	<MRL
18	<MRL	22.3		<MRL		<MRL	<MRL
19	<MRL	<MRL		<MRL		<MRL	<MRL
20	<MRL	41.1		<MRL		<MRL	<MRL
21	<MRL	<MRL		<MRL		<MRL	<MRL
22	<MRL	<MRL		<MRL		<MRL	<MRL
23	<MRL	<MRL		<MRL		<MRL	<MRL
24	<MRL	625		64.3	S	<MRL	3.86
25	<MRL	<MRL		<MRL		<MRL	<MRL
MRL	6.00	13.3		1.50		3.00	1.50

Table 4 Continued...

Sample number	Androstenedione	Androsterone	Desogestrel	17 alpha-Dihydroequilin	Equilenin	Equilin	17 alpha-Estradiol	17 beta-Estradiol	beta-Estradiol 3-benzoate	17 alpha-Ethinyl-Estradiol	Estriol	Estrone
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
1	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
2	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
3	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
4	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
5	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
6	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
7	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
8	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
9	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
10	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
11	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
12	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
13	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
14	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
15	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
16	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
17	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
18	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
19	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
20	< MRL	< MRL	< MRL	9.08 V	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
21	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
22	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
23	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
24	< MRL	< MRL	< MRL	100	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	27.5
25	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
MRL	20.6	8.32	8.56	8.08	8.31	7.85	7.92	8.16	8.64	8.56	7.92	9.28

Table 4 Continued...

Sample number	Mestranol	Norethindrone	Norgestrel	Progesterone	Testosterone	Campesterol	Cholestanol	Cholesterol	Coprostanol	Epicoprostanol	Desmosterol
	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
1	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	78.9	195 B (232)	< MRL	< MRL	< MRL
2	< MRL	< MRL	< MRL	< MRL	< MRL	62.6	60.7	516 B (232)	< MRL	< MRL	< MRL
3	< MRL	< MRL	< MRL	< MRL	< MRL	130	522	1710 B (232)	1060	< MRL	K
4	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	65.6	676 B (232)	< MRL	< MRL	< MRL
5	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
6	< MRL	< MRL	< MRL	< MRL	< MRL	63.4	< MRL	278 B (232)	< MRL	< MRL	82.5
7	< MRL	< MRL	< MRL	< MRL	< MRL	186	277	1570 B (232)	818	< MRL	199
8	< MRL	< MRL	< MRL	< MRL	< MRL	52.3	52.2	449 B (232)	< MRL	< MRL	112
9	< MRL	< MRL	< MRL	< MRL	< MRL	140	69.3	503 B (232)	< MRL	< MRL	K
10	< MRL	< MRL	< MRL	< MRL	< MRL	67.9	< MRL	741 B (232)	< MRL	< MRL	K
11	< MRL	< MRL	< MRL	< MRL	< MRL	200	214	1830 B (232)	602	< MRL	K
12	< MRL	< MRL	< MRL	< MRL	< MRL	587	204	911 B (232)	< MRL	< MRL	K
13	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL
14	< MRL	< MRL	< MRL	< MRL	< MRL	189	118	834 B (232)	< MRL	< MRL	K
15	< MRL	< MRL	< MRL	< MRL	< MRL	117	125	955 B (232)	< MRL	< MRL	219
16	< MRL	< MRL	< MRL	< MRL	< MRL	812	308	1470 B (232)	462	< MRL	K
17	< MRL	< MRL	< MRL	< MRL	< MRL	223	91.3	1120	< MRL	< MRL	K
18	< MRL	< MRL	< MRL	< MRL	< MRL	197	153	1400	624	< MRL	K
19	< MRL	< MRL	< MRL	< MRL	< MRL	165	97.5	1160	< MRL	< MRL	K
20	< MRL	< MRL	< MRL	< MRL	< MRL	353	562	> 1890 E	1830	120	K
21	< MRL	< MRL	< MRL	< MRL	< MRL	> 800 E	259	1680 B (232)	< MRL	< MRL	867
22	< MRL	< MRL	< MRL	< MRL	< MRL	507	164	> 1890 E	< MRL	< MRL	275
23	< MRL	< MRL	< MRL	< MRL	< MRL	256	111	1050 B (128)	< MRL	< MRL	K
24	< MRL	< MRL	< MRL	< MRL	< MRL	> 800 E	507	> 1890 E	1750	121	K
25	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	< MRL	129 B (232)	< MRL	< MRL	< MRL
MRL	8.16	8.59	8.16	20.5	8.56	40.0	40.0	94.4	96.9	95.9	57.5

Table 4 Continued...

Sample number	Ergosterol	beta-Sitosterol	beta-Stigmastanol	Stigmasterol	
	ng/L	ng/L	ng/L	ng/L	
1	< MRL	98.0	< MRL	90.9	
2	< MRL	176	< MRL	213	
3	< MRL	415	< MRL	472	
4	< MRL	128	< MRL	107	
5	< MRL	< MRL	< MRL	< MRL	
6	< MRL	948	< MRL	265	
7	< MRL	629	< MRL	> 810	E
8	< MRL	231	< MRL	194	
9	< MRL	196	< MRL	374	
10	< MRL	144	< MRL	159	
11	< MRL	1440	< MRL	651	
12	< MRL	1420	213	> 810	E
13	< MRL	< MRL	< MRL	< MRL	
14	< MRL	193	< MRL	226	
15	< MRL	260	< MRL	255	
16	< MRL	794	111	> 810	E
17	< MRL	543	< MRL	662	
18	< MRL	579	137	475	
19	< MRL	397	< MRL	365	
20	< MRL	896	185	> 810	E
21	< MRL	1640	< MRL	> 810	E
22	< MRL	649	< MRL	750	
23	< MRL	481	< MRL	779	S
24	< MRL	> 1890	E 135	> 810	E
25	< MRL	< MRL	< MRL	< MRL	
MRL	96.3	94.5	96.8	40.5	

5.0 Next Steps

The results presented in this summary document will be used by various ORSANCO committees, subcommittees, workgroups and advisory committees. These groups will provide the Commission with comments on interpretation and use of the data generated by this survey such that the Commission might consider future actions. Additionally, a more thorough report summarizing the results of the study will be prepared by ORSANCO and EPA staff in the coming months.

6.0 References

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APPENDIX 1

METHOD SUMMARIES

Perfluorinated Organic Compounds MLA-060
Pharmaceutical and personal care Compounds MLA-075
Sterols and Hormones MLA-068

AXYS Analytical Services Ltd.

Method Summary

Analytical Procedure for the Analysis of Perfluorinated Organic Compounds in Aqueous Samples by LC-MS/MS by AXYS Method MLA-060 Rev 09

Method MLA-060 describes the analysis of perfluorinated organic compounds (PFC) in aqueous samples. Typical quantification limits are in the range of 1 - 2 ng/L for a 0.5 L sample size.

ANALYTES OF INTEREST

Perfluorobutanoate (PFBA) Perfluoropentanoate (PFPeA) Perfluorohexanoate (PFHxA) Perfluoroheptanoate (PFHpA) Perfluorooctanoate (PFOA) Perfluorononanoate (PFNA) Perfluorodecanoate (PFDA) Perfluoroundecanoate (PFUnA) Perfluorododecanoate (PFDoA)	Perfluorobutanesulfonate (PFBS) Perfluorohexanesulfonate (PFHxS) Perfluorooctanesulfonate (PFOS) Perfluorooctane sulfonamide (PFOSA) [1] [1] Optional analyte, not always required by client.

EXTRACTION AND CLEANUP

Sample size may be up to 1000 mL. Samples are stored in HDPE (high density polyethylene) containers. Samples are filtered, adjusted to pH 6.5, spiked with surrogate standards and extracted by solid phase extraction (SPE) using weak anion exchange cartridges. Wash and elution procedures are chosen to meet various analysis requirements. The eluates are spiked with recovery standards and analyzed by HPLC-MS/MS. Calibration solutions are processed through SPE in the same way as the samples.

QUALITY ASSURANCE / QUALITY CONTROL

All samples are analyzed in batches. The composition of a batch is detailed on a batch sheet. Each batch has the following composition:

- Batch Size - Each batch consists of test samples and additional QC samples.
- Blanks – 5% of the samples within a batch are procedural blanks.
- Duplicates – With each analysis batch containing greater than six (6) test samples, or as required by contract, a duplicate sample is analyzed, provided there is sufficient sample.
- Matrix Spike/Matrix Spike Duplicate analyzed upon client request.

- OPR (Spiked Reference Sample) – 5% of the samples within a batch are spiked reference samples.

QC Specification: Procedural Blank Levels and OPR Recoveries

Analyte		Procedural Blank Level ng/sample [2]	OPR Recovery Range (%)[1]
Perfluorobutanoate	(PFBA)	<0.25	80 – 120 [1]
Perfluoropentanoate	(PFPeA)	<0.25	80 – 120 [1]
Perfluorohexanoate	(PFHxA)	<0.25	80 – 120 [1]
Perfluoroheptanoate	(PFHpA)	<0.25	80 – 120 [1]
Perfluorooctanoate	(PFOA)	<0.25	80 – 120 [1]
Perfluorononanoate	(PFNA)	<0.25	80 – 120 [1]
Perfluorodecanoate	(PFDA)	<0.25	80 – 120 [1]
Perfluoroundecanoate	(PFUnA)	<0.25	80 – 120 [1]
Perfluorododecanoate	(PFDoA)	<0.25	80 – 120 [1]
Perfluorobutanesulfonate	(PFBS)	<0.25	70 - 130
Perfluorohexanesulfonate	(PFHxS)	<0.25	70 – 130
Perfluorooctanesulfonate	(PFOS)	<0.25	70 – 130
Perfluorooctane sulfonamide	(PFOSA)	<0.25	70 – 130

[1] Marginal exceedance allowance – recovery for 2 compounds may be 75-125% and for one compound 70-130%.

[2] Reporting limits (based on the lowest calibration standard and routine final extract volume of 4 mL) may exceed the stated blank criteria.

QC Specification: Surrogate Standard Recoveries (Calibration Solutions and Samples)

Surrogate Standard	Recovery Range [1]
$^{13}C_4$-Heptafluorobutyric acid ($^{13}C_4$-PFBA)	20 - 150%
$^{13}C_2$-Perfluorocaproic acid ($^{13}C_2$-PFHxA)	40 - 150%
$^{13}C_2$- Perfluorooctanoic acid ($^{13}C_2$-PFOA)	40 - 150%
$^{13}C_5$-Heptadecafluorononanoic acid ($^{13}C_5$-PFNA)	40 - 150%
$^{13}C_2$- Perfluorodecanoic acid ($^{13}C_2$-PFDA)	40 - 150%
$^{13}C_2$-Perfluoro-n-(1,2)decanoic acid ($^{13}C_2$-PFDoA)	40 - 150%
$^{13}C_4$-Perfluorooctanesulfonate ($^{13}C_4$-PFOS)	40 - 150%
d7-N-Me-Perfluoro-1-octanesulfonamidoethanol (d7-MeFOSE)	40 - 150%

[1] Lower recoveries may be accepted based on application and professional judgment

QC Specification Table: Other Parameters

QC Parameter	Specification
Instrument Sensitivity	Daily, S:N ≥ 3:1 for all analytes for lowest calibration standard.
Initial Calibration (native compounds)	Run initially, and as required to maintain calibration verification and instrument sensitivity. (1/x) weighted quadratic, exclude origin. Calculated conc. 75-125 % of actual (lowest cal may be 70-130%), R^2 > 0.990
Continuing Calibration Verification (native compounds)	Run every 20 samples or more frequently, quantify against I-CAL. Calculated conc. 70-130% actual for a maximum of three compounds with the remainder 80–120 % of actual
Instrumental Carryover and Instrument Background	Every Initial Calibration, Cal/Ver, or SPM: < 0.3 % carryover and area response of analytes in instrument blank < 800 judged following two previous methanol blank injections.
Duplicate Samples or MS/MSD	If conc. > 5 times R.L., RPD < 40% If conc. < 5 times R.L., difference between pairs < R.L.

ANALYSIS BY LC-MS/MS

Analysis of sample extracts for perfluorinated organics by HPLC-MS/MS is performed on a high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer. The MS is run at unit mass resolution in the Multiple Reaction Monitoring (MRM) mode.

Instrument specifications:

Instrument	Waters 2690 or Waters 2795 HPLC, Micromass Quattro Ultima MS/MS
LC Column	Waters Xtera C18MS Reverse Phase C18, 10.0 cm, 2.1 mm i.d., 3.5 µm particle size (or equivalent)
Ionization	Negative Ion Electrospray
Acquisition	MRM mode, unit resolution
Injection Volume	15 µL

LC-MS/MS Operating Conditions:

LC Gradient Program				General LC Conditions	
Time (min)	**Flow mixture[1]**	**LC Flow Rate Program**	**Gradient Curve**	Column Temp (℃)	40
0.0	15% solvent A 85% solvent B	0.15 mL/min	1	Max Pressure (bar)	300.0
1.0	15% solvent A 85% solvent B	0.15 mL/min	1	**MS Conditions**	
5.0	70% solvent A 30% solvent B	0.20 mL/min	4	Source Temp (℃)	120
8.5	100% solvent A	0.20 mL/min	4	Desolvation Temp (℃)	300
11	100% solvent A	0.20 mL/min	4	Capillary Voltage	2.75
11.3 - 14.5	15% solvent A 85% solvent B	0.20 mL/min	2	Gases	~70L/hr cone ~300L/hr desolvation

[1] Eluent A = 90% CH_3CN (aqueous)
Eluent B = 12.1 mM NH_4OAc in 0.1% acetic acid (aqueous)

Initial calibration of the LC-MS/MS instrument is performed by the analysis of six or more calibration solutions. A mid-level calibration standard is analyzed to verify the initial calibration after every 20th sample (including QC samples) injected at a minimum. All calibration solutions go through the same SPE extraction/cleanup procedures as the samples.

ANALYTE IDENTIFICATION

Positive identification of target PFC, surrogate standard and recovery standards require:

- > 3:1 S:N for parent ion to daughter ion transition.
- Compound retention time falls within 0.4 minutes of the predicted retention times from the mean determined from the Initial Calibration. Natives with labelled surrogate standards must elute within 0.1 minutes of the associated labelled surrogates.

QUANTIFICATION AND DATA REPORTING PROCEDURES

Target compounds are quantified using the internal standard method, comparing the area of the quantification ion to that of the ^{13}C-labelled standard and correcting for response factors.

Quadratic calibration equations are determined from a multi-point calibration series with 1/X weighing fit as described by the following general equation:

$Y= a + bX + cX^2$ (general quadratic equation)

Where Y = (area target/area surr) x weight surr
X = weight target
a,b,c are empirical constants

Concentrations in samples are determined as:

$$Sample\ Conc = \frac{-b \pm \sqrt{b^2 - 4c\left(a - \left(\frac{area\ of\ target}{area\ of\ sur}\ x\ weight\ sur\right)\right)}}{2c\ x\ sample\ size}$$

The recovery of the surrogate standard is calculated **(by internal standard quantification against the recovery standard using an average RRF)** and monitored as an indication of overall data quality. Final target concentrations are recovery corrected by this method of quantification.

Sample Specific Detection Limits (SDL) are determined .by converting the area equivalent of 3.0 times the estimated chromatographic noise height to a concentration in the same manner that target peak responses are converted to final concentrations. The SDL accounts for any effect of matrix on the detection system and for recovery achieved through the analytical work-up.

Results are reported to the greater of the SDL or the concentration equivalent to the lowest calibration standard analyzed.

Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (minutes)	Parent Ion Mass	Daughter Ion Mass	Quantified Against
Target Analytes				
Perfluorobutanoate (PFBA)	5.0	213	169	$^{13}C_4$-PFBA
Perfluoropentanoate (PFPeA)	5.8	263	219	$^{13}C_2$-PFHxA
Perfluorohexanoate (PFHxA)	6.2	313	269	$^{13}C_2$-PFHxA
Perfluoroheptanoate (PFHpA)	6.6	363	319 (169)[1]	$^{13}C_2$-PFHxA
Perfluorooctanoate (PFOA)	7.0	413	369 (169/219)[1]	$^{13}C_2$-PFOA
Perfluorononanoate (PFNA)	7.4	463	419 (219)[1]	$^{13}C_5$-PFNA
Perfluorodecanoate (PFDA)	7.9	513	469 (269)[1]	$^{13}C_2$-PFDA

Perfluoroundecanoate (PFUnA)	8.5	563	519 (269)[1]	$^{13}C_2$-PFDA
Perfluorododecanoate (PFDoA)	9.0	613	569 (319)[1]	$^{13}C_2$-PFDoA
Perfluorobutanesulfonate (PFBS)	6.3	299	80 (99)[1]	$^{13}C_4$-PFOS
Perfluorohexanesulfonate (PFHxS)	7.2	399	80 (99/119)[1]	$^{13}C_4$-PFOS
Perfluorooctane sulfonate (PFOS)	8.2	499	80 (99/130)[1]	$^{13}C_4$-PFOS
Perfluorooctane sulfonamide (PFOSA) [2]	9.9	498	78 (478)[1]	$^{13}C_4$-PFOS
Surrogate Standard				
$^{13}C_4$-Heptafluorobutyric acid ($^{13}C_4$-PFBA)	5.0	217	172	$^{13}C_2$-PFOUEA
$^{13}C_2$-Perfluorocaproic acid ($^{13}C_2$-PFHxA)	6.2	315	270	$^{13}C_2$-PFOUEA
$^{13}C_2$-Perfluorooctanoic acid ($^{13}C_2$-PFOA)	7.0	415	370	$^{13}C_4$-PFOA
$^{13}C_5$-Heptadecafluorononanoic acid ($^{13}C_5$-PFNA)	7.4	470	423	$^{13}C_2$-PFOUEA
$^{13}C_2$-Perfluorodecanoic acid ($^{13}C_2$-PFDA)	7.9	515	470	$^{13}C_2$-PFOUEA
$^{13}C_2$-Perfluoro-n-(1,2)decanoic acid ($^{13}C_2$-PFDoA)	9.0	615	570	$^{13}C_2$-PFOUEA
$^{13}C_4$–Perfluorooctanesulfonate ($^{13}C_4$-PFOS)	8.2	503	80 (99)[1]	$^{13}C_2$-PFOUEA
d7-N-Me-Perfluoro-1-octanesulfonamidoethanol (d7-Me-FOSE)	~10.6	623	59	$^{13}C_2$-PFOUEA
Recovery Standard				
$^{13}C_2$-2H-Perfluoro-2-decenoic acid ($^{13}C_2$-PFOUEA)	7.3	459	394	-
$^{13}C_4$-Perfluorooctanoic acid ($^{13}C_4$-PFOA)	6.9	417	372	-

[1] Alternate transition within brackets, may be used if necessary to avoid interference.

[2] PFOSA quantified against d7-Me-FOSE if collected in separate fraction under option C.

AXYS Analytical Services Ltd.

SUMMARY OF AXYS METHOD MLA-075:

ANALYTICAL PROCEDURES FOR THE ANALYSIS OF PHARMACEUTICAL AND PERSONAL CARE COMPOUNDS IN SOLID AND AQUEOUS SAMPLES BY LC-MS/MS

ANALYTE LISTS

List 1 (Acid extraction, positive ESI)	
Acetaminophen	Norfloxacin
Ampicillin [1]	Norgestimate
Azithromycin	Ofloxacin
Caffeine	Ormetoprim
Carbadox	Oxacillin
Carbamazepine	Oxolinic acid
Cefotaxime	Penicillin G
Ciprofloxacin	Penicillin V
Clarithromycin	Roxithromycin
Clinafloxacin	Sarafloxacin
Cloxacillin	Sulfachloropyridazine
Dehydronifedipine	Sulfadiazine
Digoxigenin	Sulfadimethoxine
Digoxin	Sulfamerazine
Diltiazem	Sulfamethazine
1,7-Dimethylxanthine	Sulfamethizole
Diphenhydramine	Sulfamethoxazole
Enrofloxacin	Sulfanilamide
Erythromycin	Sulfathiazole
Flumequine	Thiabendazole
Fluoxetine	Trimethoprim
Lincomycin	Tylosin
Lomefloxacin	Virginiamycin
Miconazole	
List 2 (Tetracyclines, positive ESI)	
Anhydrochlortetracycline (ACTC)	4-Epichlortetracycline (ECTC)
Anhydrotetracycline (ATC)	4-Epioxytetracycline (EOTC)
Chlortetracycline (CTC)	4-Epitetracycline (ETC)
Demeclocycline	Isochlortetracycline (ICTC)
Doxycycline	Minocycline
4-Epianhydrochlortetracycline (EACTC)	Oxytetracycline (OTC)
4-Epianhydrotetracycline (EATC)	Tetracycline (TC)

List 3 (Acid extraction, negative ESI)	
Bisphenol A	2-hydroxy-ibuprofen
Furosemide	Ibuprofen
Gemfibrozil	Naproxen
Glipizide	Triclocarban
Glyburide	Triclosan
Hydrochlorothiazide	Warfarin
List 4 (Base extraction, positive ESI)	
Albuterol	Cotinine
Amphetamine	Enalapril
Atenolol	Hydrocodone
Atorvastatin	Metformin
Cimetidine	Oxycodone
Clonidine	Ranitidine
Codeine	Triamterene
List 5 (Acid Extraction, positive ESI)	
Alprazolam	Metoprolol
Amitriptyline	Norfluoxetine
Amlodipine	Norverapamil
Benzoylecgonine	Paroxetine
Benztropine	Prednisolone
Betamethasone	Prednisone
Cocaine	Promethazine
DEET (N,N-diethyl-m-toluamide)	Propoxyphene
Desmethyldiltiazem	Propranolol
Diazepam	Sertraline
Fluocinonide	Simvastatin
Fluticasone propionate	Theophylline
Hydrocortisone	Trenbolone
10-hydroxy-amitriptyline	Trenbolone acetate
Meprobamate	Valsartan
Methylprednisolone	Verapamil

[1] Due to instability accuracy of Ampicillin data is unknown.

EXTRACTION AND CLEANUP PROCEDURES

The analysis requires extraction at two different pH conditions: At pH 10 for analysis of fourteen analytes (List 4); and at pH 2.0 for the analysis of the other analytes (Lists 1, 2, 3, and 5). Prior to extraction and/or clean-up samples are adjusted to the required pH and spiked with surrogates.

Solid samples are repeatedly extracted by sonication with aqueous buffered acetonitrile and pure acetonitrile, concentrated by rotary evaporation, and diluted with ultra pure water to 200 mL. The acidic extract is treated with EDTA. The extracts are cleaned up by solid phase extraction (SPE), filtered, and analyzed by LC/ESI-MS/MS in positive and negative ionization modes requiring a total of five runs to analyze the complete list of analytes.

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All aqueous samples are filtered and the aqueous portion is cleaned up by solid phase extraction before analysis by LC/ESI-MS/MS.

Aqueous samples with no or limited visible particulate (e.g. surface water, ground water, wastewater treatment final effluent, typically with < 100 mg/L TSS) normally can be processed with up to 1L samples sizes. The sample is filtered and routinely only the aqueous phase is analyzed. However, upon specific agreement a separate extraction may be performed on the solids phase. The solids extract may in this case either be carried through the analysis individually as a separate sample that is reported separately, or the aqueous extract and the solids extract may be combined just prior to clean-up and reported as a combined aqueous/solids phase result.

For mixed phase aqueous/solids samples with significant solids and distinct aqueous and solids phases such as wastewater influent or process streams the sample may either be analyzed as an aqueous phase only or as two separate samples, one aqueous and one solid.

ANALYSIS BY LC-MS/MS

The analysis is performed on a high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer. Instrument calibration is performed using a series of calibration solutions (7 points) covering the working concentration range of the instrument specific for the individual compounds of interest. The LC/MS/MS is run in MRM (Multiple Reaction Monitoring) mode and quantification is performed by recording the peak areas of the applicable parent ion/daughter ion transitions. Some analytes are analyzed in the ESI positive mode and some are analyzed in the ESI negative mode.

List 1 – Acid Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Sulfanilamide	2.5	190.0	155.8	$^{13}C_6$-Sulfamethazine
Acetaminophen	4.6	152.2	110.0	$^{13}C_2$-^{15}N-Acetaminophen
Sulfadiazine	6.0	251.2	156.1	$^{13}C_6$-Sulfamethazine
1,7-Dimethylxanthine	6.9	181.2	124.0	$^{13}C_3$-Caffeine
Sulfathiazole	7.7	256.3	156.0	$^{13}C_6$-Sulfamethoxazole
Sulfamerazine	8.7	265.0	156.0	$^{13}C_6$-Sulfamethazine
Caffeine	9.3	195.0	138.0	$^{13}C_3$-Caffeine
Lincomycin	9.3	407.5	126.0	$^{13}C_3$-Trimethoprim
Sulfamethizole	10.0	271.0	156.0	$^{13}C_6$-Sulfamethoxazole
Thiabendazole	10.0	202.1	175.1	d_6-Thiabendazole
Trimethoprim	10.0	291.0	230.0	$^{13}C_3$-Trimethoprim
Sulfamethazine	10.1	279.0	156.0	$^{13}C_6$-Sulfamethazine
Cefotaxime	10.2	456.4	396.1	$^{13}C_3$-Trimethoprim
Carbadox	10.5	263.2	231.2	$^{13}C_3$-Trimethoprim
Ormetoprim	10.5	275.3	259.1	$^{13}C_3$-Trimethoprim
Norfloxacin	10.7	320.0	302.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Ofloxacin	10.8	362.2	318.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sulfachloropyridazine	10.8	285.0	156.0	$^{13}C_6$-Sulfamethazine
Ciprofloxacin	10.9	332.2	314.2	$^{13}C_3$, ^{15}N-Ciprofloxacin
Lomefloxacin	11.2	352.2	308.1	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sulfamethoxazole	11.2	254.0	156.0	$^{13}C_6$-Sulfamethoxazole

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Enrofloxacin	11.5	360.0	316.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sarafloxacin	11.9	386.0	299.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Clinafloxacin	12.1	366.3	348.1	$^{13}C_3$, ^{15}N-Ciprofloxacin
Digoxigenin	12.6	391.2	355.2	$^{13}C_3$-Trimethoprim
Oxolinic Acid	13.1	261.8	243.8	$^{13}C_3$-Trimethoprim
Sulfadimethoxine	13.2	311.0	156.0	$^{13}C_6$-Sulfamethoxazole
Diphenhydramine	14.5	256.8	168.1	$^{13}C_3$-Trimethoprim
Penicillin G	14.6	367.5	160.2	$^{13}C_3$-Trimethoprim
Azithromycin	14.8	749.9	591.6	$^{13}C_3$-Trimethoprim
Flumequine	15.2	262.0	173.7	$^{13}C_3$-Trimethoprim
Ampicillin	15.3	350.3	160.2	$^{13}C_3$-Trimethoprim
Carbamazepine	15.3	237.4	194.2	$^{13}C_3$-Trimethoprim
Diltiazem	15.3	415.5	178.0	$^{13}C_3$-Trimethoprim
Penicillin V	15.4	383.4	160.2	$^{13}C_3$-Trimethoprim
Erythromycin [1]	15.9	734.4	158	not quantified
Tylosin	16.3	916.0	772.0	$^{13}C_6$-Sulfamethazine
Oxacillin	16.4	434.3	160.1	$^{13}C_3$-Trimethoprim
Dehydronifedipine	16.5	345.5	284.1	$^{13}C_3$-Trimethoprim
Digoxin	16.6	803.1	283.0	$^{13}C_3$-Trimethoprim
Cloxacillin	16.9	469.1	160.1	$^{13}C_3$-Trimethoprim
Fluoxetine	16.9	310.3	148.0	d_5-Fluoxetine
Virginiamycin	17.3	508.0	355.0	$^{13}C_3$-Trimethoprim
Clarithromycin	17.5	748.9	158.2	$^{13}C_6$-Sulfamethazine
Erythromycin - H_2O [1]	17.7	716.4	158	$^{13}C_2$-Erythromycin - H_2O
Roxithromycin	17.8	837.0	679.0	$^{13}C_6$-Sulfamethazine
Miconazole	20.1	417.0	161.0	$^{13}C_3$-Trimethoprim
Norgestimate	21.7	370.5	124.0	$^{13}C_3$-Trimethoprim
Surrogate Standard				
$^{13}C_2$-^{15}N-Acetaminophen	4.5	155.2	111.0	$^{13}C_3$-Atrazine
$^{13}C_3$-Caffeine	9.3	198.0	140.0	$^{13}C_3$-Atrazine
d_6-Thiabendazole	9.8	208.1	180.1	$^{13}C_3$-Atrazine
$^{13}C_3$-Trimethoprim	10.0	294.0	233.0	$^{13}C_3$-Atrazine
$^{13}C_6$-Sulfamethazine	10.1	285.1	162.1	$^{13}C_3$-Atrazine
$^{13}C_3$, ^{15}N-Ciprofloxacin	10.9	336.1	318.2	$^{13}C_3$-Atrazine
$^{13}C_6$-Sulfamethoxazole	11.2	260.0	162.0	$^{13}C_3$-Atrazine
$^{13}C_2$-Erythromycin [1]	15.9	736.4	160.0	monitor for less than 5%
d_5-Fluoxetine	16.8	315.3	153.0	$^{13}C_3$-Atrazine
$^{13}C_2$-Erythromycin - H_2O [1]	17.7	718.4	160.0	$^{13}C_3$-Atrazine
Recovery Standard				
$^{13}C_3$-Atrazine	15.9	219.5	176.9 (134.0)	External Standard

[1] Because of intramolecular dehydration during the analytical procedure erythromycin is quantified as the dehydration product "erythromycin – H_2O" [5]. The peak area of the $^{13}C_2$-Erythromycin is monitored and must be less than 5% of the $^{13}C_2$-Erythromycin - H_2O peak area. If it is greater, the Erythromycin - H_2O result is flagged as 'accuracy unknown'.

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List 2 – Acid Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Minocycline	5.1	458.0	441.0	d_6-Thiabendazole
Epitetracycline (ETC)	8.1	445.2	410.2	d_6-Thiabendazole
Epioxytetracycline (EOTC)	8.6	461.2	426.2	d_6-Thiabendazole
Oxytetracycline (OTC)	9.4	461.2	426.2	d_6-Thiabendazole
Tetracycline (TC)	9.9	445.2	410.2	d_6-Thiabendazole
Demeclocycline	11.7	465.0	430.0	d_6-Thiabendazole
Isochlortetracycline (ICTC) [1]	11.9	479.0	462.0	d_6-Thiabendazole
Epichlortetracycline (ECTC)	12.0	479.0	444.0	d_6-Thiabendazole
Chlortetracycline (CTC)	14.1	479.0	444.0	d_6-Thiabendazole
Doxycycline	16.7	445.2	428.2	d_6-Thiabendazole
Epianhydrotetracycline (EATC)	17.0	426.8	409.8	d_6-Thiabendazole
Anhydrotetracycline (ATC)	18.8	426.8	409.8	d_6-Thiabendazole
Epianhydrochlortetracycline (EACTC)	20.7	461.2	444.0	d_6-Thiabendazole
Anhydrochlortetracycline (ACTC)	22.1	461.2	444.0	d_6-Thiabendazole
Surrogate Standard				
d_6-Thiabendazole	7.1	208.1	180.1	$^{13}C_3$-Atrazine
Recovery Standard				
$^{13}C_3$-Atrazine	21.2	219.5	176.9	External Standard

[1] Isochlortetracycline (ICTC) is reported as the sum ICTC + ECTC due to a common transition ion.

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List 3 – Acid Extraction, Negative Electrospray Ionization (-)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Hydrochlorathiazide	2.3	296.0	268.0	^{13}C-d_3-Naproxen
Hydrochlorathiazide*	2.3	296.0	204.8	^{13}C-d_3-Naproxen
Furosemide	3.4	329.0	284.8	^{13}C-d_3-Naproxen
Furosemide*	3.4	329.0	204.7	^{13}C-d_3-Naproxen
2-hydroxy-ibuprofen	4.2	221.1	176.8	$^{13}C_3$-Ibuprofen
Bisphenol A	6.5	227.0	211.9	d6-Bisphenol A
Bisphenol A*	6.5	227.0	132.9	d6-Bisphenol A
Glipizide	6.9	444.2	319.0	d11-Glipizide
Glipizide*	6.9	444.2	169.8	d11-Glipizide
Naproxen	7.0	228.9	168.6	^{13}C-d_3-Naproxen
Warfarin	7.4	307.0	161.0	d_5-Warfarin
Glyburide	8.8	492.1	169.8	d3-Glyburide
Glyburide*	8.8	492.1	367.0	d3-Glyburide
Ibuprofen	8.8	205.1	161.1	$^{13}C_3$-Ibuprofen
Gemfibrozil	9.9	249.0	121.0	d_6-Gemfibrozil
Triclocarban	10.1	312.9	159.7	$^{13}C_6$-Triclocarban
Triclosan	10.2	286.8	35.0	$^{13}C_{12}$-Triclosan
Surrogate Standard				
d_6-Bisphenol A	6.5	233.0	214.8	$^{13}C_6$-2,4,5-T
d_6-Bisphenol A*	6.5	233.0	137.8	$^{13}C_6$-2,4,5-T
d_{11}-Glipizide	6.8	455.0	319.0	$^{13}C_6$-2,4,5-T
d_{11}-Glipizide*	6.8	455.0	169.8	$^{13}C_6$-2,4,5-T
^{13}C-d_3-Naproxen	7.0	232.9	168.6	$^{13}C_6$-2,4,5-T
d_5-Warfarin	7.4	312	161.0	$^{13}C_6$-2,4,5-T
d_3-Glyburide	8.7	495.0	169.9	$^{13}C_6$-2,4,5-T
d_3-Glyburide*	8.7	495.0	370.1	$^{13}C_6$-2,4,5-T
$^{13}C_3$-Ibuprofen	8.8	208.2	163.1	$^{13}C_6$-2,4,5-T
d_6-Gemfibrozil	9.9	255	121	$^{13}C_6$-2,4,5-T
$^{13}C_6$-Triclocarban	10.1	318.9	159.7	$^{13}C_6$-2,4,5-T
$^{13}C_{12}$-Triclosan	10.2	298.8	35	$^{13}C_6$-2,4,5-T
Recovery Standard				
$^{13}C_6$-2,4,5-Trichlorophen-oxyacetic acid ($^{13}C_6$-2,4,5-T)	4.9	258.8	200.7	External Standard

* Indicates secondary transition for possible diagnostic use.

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List 4 – Base Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Cotinine	4.0	177.0	98.0	d3-Cotinine
Cimetidine	4.7	253.1	159.0	d3-Cimetidine
Triamterene	5.4	254.1	236.9	d4-Clonidine
Triamterene*	5.4	254.1	103.7	d4-Clonidine
Enalapril	6.5	377.2	233.9	d5-Enalapril
Enalapril*	6.5	377.2	159.8	d5-Enalapril
Oxycodone	6.7	316.2	240.9	d6-Oxycodone
Oxycodone*	6.7	316.2	298.0	d6-Oxycodone
Clonidine	6.8	230.0	212.5	d4-Clonidine
Clonidine*	6.8	230.0	43.9	d4-Clonidine
Amphetamine	8.1	136.1	90.8	d5-Amphetamine
Amphetamine*	8.1	136.1	118.9	d5-Amphetamine
Albuterol	8.3	240.0	148.0	d_3-Albuterol
Codeine	8.4	300.0	152.0	d6-Codeine
Hydrocodone	8.6	300.2	198.8	d3-Hydrocodone
Hydrocodone*	8.6	300.2	170.6	d3-Hydrocodone
Atorvastatin	8.9	559.3	440.0	d5-Enalapril
Atorvastatin*	8.9	559.3	466.0	d5-Enalapril
Atenolol	9.0	267.2	144.7	d7-Atenolol
Atenolol*	9.0	267.2	189.7	d7-Atenolol
Metformin	9.5	131.1	60.1	d_6-Metformin
Ranitidine	18.8	315.0	175.9	d_3-Albuterol
Surrogate Standards				
d_3-Cotinine	4.0	180.0	79.9	d3-Amitriptyline
d_3-Cotinine*	4.0	180.0	101.0	d3-Amitriptyline
d_3-Cimetidine	4.7	256.0	161.8	d3-Amitriptyline
d_3-Cimetidine*	4.7	256.0	94.8	d3-Amitriptyline
d_5-Enalapril	6.5	382.0	238.8	d3-Amitriptyline
d_5-Enalapril*	6.5	382.0	164.8	d3-Amitriptyline
d_6-Oxycodone	6.7	322.1	262.0	d3-Amitriptyline
d_6-Oxycodone*	6.7	322.1	304.1	d3-Amitriptyline
d_4-Clonidine	6.8	234.0	216.7	d3-Amitriptyline
d_4-Clonidine*	6.8	234.0	47.9	d3-Amitriptyline
d_5-Amphetamine	8.1	141.1	92.9	d3-Amitriptyline
d_5-Amphetamine*	8.1	141.1	123.9	d3-Amitriptyline
d_3-Albuterol	8.3	243.0	151.0	d3-Amitriptyline
d_6-Codeine	8.4	306.0	151.8	d3-Amitriptyline
d_6-Codeine*	8.4	306.0	217.9	d3-Amitriptyline
d_3-Hydrocodone	8.6	303.1	198.9	d3-Amitriptyline
d_3-Hydrocodone*	8.6	303.1	170.8	d3-Amitriptyline
d_7-Atenolol	9.0	274.0	144.7	d3-Amitriptyline
d_7-Atenolol*	9.0	274.0	189.7	d3-Amitriptyline
d_6-Metformin	9.5	137.1	60.1	d3-Amitriptyline
Recovery Standards				

d_3-Amitriptyline	7.9	281.0	232.7	External Standard
d_3-Amitriptyline*	7.9	281.0	90.7	External Standard

* Indicates secondary transition for possible diagnostic use.

List 5 – Acid Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention	Parent Ion Mass	Daughter Ion Mass	Quantified against
Theophylline	2.7	181.1	123.8	13C-15N2-Theophylline
Theophylline*	2.7	181.1	95.8	13C-15N2-Theophylline
Benzoylecgonine	5.7	290.1	167.8	d8-Benzoylecgonine
Benzoylecgonine*	5.7	290.1	104.8	d8-Benzoylecgonine
Metoprolol	8.4	268.2	190.7	d7-Metoprolol
Metoprolol*	8.4	268.2	115.7	d7-Metoprolol
Cocaine	9.2	304.1	181.8	d3-Cocaine
Cocaine*	9.2	304.1	81.9	d3-Cocaine
Meprobamate	11.1	219.0	157.8	d7-Metoprolol
Meprobamate*	11.1	219.0	96.9	d7-Metoprolol
10-hydroxy-amitriptyline	12.0	294.2	215.0	d7-Propranolol
10-hydroxy-amitriptyline*	12.0	294.2	276.0	d7-Propranolol
Propranolol	14.6	260.2	115.8	d7-Propranolol
Propranolol*	14.6	260.2	182.7	d7-Propranolol
Prednisone	16.6	359.2	341.0	d7-Propranolol
Prednisone*	16.6	359.2	146.7	d7-Propranolol
Prednisolone	17.5	361.2	343.0	d7-Propranolol
Prednisolone*	17.5	361.2	324.7	d7-Propranolol
Hydrocortisone	17.6	363.2	120.7	d4-Hydrocortisone
Hydrocortisone*	17.6	363.2	326.7	d4-Hydrocortisone
Desmethyldiltiazem	18.8	401.2	177.8	d4-Promethazine
Desmethyldiltiazem*	18.8	401.2	149.5	d4-Promethazine
Promethazine	18.8	285.1	197.8	d4-Promethazine
Promethazine*	18.8	285.1	85.7	d4-Promethazine
DEET	20.6	192.0	118.6	d7-DEET
DEET	20.6	192.0	90.7	d7-DEET
Paroxetine	20.6	330.2	191.8	d6-Paroxetine
Paroxetine*	20.6	330.2	69.8	d6-Paroxetine
Norverapamil	21.0	441.3	164.7	d7-Propranolol
Norverapamil*	21.0	441.3	149.7	d7-Propranolol
Methylprednisolone	21.4	375.2	357.0	d2-Methylprednisolone
Methylprednisolone*	21.4	375.2	339.0	d2-Methylprednisolone
Verapamil	21.4	455.3	164.8	d6-Amitriptyline
Verapamil*	21.4	455.3	149.8	d6-Amitriptyline
Betamethasone	21.7	393.2	355.1	d6-Amitriptyline
Betamethasone*	21.7	393.2	373.0	d6-Amitriptyline
Propoxyphene	21.8	340.2	57.9	d5-Propoxyphene
Propoxyphene*	21.8	340.2	266.1	d5-Propoxyphene
Amitriptyline	22.4	278.2	232.8	d6-Amitriptyline
Amitriptyline*	22.4	278.2	90.7	d6-Amitriptyline
Trenbolone	22.5	271.2	198.7	d5-Alprazolam

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Trenbolone*	22.5	271.2	252.8	d5-Alprazolam
Benztropine	22.9	308.2	166.7	d3-Benztropine
Benztropine*	22.9	308.2	151.7	d3-Benztropine
Alprazolam	23.3	309.1	280.9	d5-Alprazolam
Alprazolam*	23.3	309.1	204.9	d5-Alprazolam
Amlodipine	23.8	409.1	237.8	d5-Norfluoxetine
Amlodipine*	23.8	409.1	293.8	d5-Norfluoxetine
Norfluoxetine	24.7	296.1	133.7	d5-Norfluoxetine
Sertraline	26.4	306.1	274.8	d7-Propranolol
Sertraline*	26.4	306.1	158.7	d7-Propranolol
Diazepam	29.1	285.1	192.8	d5-Diazepam
Diazepam*	29.1	285.1	153.8	d5-Diazepam
Valsartan	31.8	436.2	235.0	d5-Propoxyphene
Valsartan*	31.8	436.2	291.0	d5-Propoxyphene
Fluocinonide	34.8	495.2	337.0	d5-Alprazolam
Fluocinonide*	34.8	495.2	475.0	d5-Alprazolam
Trenbolone acetate	37.8	313.2	253.0	d5-Alprazolam
Trenbolone acetate*	37.8	313.2	271.0	d5-Alprazolam
Fluticasone propionate	37.9	501.2	293.0	d7-Metoprolol
Fluticasone propionate*	37.9	501.2	313.0	d7-Metoprolol
Simvastatin	40.1	419.3	285.0	d5-Propoxyphene
Simvastatin*	40.1	419.3	198.9	d5-Propoxyphene
Surrogate Standards				
^{13}C, $^{15}N_2$-Theophylline	2.7	184.0	124.7	$^{13}C_3$-Atrazine
^{13}C, $^{15}N_2$-Theophylline*	2.7	184.0	96.8	$^{13}C_3$-Atrazine
d_8-Benzoylecgonine	5.6	298.1	170.9	$^{13}C_3$-Atrazine
d_8-Benzoylecgonine*	5.6	298.1	109.8	$^{13}C_3$-Atrazine
d_7-Metoprolol	8.3	275.0	190.8	$^{13}C_3$-Atrazine
d_7-Metoprolol*	8.3	275.0	122.7	$^{13}C_3$-Atrazine
d_3-Cocaine	9.2	307.1	184.9	$^{13}C_3$-Atrazine
d_3-Cocaine*	9.2	30.7.1	84.8	$^{13}C_3$-Atrazine
d_7-Propranolol	14.4	267.0	116.0	$^{13}C_3$-Atrazine
d_7-Propranolol*	14.4	267.0	188.7	$^{13}C_3$-Atrazine
d_4-Hydrocortisone	17.6	367.0	120.8	$^{13}C_3$-Atrazine
d_4-Hydrocortisone*	17.6	367.0	331.0	$^{13}C_3$-Atrazine
d_4-Promethazine	18.6	289.0	201.8	$^{13}C_3$-Atrazine
d_4-Promethazine*	18.6	289.0	86.0	$^{13}C_3$-Atrazine
d_7-DEET	20.6	199.1	125.8	$^{13}C_3$-Atrazine
d_7-DEET*	20.6	199.1	97.8	$^{13}C_3$-Atrazine
d_6-Paroxetine	20.6	336.0	197.8	$^{13}C_3$-Atrazine
d_6-Paroxetine*	20.6	336.0	75.8	$^{13}C_3$-Atrazine
d_2-Methylprednisolone	21.4	377.0	359.0	$^{13}C_3$-Atrazine
d_2-Methylprednisolone*	21.4	377.0	341.0	$^{13}C_3$-Atrazine
d_5-Propoxyphene	21.8	245.2	266.1	$^{13}C_3$-Atrazine
d_5-Propoxyphene*	21.8	345.2	57.9	$^{13}C_3$-Atrazine
d_6-Amitriptyline	22.4	284.0	233.0	$^{13}C_3$-Atrazine
d_6-Amitriptyline*	22.4	284.0	90.8	$^{13}C_3$-Atrazine
d_3-Benztropine	22.9	311.0	166.7	$^{13}C_3$-Atrazine
d_3-Benztropine*	22.9	311.0	151.7	$^{13}C_3$-Atrazine
d_5-Alprazolam	23.1	314.1	285.9	$^{13}C_3$-Atrazine
d_5-Alprazolam*	23.1	314.1	209.9	$^{13}C_3$-Atrazine

d_5-Norfluoxetine	24.7	301.0	138.7	$^{13}C_3$-Atrazine
d_5-Diazepam	29.1	290.1	197.9	$^{13}C_3$-Atrazine
d_5-Diazepam*	29.1	290.1	153.8	$^{13}C_3$-Atrazine
Recovery Standards				
$^{13}C_3$-Atrazine	18.8	219.5	176.9	External Standard
$^{13}C_3$-Atrazine *	18.8	219.5	134.0	External Standard

* Indicates secondary transition for possible diagnostic use.

QUALITY ACCEPTANCE CRITERIA

QC Acceptance Limits

	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
List 1 Compounds (APOS)						
Acetaminophen	70	140	70	140	30	≤60
Ampicillin [2]						
Azithromycin	10	130	10	130	130	≤1.5
Caffeine	25	160	35	150	60	≤15
Carbadox	25	180	35	180	40	≤1.5
Carbamazepine	25	200	35	200	40	≤1.5
Cefotaxime	10	300	10	300	60	≤6
Ciprofloxacin	25	180	35	180	40	≤6
Clarithromycin	50	160	50	160	30	≤1.5
Clinafloxacin	25	300	35	300	70	≤6
Cloxacillin	35	160	40	150	50	≤3
Dehydronifedipine	35	160	40	160	30	≤0.6
Digoxigenin	50	150	60	140	30	≤6
Digoxin	10	300	10	300	30	≤15
Diltiazem	20	160	25	160	50	≤0.3
1,7-Dimethylxanthine	30	300	40	300	60	≤150
Diphenhydramine	30	200	35	180	50	≤0.6
Enrofloxacin	30	220	40	220	40	≤3
Erythromycin - H_2O	70	130	70	130	30	≤0.3
Flumequine	40	160	50	160	30	≤1.5
Fluoxetine	60	150	70	140	30	≤1.5
Lincomycin	10	300	10	300	70	≤3
Lomefloxacin	50	250	60	250	30	≤3
Miconazole	35	130	40	130	30	≤1.5
Norfloxacin	10	250	25	220	40	≤15
Norgestimate	35	130	40	130	30	≤3
Ofloxacin	60	250	70	250	30	≤1.5
Ormetoprim	70	150	70	150	30	≤0.6
Oxacillin	20	130	20	130	40	≤3
Oxolinic Acid	60	150	70	150	30	≤0.6
Penicillin G	10	130	10	130	40	≤3
Penicillin V	40	140	50	140	30	≤3
Roxithromycin	50	140	50	140	30	≤0.3

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Sarafloxacin	50	200	60	180	30	≤15
Sulfachloropyridazine	60	160	70	160	30	≤1.5
Sulfadiazine	70	130	70	130	30	≤1.5
Sulfadimethoxine	35	160	40	160	30	≤0.3
Sulfamerazine	60	140	60	140	30	≤0.6
Sulfamethazine	70	130	70	130	30	≤0.6
Sulfamethizole	30	140	35	140	30	≤0.6
Sulfamethoxazole	70	130	70	130	30	≤0.6
Sulfanilamide	2	160	3	150	150	≤15
Sulfathiazole	30	180	30	160	50	≤1.5
Thiabendazole	60	150	60	150	30	≤1.5
Trimethoprim	50	150	60	150	30	≤1.5
Tylosin	10	180	20	180	40	≤6
Virginiamycin	15	300	15	250	90	≤3
Surrogate Standard						
$^{13}C_2$, ^{15}N-Acetaminophen	30	160	40	150	30	
$^{13}C_3$-Caffeine	40	140	50	140	30	
d_{10}-Carbamazepine-10,11-epoxide						
$^{13}C_3$, ^{15}N-Ciprofloxacin	7	150	9	140	70	
$^{13}C_2$-Erythromycin - H_2O	35	130	35	130	30	
d_5-Fluoxetine	40	130	50	130	30	
$^{13}C_6$-Sulfamethazine	30	160	35	150	40	
$^{13}C_6$-Sulfamethoxazole	30	140	40	130	30	
d_6-Thiabendazole	25	180	30	160	50	
$^{13}C_3$-Trimethoprim	30	140	40	130	30	
Recovery Standard						
$^{13}C_3$-Atrazine						
List 2 Compounds (TCYS)						
Anhydrochlortetracycline (ACTC)	15	200	20	180	70	≤15
Anhydrotetracycline (ATC)	20	160	20	150	50	≤15
Chlortetracycline (CTC)	30	250	35	250	60	≤6
Demeclocycline	35	180	35	160	50	≤15
Doxycycline	35	180	40	180	40	≤6
Epianhydrochlortetracycline (EACTC)	6	130	7	130	70	≤60
Epianhydrotetracycline (EATC)	15	200	20	200	60	≤15
Epichlortetracycline (ECTC)	25	180	30	160	50	≤15
Epioxytetracycline (EOTC)	25	180	35	160	40	≤6
Epitetracycline (ETC)	35	200	40	180	40	≤6
Isochlortetracycline (ICTC)	25	180	35	160	40	≤6
Minocycline	1	250	2	200	110	≤60
Oxytetracycline (OTC)	20	200	30	200	40	≤6
Tetracycline (TC)	20	200	30	180	40	≤6
Surrogate Standard						
d_6-Thiabendazole	25	140	25	130	50	

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Recovery Standard						
$^{13}C_3$-Atrazine						
	OPR Recovery and surrogate recovery in sample		**IPR**			**Blank Level (ng)**
			Average Recovery (%)		**RSD (%)**	
	Low	**High**	**Low**	**High**		
List 3 Compounds (ANEG)						
Bisphenol A	70	130	70	130	30	≤2500
Furosemide	65	130	70	130	30	≤40
Gemfibrozil	60	140	70	130	30	≤1.5
Glipizide	55	170	60	160	30	≤6
Glyburide	50	180	55	170	30	≤3
Hydroxychlorothiazide	70	200	70	180	30	≤20
2-hydroxy-ibuprofen	70	130	70	130	30	≤80
Ibuprofen	70	130	70	130	30	≤15
Naproxen	50	150	60	150	30	≤3
Triclocarban	60	140	70	130	30	≤3
Triclosan	70	130	70	130	30	≤60
Warfarin	70	140	70	140	30	≤1.5
Surrogate Standards						
d_6-Bisphenol A	50	170	60	160	30	
d_6-Gemfibrozil	50	150	55	140	30	
d_3-Glyburide	20	160	25	150	40	
d_{11}-Glipizide	30	180	35	170	50	
$^{13}C_3$-Ibuprofen	50	140	55	140	30	
^{13}C-d_3-Naproxen	30	150	35	140	30	
$^{13}C_6$-Triclocarban	20	160	25	150	50	
$^{13}C_{12}$-Triclosan	20	160	30	150	40	
d_5-Warfarin	35	250	50	250	30	
Recovery Standard						
$^{13}C_6$-2,4,5-Trichloro-phenoxyacetic acid						
List 4 Compounds (BPOS)						
Albuterol	50	160	50	160	30	≤0.3
Amphetamine	50	160	60	150	30	≤1.5
Atenolol	70	130	70	130	30	≤0.6
Atorvastatin	20	130	25	130	40	≤1.5
Cimetidine	15	130	20	130	50	≤0.6
Clonidine	70	130	70	130	30	≤1.5
Codeine	70	130	70	130	30	≤3
Cotinine	70	130	70	130	30	≤1.5
Enalapril	70	130	70	130	30	≤0.3
Hydrocodone	70	130	70	130	30	≤1.5
Metformin	70	160	70	160	30	≤30
Oxycodone	65	130	70	130	30	≤0.6
Ranitidine	25	140	30	140	50	≤0.6

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Triamterene	70	140	70	140	30	≤0.3
Surrogate Standards						
d_3-Albuterol	20	140	30	130	30	
d_5-Amphetamine	20	130	25	130	40	
d_7-Atenolol	70	130	70	130	30	
d_3-Cimetidine	15	130	15	130	50	
d_4-Clonidine	70	130	70	130	30	
d_6-Codeine	70	130	70	130	30	
d_3-Cotinine	70	140	70	135	30	
d_5-Enalapril	65	130	70	130	30	
d_3-Hydrocodone	70	130	70	130	30	
d_6-Metformin	3	130	4	130	130	
d_6-Oxycodone	50	150	60	140	30	
Recovery Standards						
d_3-Amitriptyline						
List 5 Compounds (APOS)						
Alprazolam	70	130	70	130	30	≤0.3
Amitriptyline	70	130	70	130	30	≤0.3
Amlodipine	45	130	50	130	30	≤1.5
Benzoylecgonine	70	130	70	130	30	≤0.3
Benztropine	70	130	70	130	30	≤0.3
Betamethasone	20	240	30	220	40	≤1.5
Cocaine	70	130	70	130	30	≤0.15
DEET	70	130	70	130	30	≤0.15
Desmethyldiltiazem	3	350	5	320	80	≤0.15
Diazepam	70	130	70	130	30	≤0.3
Fluocinonide	7	230	9	220	70	≤6
Fluticasone propionate	20	160	25	150	50	≤2
Hydrocortisone	15	220	20	200	80	≤60
10-hydroxy-amitriptyline	70	130	70	130	30	≤0.15
Meprobamate	65	150	70	140	30	≤4
Methylprednisolone	35	240	40	220	50	≤4
Metoprolol	70	130	70	130	30	≤1.5
Norfluoxetine	70	130	70	130	30	≤1.5
Norverapamil	55	130	60	130	30	≤0.15
Paroxetine	70	130	70	130	30	≤4
Prednisolone	35	240	40	220	50	≤6
Prednisone	50	180	60	170	30	≤20
Promethazine	70	130	70	130	30	≤0.4
Propoxyphene	70	130	70	130	30	≤0.3
Propranolol	70	150	70	150	30	≤2
Sertraline	50	130	55	130	30	≤0.4
Simvastatin	1	150	1	140	100	≤20
Theophylline	10	1000	70	900	50	≤60
Trenbolone	70	140	70	135	30	≤4

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Trenbolone acetate	55	130	60	130	30	≤0.3
Valsartan	70	130	70	130	30	≤4
Verapamil	70	145	70	140	30	≤0.15
Surrogate Standards						
d_5-Alprazolam	45	130	45	130	30	
d_6-Amitriptyline	10	130	20	130	40	
d_8-Benzoylecgonine	10	170	20	160	40	
d_3-Benztropine	20	140	25	130	40	
d_3-Cocaine	25	140	30	130	50	
d_7-DEET	15	160	20	150	40	
d_5-Diazepam	15	160	25	150	40	
d_4-Hydrocortisone	40	240	45	230	50	
d_2-Methylprednisolone	15	160	20	150	60	
d_7-Metoprolol	25	140	30	140	30	
d_5-Norfluoxetine	20	130	20	130	50	
d_6-Paroxetine	7	150	9	140	60	
d_4-Promethazine	3	140	5	130	80	
d_5-Propoxyphene	30	130	40	130	30	
d_7-Propranolol	25	140	30	130	30	
$^{13}C_1$, $^{15}N_2$-Theophylline	20	200	25	180	60	
Recovery Standards						
$^{13}C_3$-Atrazine						

[1] OPR and IPR limits derived from actual method performance data according to EPA 821B98003, appendix D.

[2] Because of very low stability the accuracy of Ampicillin is not known. The analysis result is classified as "Information Value" only.

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Instrumental Acceptance Specifications

QC Parameter	Specification
Instrument Sensitivity	Daily, S:N ≥ 3:1 for all analytes for lowest calibration point.
Initial Calibration (native compounds)	Initial, (1/X) weighted linear regression (followed by regular Cal/Ver procedures and repeated as necessary to maintain Cal/Ver results within established acceptance ranges. Calculated concentrations 70-130%, one point per compound may be 60-140% Internal guideline - correlation coefficient >0.985. Calibration curves with lower correlation coefficient values meeting all above criteria may be accepted based on batch specific QC results and professional judgement
OPENING Calibration Verification	Every 20 samples, determined concentrations within 30% of actual concentrations. Professional judgment allowed for wider acceptance limits.
CLOSING Calibration Verification	Within OPENING Calibration Verification specifications. Allowable exception: results for the greater of 1 compound or 10% of the compounds on a Compound List (1,2,3,4,5) may fall outside the Opening Calibration Verification specification provided the RPD between the CLOSING result and the OPENING result is <30%.
Instrumental Carryover And Instrument Background	Every Initial Calibration, Cal/Ver, or SPM: < 0.3 % carryover and area response of analytes in instrument blank < 800 judged following two previous methanol blank injections.

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QUANTIFICATION AND DATA REPORTING PROCEDURES

Positive identification of target natives, surrogate standard and recovery standards require:

- ≥ 3:1 S:N for parent ion to daughter ion transition, on condition that the result is above the lowest calibration standard level.
- Compound retention time falls within 0.4 minutes of the predicted retention times from the daily calibration standard. Natives with labelled surrogate standards must elute within 0.1 minutes of the associated labelled surrogates.

Concentrations of the targets and surrogates are calculated by isotope dilution or internal standard quantification with linear regression calibration, using a 1/X weighting type, excluding origin. Sample specific detection limits (SDLs) are calculated by QuanLynx software using 3 times the signal of the noise in the target channel converted to an equivalent sample concentration.

General equation : $Y = \text{slope} \times X + \text{intercept}$

Where: $Y = \text{Response ratio} = \left(\frac{\text{area of Target}}{\text{area of Quant. Std}} \times \text{weight of Quant. std (ng)} \right)$

X = weight of target (ng)

Quant. Std = labelled surrogate or recovery standard

The slope and intercept are used to convert raw peak areas in sample chromatograms to final concentrations as follows:

$$\text{Sample Conc.} = \left(\frac{\text{area of Target}}{\text{area of Quant. Std}} \times \text{weight of Quant. Std (ng)} - \text{intercept} \right) \text{x} \left(\frac{1}{\text{slope}} \right) \text{x} \left(\frac{1}{\text{samplesize(L)}} \right)$$

The recovery of surrogate standards, calculated from the determined concentration of the surrogate in the extract relative to the amount spiked, are monitored as an indication of overall data quality.

The lower reporting limit for each target compound is defined as the concentration equivalent to the lowest calibration standard analyzed or the SDL, whichever is greater.

METHOD SUMMARY

ANALYSIS OF STEROLS AND HORMONES IN WATER AND SOLIDS SAMPLES BY METHOD MLA-068 Rev 03

List of Analytes (Sterols and Hormones)

Compound	*Type*	*Function*	*Expected Levels*	
			Environmental	**POTW**
Androstenedione	Androgen	Reproductive hormone, also from plant sources	Low	Low
Androsterone	Androgen	Reproductive hormone, testosterone metabolite	Low	Low
Testosterone	Androgen	Reproductive hormone	Low	Low
17α-estradiol	Estrogen	Reproductive hormone, some plant sources, inactive isomer of 17b-estradiol	Low	Low
17β-estradiol	Estrogen	Reproductive hormone	Low	Low
17α-ethinylestradiol	Estrogen	Synthetic ovulation inhibitor (human use)	Low	Low
Estrone	Estrogen	Reproductive hormone; (17β-estradiol metabolite)	Low	Low
Estriol	Estrogen	Reproductive hormone (17β-estradiol metabolite)	Low	Low
β-Estradiol-3-Benzoate	Estrogen	Bovine estrus stimulator	Low	Low
17α-Dihydroequilin	Estrogen (Equine)	Constituent of mare urine	Low	Low
Equilenin	Estrogen (Equine)	Equine hormone, constituent of HRT drug (Primarin)	Low	Low
Equilin	Estrogen (Equine)	Equine hormone, constituent of HRT drug (Primarin)	Low	Low
Mestranol	Progestagen	Synthetic ovulation inhibitor (human use)	Low	Low
Norethindrone	Progestagen	Synthetic ovulation inhibitor (human use)	Low	Low
Progesterone	Progestagen	reproductive hormone	Low	Low
Desogestrel	Progestagen	Ovulation inhibitor (human use)	Low	Low
Norgestrel	Progestagen	Synthetic ovulation inhibitor (human use)	Low	Low
Cholestanol	Stanol	Stanol, fecal matter biomarker	Low/Medium	High
Coprostanol	Stanol	Stanol, fecal matter biomarker	Low/Medium	Very High
Epicoprostanol	Stanol	Stanol, fecal matter biomarker	Low/Medium	High
b-Stigmastanol	Stanol	Stanol, fecal matter biomarker	Low/medium	High
Campesterol	Sterol	Plant sterol	Medium	High
Cholesterol	Sterol	Animal Sterol	High	Very High
Desmosterol	Sterol	Animal Sterol, cholesterol precursor	Low/Medium	High
Ergosterol	Sterol	Fungal sterol	Information only	Information only
β-Sitosterol	Sterol	Plant Sterol	High	Very High
Stigmasterol	Sterol	Plant Sterol	Medium/High	Very High

Ergosterol is not stable through the work up procedure and does not have a defined accuracy and precision through the procedure. It is tagged as an “information only” analyte.

This method describes the determination of concentrations of a selected suite of sterols and hormones in solid (sediment/soil/biosolids) and aqueous (potable water, groundwater, surface water, final effluent, influent) samples. The method is primarily intended for the low level detection of hormones and sterols in aqueous samples (low organic particulate content), and

low organic solids not associated with wastewater treatment. The method provides additional options for the analysis of samples from wastewater treatment.

High Resolution mass Spectrometry (HRMS) is used as the default option. Low Resolution Mass Spectrometry (LRMS) is also available as an analytical option. For hormones, HRMS offers approximately five times lower reporting limits. For sterols, reporting limits are identical in low resolution and high resolution, with LRMS extending the upper calibrated range by a factor of five.

Sample size selection and treatment varies depending on source and expected organic matter content. Aqueous samples are filtered and typically, only the filtrate is extracted and analyzed. Some aqueous samples of environmental origin with limited visible particulate (e.g. surface water, ground water), and final effluent samples from POTW sources can be filtered and processed using a Whole Water scheme. Here, the filtrate and solid phases are extracted separately and combined after extraction to result in a single analysis. Water samples from POTW streams other than final effluent are processed using limited sample volumes and are analyzed from a subsample of the extract.

For mixed phase aqueous/solids samples with distinct aqueous and solids phases such as wastewater influent or process streams, the sample is typically analyzed as an aqueous filtered phase. Optionally, they can be analyzed as two separate samples, one filtrate and one solid.

Solids of environmental origin which are not potentially affected by POTW biosolids/outfall application are processed from the entire extract. Solids from POTW sources are processed using small sample sizes and using smaller fractions of the extract. Sewage markers such as cholesterol, coprostanol, and stigmastanol may be reported as estimated values in the sample if their reported concentrations exceed the calibration range of the instrument.

Cholesterol and sitosterol are present at high levels in POTW samples and may be present at high levels in environmental samples. The presence of these analytes at high levels can potentially interfere with the low detection limit analysis of hormones and some sterols. In general, the accurate quantification of trace levels of hormones and some sterols is not compatible with the accurate quantification of cholesterol and sitosterol present at orders of magnitude higher than levels in the samples. Analyses designed for the accurate measurement of cholesterol, sitosterol, and most of the sterols in POTW influenced samples are typically carried out under options that involve instrumental analysis from a small fraction of the extract.

Interferences

Interferences co-extracted from samples may vary considerably from sample to sample. Interfering compounds may be present at concentrations several orders of magnitude higher than the analytes. The most frequently encountered interferences are humic acids in water and soil and fatty acids, particularly in biosolids. High levels of sterols in the extract can interfere with the analysis of hormones. Cholesterol and sitosterol are present at high levels in samples and in ambient background. The presence of these analytes at high levels can potentially interfere with the low detection limit analysis of hormones and some sterols. In general, the accurate quantification of trace levels of hormones and some sterols is not compatible with the accurate quantification of cholesterol and sitosterol present at orders of magnitude higher than levels in the samples. Analyses designed for the accurate measurement of cholesterol, sitosterol in environmental samples, and most of the sterols in wastewater influenced samples, are

typically carried out under options that involve instrumental analysis from a small fraction of the original extract. See the table below for appropriate choice of method options.

Sample handling and analysis recommendations:

Sample	***Analysis & Options***	***Analyte Notes***
Potable water	1) Filtrate only 2) Whole Water	Cholesterol and sitosterol may require dilution analysis
Groundwater	1) Filtrate Only 2) Whole Water if limited particulate and not opaque prior to filtration	Cholesterol and sitosterol may require dilution analysis.
Surface water	1) Filtrate Only 2) Whole Water if limited particulate and not opaque prior to filtration	Cholesterol and sitosterol may require dilution analysis.
Leachates (Non TCLP)	Depends on source and organic content	Depends on source and organic content.
Mixed phase environmental samples (non POTW)	Filtrate only. Solids require separate analysis	Cholesterol and plant sterols may require dilution analysis
Soil/Sediment samples (non POTW impacted)	Standard sample size	Cholesterol and plant sterols may require dilution analysis
POTW final effluent	1) Filtrate only 2) Whole water if limited particulate and not opaque prior to filtration	Many sterols may require dilution analysis.
POTW influent	Filtrate only – Solids require separate analysis	1:5 dilution for hormones, 1:100 for sterols. Several sterols will require dilutions. Cholesterol, coprostanol and some other sterols may be reported as minimum values if above calibrated range
POTW biosolids	Low sample size	1:5 dilution for hormones, 1:100 for sterols. Several sterols will require dilutions. Cholesterol, coprostanol and some other sterols may be reported as minimum values if above calibrated range

Extraction and Cleanup Procedures

Dependent on origin and suspended matter content samples may be pre-treated and separated into aqueous and solid phases as necessary to ensure that the sub-samples are representative and that the analytes are efficiently extracted. Before extraction the samples are spiked with a suite of isotopically labelled surrogate standards. A solid sample is extracted by sonication with aqueous buffered methanol, concentrated by rotary evaporation, and diluted with ultra pure water to 200 mL. Aqueous samples and the diluted extracts of solid samples are cleaned up by solid phase extraction (SPE) on HLB cartridges. Extracts are then dried using sodium sulphate, split as necessary and derivatized with BSTFA prior to analysis by capillary gas chromatography and low resolution mass spectrometry (LRMS) or high resolution mass spectrometry (HRMS). All analytes except androsterone, androstenedione, desogestrel and testosterone are quantified against surrogate standards added prior to extraction and incorporate recovery correction. Androsterone, androstenedione, desogestrel and testosterone are quantified against the labelled injection internal standard (recovery standard), PCB-101-13C, added prior to instrumental analysis as data indicates inadequate tracking of non-estrogen analytes by the estrogen surrogate standard 17β-estradiol-d4 which is added prior to extraction. Ergosterol, due to its instability, is reported on a semi-quantitative basis for information purposes only.

Instrumental Analysis

Instrumental analysis of the final derivatized extracts is performed by capillary gas chromatography and either low-resolution (LRMS) or high-resolution (HRMS) mass spectrometric detection. The extract is injected by split/splitless injection on a high-resolution gas chromatograph (HRGC) equipped with a Restek RTx-5 capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness). The LRMS is operated at unit mass resolution in the EI mode using multiple ion detection (MID). The HRMS is operated at a static (5000) mass resolution in the electron ionization (EI) mode using Voltage SIR detection. Two characteristic ions for each target analyte and surrogate standard are acquired.

Initial calibration is performed using a multi-point series of derivatized calibration solutions that encompass the working concentration range. Calibration is verified at least once every twelve hours by analysis of a mid-level calibration solution.

Quantification Procedures

$$\text{Concentration of Target (ng/g or ng/L)} = \left(\frac{\text{area of Target}}{\text{area of Surr Std}}\right) \times \left(\frac{\text{weight of Surr Std (ng)}}{\text{RRF}}\right) \times \left(\frac{1}{\text{weight of sample (g or L)}}\right)$$

$$\textit{where} \quad \text{RRF} = \left(\frac{\text{area of Target}}{\text{area of Surr Std}}\right) \times \left(\frac{\text{concentration of Surr Std}}{\text{concentration of Target}}\right)$$

and the Surr Std is either the surrogate or the internal standard

Table 1. Analyte Ions Monitored, Surrogates Used, and RRF Determination for Sterols and Hormones by GC/LRMS

Analyte Name	Quantification Standard	Typical Retention Time (min)	RT Window (sec)	RT Standard	mass 1	mass 2	m1/m ratio
Androsterone	PCB-101-$^{13}C_{12}$	21:17	6	17β-Estradiol -d4	347	348	3.56
Desogestrel	PCB-101-$^{13}C_{12}$	21:32	6	17β-Estradiol -d4	353	354	2.97
17α-Estradiol	17β-Estradiol -d4	22:25	6	17β-Estradiol -d4	416	417	2.80
Estrone	17β-Estradiol -d4	22:25	6	17β-Estradiol -d4	342	343	3.50
Equilin	17β-Estradiol -d4	22:31	6	17β-Estradiol -d4	340	341	3.36
Androstenedione	PCB-101-13C12	22:37	6	17β-Estradiol -d4	286	287	4.75
17β-Estradiol	17β-Estradiol -d4	22:52	6	17β-Estradiol -d4	416	417	2.71
Testosterone	PCB-101-$^{13}C_{12}$	22:58	8	17β-Estradiol -d4	360	361	3.37
Equilenin	17β-Estradiol -d4	23:22	8	17β-Estradiol -d4	338	339	3.48
Mestranol	Mestranol -d4	23:26	6	Mestranol -d4	367	368	3.35
Norethindrone	Norethindrone -d6	23:33	6	Norethindrone -d6	355	356	3.49
17α-Dihydroequilin	17β-Estradiol -d4	22:46	6	17β-Estradiol -d4	309	310	3.52
17α-Ethinyl-Estradiol	17α-Ethinyl-Estradiol -d4	24:09	6	17α-Ethinyl-Estradiol -d4	425	426	2.70
Progesterone	Progesterone -d9	24:40	6	Progesterone -d9	314	315	4.45
Norgestrel	Norgestrel -d6	24:53	6	Norgestrel -d6	355	356	2.91
Estriol	Norgestrel -d6	25:27	6	Norgestrel -d6	504	505	2.28
Coprostanol	Cholesterol -d7	27:48	6	Cholesterol -d7	370	371	3.43
Epicoprostanol	Cholesterol -d7	27:59	6	Cholesterol -d7	370	371	3.49
Cholesterol	Cholesterol -d7	29:36	10	Cholesterol -d7	368	369	3.30
Cholestanol	Cholesterol -d7	29:46	6	Cholesterol -d7	445	446	2.65
Desmosterol	Cholesterol -d7	30:14	8	Cholesterol -d7	343	344	3.03
Ergosterol	Cholesterol -d7	30:54	6	Cholesterol -d7	363	364	3.51
Campesterol	Cholesterol -d7	31:08	6	Cholesterol -d7	382	383	3.19
Stigmasterol	Cholesterol -d7	31:31	6	Cholesterol -d7	484	485	2.43
β-Sitosterol	Cholesterol -d7	32:10	6	Cholesterol -d7	486	487	2.38
β-Stigmastanol	Cholesterol -d7	32:17	6	Cholesterol -d7	488	489	2.29
β-Estradiol-3-Benzoate	Norgestrel -d6	34:56	12	Norgestrel -d6	105	106	13.07
17β-Estradiol -d4	PCB-101-$^{13}C_{12}$	22:50	20	PCB-101-$^{13}C_{12}$	420	421	2.78
Mestranol -d4	PCB-101-$^{13}C_{12}$	23:25	20	PCB-101-$^{13}C_{12}$	371	372	3.38
Norethindrone -d6	PCB-101-$^{13}C_{12}$	23:29	20	PCB-101-$^{13}C_{12}$	361	362	3.47
17α-Ethinyl-Estradiol-d4	PCB-101-$^{13}C_{12}$	24:07	20	PCB-101-$^{13}C_{12}$	429	430	2.70
Progesterone -d9	PCB-101-$^{13}C_{12}$	24:32	20	PCB-101-$^{13}C_{12}$	323	324	4.39
Norgestrel -d6	PCB-101-$^{13}C_{12}$	24:49	20	PCB-101-$^{13}C_{12}$	361	362	3.03
Cholesterol -d7	PCB-101-$^{13}C_{12}$	29:30	20	PCB-101-$^{13}C_{12}$	375	376	3.34
PCB-101-$^{13}C_{12}$		17:42	100		338	340	1.55

Table 2. Analyte Ions Monitored, Surrogates Used, and RRF Determination for Sterols and Hormones by GC/HRMS

Analyte Name	Quantification Standard	Typical Retention Time	RT Win. (sec)	RT Standard	Funct.	mass1	mass2	m1/m2 ratio (theoretical)
Androsterone	PCB-101-$^{13}C_{12}$	21:08	6	17β-Estradiol -d4	2	347.2406	348.2440	3.44
Desogestrel	PCB-101-$^{13}C_{12}$	21:26	6	17β-Estradiol -d4	2	353.2301	354.2335	3.21
17α-Estradiol	17β-Estradiol -d4	22:29	6	17β-Estradiol -d4	2	416.2567	417.2601	2.67
Estrone	17β-Estradiol -d4	22:34	6	17β-Estradiol -d4	2	342.2015	343.2049	3.45
Equilin	17β-Estradiol -d4	22:40	6	17β-Estradiol -d4	2	340.1859	341.1893	3.46
Androstenedione	PCB-101-$^{13}C_{12}$	22:36	6	17β-Estradiol -d4	2	286.1933	287.1967	4.64
17β-Estradiol	17β-Estradiol -d4	23:02	6	17β-Estradiol -d4	2	416.2567	417.2601	2.67
Testosterone	PCB-101-$^{13}C_{12}$	23:16	8	17β-Estradiol -d4	2	360.2485	361.2519	3.32
Equilenin	17β-Estradiol -d4	23:45	8	17β-Estradiol -d4	3	338.1702	339.1736	3.46
Mestranol	Mestranol -d4	23:47	6	Mestranol -d4	3	367.2093	368.2127	3.21
Norethindrone	Norethindrone –d6	23:58	6	Norethindrone -d6	3	355.2093	356.2127	3.32
17α-Dihydroequilin	17β-Estradiol -d4	23:08	6	17β-Estradiol -d4	4	309.1706	310.1740	3.52
17α-Ethinyl-Estradiol	17α-Ethinyl-Estradiol -d4	24:38	6	17α-Ethinyl-Estradiol -d4	4	425.2332	426.2366	2.59
Progesterone	Progesterone -d9	24:40	6	Progesterone -d9	4	314.2246	315.2280	4.19
Norgestrel	Norgestrel -d6	25:40	6	Norgestrel -d6	4	355.2093	356.2127	3.32 (2.66)
Estriol	Norgestrel -d6	26:15	6	Norgestrel -d6	5	504.2911	505.2945	2.17
Coprostanol	Cholesterol -d7	28:57	6	Cholesterol -d7	5	370.3600	371.3634	3.26
Epicoprostanol	Cholesterol -d7	29:05	6	Cholesterol -d7	5	370.3600	371.3634	3.26
Cholesterol	Cholesterol -d7	30:21	10	Cholesterol -d7	5	368.3443	369.3477	3.26
Cholestanol	Cholesterol -d7	30:29	6	Cholesterol -d7	5	445.3866	446.3900	2.62
Desmosterol	Cholesterol -d7	30:51	8	Cholesterol -d7	5	343.2457	344.2491	3.32
Ergosterol	Cholesterol -d7	31:26	6	Cholesterol -d7	5	363.3052	364.3086	3.27
Campesterol	Cholesterol -d7	31:41	6	Cholesterol -d7	5	382.3600	383.3634	3.14
Stigmasterol	Cholesterol -d7	32:05	6	Cholesterol -d7	5	484.4100	485.4134	2.41
β-Sitosterol	Cholesterol -d7	32:54	6	Cholesterol -d7	5	486.4257	487.4291	2.41
β-Stigmastanol	Cholesterol -d7	33:03	6	Cholesterol -d7	5	488.4413	489.4447	2.4
β-Estradiol-3-Benzoate	Norgestrel -d6	36:16	12	Norgestrel -d6	6	105.0340	106.0374	12.7
17β–Estradiol-d4	PCB-101-$^{13}C_{12}$	23:00	20	PCB-101-$^{13}C_{12}$	2	420.2818	421.2852	2.67
Mestranol-d4	PCB-101-$^{13}C_{12}$	23:44	20	PCB-101-$^{13}C_{12}$	3	371.2344	372.2378	3.21
Norethindrone-d6	PCB-101-$^{13}C_{12}$	23:54	20	PCB-101-$^{13}C_{12}$	3	361.2470	362.2504	3.33
17α-Ethinyl-Estradiol-d4	PCB-101-$^{13}C_{12}$	24:36	20	PCB-101-$^{13}C_{12}$	4	429.2583	430.2617	2.6
Progesterone-d9	PCB-101-$^{13}C_{12}$	24:56	20	PCB-101-$^{13}C_{12}$	4	323.2811	324.2845	4.21
Norgestrel-d6	PCB-101-$^{13}C_{12}$	25:35	20	PCB-101-$^{13}C_{12}$	4	361.2470	362.2504	3.33
Cholesterol-d7	PCB-101-$^{13}C_{12}$	30:15	20	PCB-101-$^{13}C_{12}$	5	375.3882	376.3916	3.27
PCB-101-$^{13}C_{12}$		17:42	100		2	337.9210	339.9180	1.55

Note: In most cases, empirical m1/m2 ratios derived from observational calibration data are used to assess m1/m2 ratios for samples. Norgestrel has an interference to one of its ions that causes the observed ratio (2.66) to be substantially and consistently different from the theoretical ratio.

Typical Detection Limits

Results are typically reported down to the sample specific detection limit, which is the sample concentration equivalent to an analyte peak 2.5 times the height of the noise in the m/z channel of interest. Upon client request alternate reporting limits may be used, e.g. based on the sample equivalent of the lowest calibration solution concentration.

Cholesterol and sitosterol reporting limits are dictated by background levels.

QA/QC

Samples are analyzed in batches containing at least one procedural blank and at least one spiked matrix (OPR) sample per 20 client samples. Sample duplicates may be analyzed on an individual contract basis. The batch is carried through the complete analytical process as a unit. For sample data to be reportable, the batch QC data must meet the established acceptance criteria presented on the analysis reports.

Table 3. General QC Acceptance Criteria for the Analysis of Sterols and Hormones

Parameter	Acceptance Specification
Blanks	Less than Table 6 limits for both high resolution and low resolution analysis; Sample batches that have been split 1:100 have an acceptance level 100 times higher than Table 6 for sterols. Higher blank levels are acceptable based on professional judgement where sample concentrations significantly exceed the blank level (x5 recommended).
Analysis Duplicate	Guideline: ≤ 40% RPD, depending on sample characteristics and professional judgement
GC Resolution	1. The retention time for β-Estradiol-3-Benzoate must be greater than 34 minutes. 2. Coprostanol and Epicoprostanol must be uniquely resolved to a valley height less than 30 % of the shorter of the two peaks.
Instrument Sensitivity	HRMS ≥ S:N 3:1 for injection for 1 µL of a 'Level A' calibration solution LRMS ≥ S:N 3:1 for injection for 2 µL of a 'Level B' calibration solution
Initial Calibration (I-CAL)	≤ 20 % RSD for native compounds quantified against exact labelled standard [1] ≤ 35 % RSD for native compounds NOT quantified against exact labelled standard ≤ 35 % RSD for labelled standards
CAL/VER	70 – 130 % of actual, except for Equilin which is 65 - 135 % and Ergosterol which is 50 - 150 %
Retention Time Window (RRT)	CAL VER: Absolute: Labelled within ±15 sec. absolute of the mid-levelI-CAL Within Table 10 RT windows compared to mid-level I-CAL RRTs Samples : Within Table 10 RT windows compared to CAL VER RRTs
Ion Abundance Ratios (quant ion vs. primary conf. ion)	I-CAL: Within 30 % of mid-level I-CAL CAL VER: Within 30 % of the mid-level I-CAL ratio Samples: Within 30% of the CAL VER ratio

[1] Wider limits may be acceptable based on professional judgement.

Table 4. OPR Recovery Limits for Hormones and Sterols

Hormones	OPR % Recovery
Androsterone	50 - 150
Desogestrel	29 - 150
17α-Estradiol	50 - 150
Estrone	50 - 150
Equilin	50 - 150
Androstenedione	50 - 193
17α-Dihydroequilin bis	50 - 150
17β-Estradiol	70 - 130
Testosterone	50 - 150
Equilenin	50 - 150
Mestranol	50 - 150
Norethindrone	70 - 130
17α-Ethinyl Estradiol	70 - 130
Progesterone	70 - 130
Norgestrel	70 - 130
Estriol-tris	6 - 169
β-Estradiol-3-benzoate	5 - 189
17β-Estradiol-d4	30 - 150
Mestranol-d4	30 - 150
Norethindrone-d6	30 - 150
17α-Ethinyl-estradiol-d4	30 - 150
Progesterone-d9	30 - 200
Norgestrel-d6	30 - 150
Sterols	**OPR % Recovery**
Coprostanol	50 - 150
Epicoprostanol	50 - 150
Cholesterol	50 - 237
Cholestanol	50 - 150
Desmosterol	47 - 150
Ergosterol	N/A Information Only
Campesterol	50 - 156
Stigmasterol	46 - 152
β-sitosterol	5 - 200
β-stigmastanol	50 - 150
Cholesterol-d7	13 - 150

Table 5. Sample Surrogate Recovery and Blank Acceptance Criteria

	Labeled Surrogate Recovery in Samples (%)	Blank Levels (ng) *
Native Analytes		
Androsterone		≤ 1
Desogestrel		≤ 1
17α-Estradiol		≤ 1
Estrone		≤ 1
Equilin		≤ 1
Androstenedione		≤ 5
17β-Estradiol		≤ 1
Testosterone		≤ 1
Equilenin		≤ 1
Mestranol		≤ 1
Norethindrone		≤ 1
17α-Dihydroequilin		≤ 1
17α-Ethinyl Estradiol		≤ 1
Progesterone		≤ 5
Norgestrel		≤ 2
Estriol		≤ 1
Coprostanol		≤ 1
Epicoprostanol		≤ 1
Cholesterol		≤ 500
Cholestanol		≤ 10
Desmosterol		≤ 20
Ergosterol		≤ 5
Campesterol		≤ 5
Stigmasterol		≤ 10
β-Sitosterol		≤ 200
β-Stigmastanol		≤ 5
β-Estradiol-3-Benzoate		≤ 1
Surrogate Standard		
17β-Estradiol -d4	30 - 150	
Mestranol -d4	30 - 150	
Norethindrone -d6	30 - 150	
17α-Ethinyl Estradiol -d4	30 - 150	
Progesterone -d9	30 - 200	
Norgestrel -d6	30 - 150	
Cholesterol -d7	13 - 150	

* Matrix specific detection limits apply to blank analyses and may exceed the values shown

APPENDIX 2

CASE NARRATIVES

Prepared by AXYS Analytical Services, Ltd.

PERFLUORINATED ORGANIC ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-060

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG30835

Analysis WG30329

10 November 2009

PERFLUORINATED ORGANIC ANALYSIS

AQUEOUS SAMPLES

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Prepared for:
ORSANCO

Prepared by:
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CANADA

Contact: Candice Navaroli
Project Manager

10 November 2009

OHIO RIVER VALLEY WATER SANITATION COMMISSION
WATER SAMPLES **PERFLUORINATED ORGANIC ANALYSIS**
AXYS METHOD: MLA-060

Project: EMERGING CONTAMINANTS IN MAINSTEM OHIO

4562: L13603-1 to -8

10 November 2009

NARRATIVE

This narrative describes the analysis of eight water samples for the determination of perfluorinated organic compounds using high performance liquid chromatography/tandem mass spectrometry (HPLC/MS-MS).

SAMPLE RECEIPT, STORAGE AND PREPARATION

Water samples were received at AXYS on the 23rd of September 2009. Details of sample conditions upon receipt are provided on the Sample Receiving Record forms included in the sample documentation section of this data package. Some samples were received at Axys above (6° Celsius) the control temperature described in the method and as the effect of this discrepancy is unknown, and as a result of discussions with the client, analysis was allowed to proceed. The samples were stored at 4°C prior to sample preparation and analysis.

SAMPLE EXTRACTION AND ANALYSIS

The samples were analyzed in one analysis batch named WG30329. Composition of the batch is shown on the Cover pages and the Batch List forms included in this data package.

Sample extraction, instrumental analysis and analyte quantification procedures were in accordance with AXYS Method MLA-060: ***Analytical Procedure for the Analysis of Perfluorinated Organic Compounds in Aqueous Samples by LC-MS/MS.***

The samples were filtered and accurately weighed. After spiked with ^{13}C-labelled quantification standards, the samples were extracted and cleaned up using SPE cartridges. The resulted extracts were instrumentally analyzed using liquid chromatography/mass spectrometry (LC-MS/MS). Analyte concentrations were determined by isotope dilution/internal standard quantification, comparing the area response of the quantification ion to that of the ^{13}C-labelled standards and correcting for response factors. Quadratic quantification equations with 1/X weighting fit were determined from a multi-point calibration series prepared alongside the samples.

The reporting limit (RL) was defined as the concentration equivalent to the lowest calibration standard analyzed in the initial calibration (CS0) or the sample specific detection limits, whichever was greater.

REPORTING CONVENTIONS

The AXYS contract number assigned for internal tracking was 4562. Samples were assigned a unique laboratory identifier of the form L13603-XX, where X = numeral. All data reports reference these unique AXYS IDs plus the client's sample identifier. To assist with locating data, a table correlating AXYS ID with the client sample number is included in this data package. The report forms were generated using Laboratory Information Management System (LIMS).

Any extra work required and performed after the initial instrumental analysis of the sample's extract is given an extra "test suffix" code. The single letter code per extra work performed is added to the AXYS sample ID as a suffix, and is combined with any other applicable test suffix codes. The extra work codes used to report data in this package include:

i	=	instrumental re-analysis performed on the sample extract

The following laboratory qualifier flags were used in this data package:

U	=	identifies a compound that was not detected
N	=	authentic analyte recovery in the OPR is not within method control limits

Results are reported in concentration units of nanograms per Litre (ng/L). Concentration and detection limits are provided to three significant figures. Analysis results for each sample are provided on Analysis Report form 1A and form 2.

QA/QC NOTE

Samples and QC samples analyzed in one analysis batch were carried intact through the entire analytical process. The sample data were reviewed and evaluated in relation to the batch QC samples.

- Sample analyte concentrations are not blank corrected.
- By virtue of the isotope dilution/internal standard quantification procedures, data are recovery corrected for possible losses during extraction and cleanup.
- All linearity, CAL/VER, OPR, duplicate and labeled compound recovery specifications were met except the following:

The percent recovery of authentic PFNA in the OPR (Axys ID: WG30329-102) was observed to be outside the method control limit and is flagged with an 'N' on the report form. The concentration of PFNA in the client samples may be similarly affected and values reported should be considered as maximums.

ANALYTICAL DISCUSSION

With concern for carryover from the sample that ran on the instrument before it, sample Site 21 (Axys ID: L13603-5) was analyzed on the instrument a second time and the re-analysis data are reported here with a test suffix 'i' on the Lab Sample I.D.

DATA PACKAGE

This data package is assigned a unique identifier, DPWG30835, shown on the cover page of the data package. Included in the data package after this narrative are the following documents:

- Sample 'Cover Page' and 'Correlation Table'
- Method Summary
- Sample receiving documentation
- Laboratory extraction logs for each sample
- Sample data reports (in order of AXYS Sample ID)
- Laboratory QC data reports
- Instrumental QC data reports (organized by analysis date)
- Sample raw data (in order of AXYS Sample ID)
- Laboratory QC raw data
- Instrumental QC raw data (organized by analysis date)

I certify that this data package is in compliance with the terms and conditions of the contract, both technically and for completeness, except for the conditions detailed above. In addition, I certify, that to the best of my knowledge and belief, the data as reported are true and accurate. The following signature, on behalf of AXYS Analytical Services Ltd, authorizes the release of the data contained in this data package.

Signed: Mark Springford, B.Sc., QA/QC Chemist

10 November 2009
Date Signed

AXYS Analytical Services Ltd.

Analytical Procedure for the Analysis of Perfluorinated Organic Compounds in Aqueous Samples by LC-MS/MS

Method MLA-060 describes the analysis of perfluorinated organic compounds (PFC) in aqueous samples. Typical quantification limits are in the range of 1 - 2 ng/L for a 0.5 L sample size.

EXTRACTION AND CLEANUP

Sample size may be up to 1000 mL. Samples are stored in HDPE (high density polyethylene) containers. Samples are filtered, adjusted to pH 7, spiked with surrogate standards and extracted by solid phase extraction (SPE) using weak anion exchange cartridges. Wash and elution procedures are chosen to meet various analysis requirements. The eluates are spiked with recovery standards and analyzed by HPLC-MS/MS. Calibration solutions are processed through SPE in the same way as the samples.

QUALITY ASSURANCE / QUALITY CONTROL

All samples are analyzed in batches. The composition of a batch is detailed on a batch sheet. Each batch has the following composition:

- Batch Size - Each batch consists of test samples and additional QC samples.
- Blanks – 5% of the samples within a batch are procedural blanks.
- Duplicates – 5% of the samples within a batch are analyzed in duplicate, provided there is sufficient sample.
- Matrix Spike/Matrix Spike Duplicate analyzed upon client request.
- OPR (Spiked Reference Sample) – 5% of the samples within a batch are spiked reference samples.

QC Specification: Procedural Blank Levels and OPR Recoveries

Analyte		Procedural Blank Level ng/sample [2]	OPR Recovery Range (%)[1]
Perfluorobutanoate	(PFBA)	<0.25	80 – 120 [1]
Perfluoropentanoate	(PFPeA)	<0.25	80 – 120 [1]
Perfluorohexanoate	(PFHxA)	<0.25	80 – 120 [1]
Perfluoroheptanoate	(PFHpA)	<0.25	80 – 120 [1]

Perfluorooctanoate	(PFOA)	<0.25	80 – 120 [1]
Perfluorononanoate	(PFNA)	<0.25	80 – 120 [1]
Perfluorodecanoate	(PFDA)	<0.25	80 – 120 [1]
Perfluoroundecanoate	(PFUnA)	<0.25	80 – 120 [1]
Perfluorododecanoate	(PFDoA)	<0.25	80 – 120 [1]
Perfluorobutanesulfonate	(PFBS)	<0.25	70 - 130
Perfluorohexanesulfonate	(PFHxS)	<0.25	70 – 130
Perfluorooctanesulfonate	(PFOS)	<0.25	70 – 130
Perfluorooctane sulfonamide	(PFOSA)	<0.25	70 – 130

1. Additional criteria– recovery for 2 compounds may be 75-125% and for one compound 70-130%
2. For results reported to higher reporting limits, the blank acceptance limit is equal to the reporting limit. Higher blank may be accepted where sample concentrations exceed blank levels by >x10.

QC Specification: Surrogate Standard Recoveries (Calibration Solutions and Samples)

Surrogate Standard	**Recovery Range [2]**
$^{13}C_4$-Heptafluorobutyric acid ($^{13}C_4$-PFBA)	20 - 150%
$^{13}C_2$-Perfluorocaproic acid ($^{13}C_2$-PFHxA)	40 - 150%
$^{13}C_2$- Perfluorooctanoic acid ($^{13}C_2$-PFOA)	40 - 150%
$^{13}C_5$-Heptadecafluorononanoic acid ($^{13}C_5$-PFNA)	40 - 150%
$^{13}C_2$- Perfluorodecanoic acid ($^{13}C_2$-PFDA)	40 - 150%
$^{13}C_2$-Perfluoro-n-(1,2)decanoic acid ($^{13}C_2$-PFDoA)	40 - 150%
$^{13}C_4$-Perfluorooctanesulfonate ($^{13}C_4$-PFOS)	40 - 150%
d7-N-Me-Perfluoro-1-octanesulfonamidoethanol (d7-MeFOSE)	40 - 150%

2. Lower recoveries may be accepted based on application and professional judgment

QC Specification Table: Other Parameters

QC Parameter	Specification
Instrument Sensitivity	Daily, S:N ≥ 3:1 for all analytes for lowest calibration standard.
Initial Calibration (native compounds)	Run initially, and as required to maintain calibration verification and instrument sensitivity. (1/x) weighted quadratic, exclude origin. Calculated conc. 75-125 % of actual (lowest cal may be 70-130%), R^2 > 0.990
Continuing Calibration Verification (native compounds)	Run every 20 samples or more frequently, quantify against I-CAL. Calculated conc. 70-130% actual for a maximum of three compounds with the remainder 80 –120 % of actual
Instrumental Carryover and Instrument Background	Every Initial Calibration, Cal/Ver, or SPM: < 0.3 % carryover and area response of analytes in instrument blank < 800 judged following two previous methanol blank injections.
Duplicate Samples or MS/MSD	If conc. > 5 times R.L., RPD < 40% If conc. < 5 times R.L., difference between pairs < R.L.

ANALYSIS BY LC-MS/MS

Analysis of sample extracts for perfluorinated organics by HPLC-MS/MS is performed on a high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer. The MS is run at unit mass resolution in the Multiple Reaction Monitoring (MRM) mode.

Instrument specifications:

Instrument	Waters 2690 or Waters 2795 HPLC, Micromass Quattro Ultima MS/MS
LC Column	Waters Xtera C18MS Reverse Phase C18, 10.0 cm, 2.1 mm i.d., 3.5 µm particle size (or equivalent)
Ionization	Negative Ion Electrospray
Acquisition	MRM mode, unit resolution
Injection Volume	15 µL

LC-MS/MS Operating Conditions:

LC Gradient Program				General LC Conditions	
Time (min)	**Flow mixture[1]**	**LC Flow Rate Program**	**Gradient Curve**	Column Temp (°C)	40
0.0	15% solvent A 85% solvent B	0.15 mL/min	1	Max Pressure (bar)	300.0
1.0	15% solvent A 85% solvent B	0.15 mL/min	1	**MS Conditions**	
5.0	70% solvent A 30% solvent B	0.20 mL/min	4	Source Temp (°C)	120
8.5	100% solvent A	0.20 mL/min	4	Desolvation Temp (°C)	300
11	100% solvent A	0.20 mL/min	4	Capillary Voltage	2.75
11.3 - 14.5	15% solvent A 85% solvent B	0.20 mL/min	2	Gases	~70L/hr cone ~300L/hr desolvation

[1] Eluent A = 90% CH_3CN (aqueous), Eluent B = 12.1 mM NH_4OAc in 0.1% AcOH (aqueous)

Initial calibration of the LC-MS/MS instrument is performed by the analysis of six or more calibration solutions. A mid-level calibration standard is analyzed to verify the initial calibration after every 20th sample (including QC samples) injected at a minimum. All calibration solutions go through the same SPE extraction/cleanup procedures as the samples.

ANALYTE IDENTIFICATION

Positive identification of target PFC, surrogate standard and recovery standards require:

- > 3:1 S:N for parent ion to daughter ion transition.
- Compound retention time falls within 0.4 minutes of the predicted retention times from the mean determined from the Initial Calibration. Natives with labelled surrogate standards must elute within 0.1 minutes of the associated labelled surrogates. Typical retention times are shown in Table 9.

QUANTIFICATION AND DATA REPORTING PROCEDURES

Target compounds are quantified using the internal standard method, comparing the area of the quantification ion to that of the ^{13}C-labelled standard and correcting for response factors.

Quadratic calibration equations are determined from a multi-point calibration series with 1/X weighing fit as described by the following general equation:

$Y = a + bX + cX^2$ (general quadratic equation)

Where Y = (area target/area surr) x weight surr
X = weight target
a,b,c are empirical constants

Concentrations in samples are determined as:

$$Sample\ Conc = \frac{-b \pm \sqrt{b^2 - 4c\left(a - \left(\frac{area\ of\ target}{area\ of\ sur}\ x\ weight\ sur\right)\right)}}{2c\ x\ sample\ size}$$

The recovery of the surrogate standard is calculated **(by internal standard quantification against the recovery standard using an average RRF)** and monitored as an indication of overall data quality. Final target concentrations are recovery corrected by this method of quantification.

Sample Specific Detection Limits (SDL) are determined .by converting the area equivalent of 3.0 times the estimated chromatographic noise height to a concentration in the same manner that target peak responses are converted to final concentrations. The SDL accounts for any effect of matrix on the detection system and for recovery achieved through the analytical work-up.

Results are reported to the greater of the SDL or the concentration equivalent to the lowest calibration standard analyzed.

Table 9. Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (minutes)	Parent Ion Mass	Daughter Ion Mass	Quantified Against
Target Analytes				
Perfluorobutanoate (PFBA)	5.0	213	169	$^{13}C_4$-PFBA
Perfluoropentanoate (PFPeA)	5.8	263	219	$^{13}C_2$-PFHxA
Perfluorohexanoate (PFHxA)	6.2	313	269	$^{13}C_2$-PFHxA
Perfluoroheptanoate (PFHpA)	6.6	363	319	$^{13}C_2$-PFHxA
Perfluorooctanoate (PFOA)	7.0	413	369 / 219	$^{13}C_2$-PFOA
Perfluorononanoate (PFNA)	7.4	463	419	$^{13}C_5$-PFNA
Perfluorodecanoate (PFDA)	7.9	513	469	$^{13}C_2$-PFDA
Perfluoroundecanoate (PFUnA)	8.5	563	519	$^{13}C_2$-PFDA
Perfluorododecanoate (PFDoA)	9.0	613	569	$^{13}C_2$-PFDoA
Perfluorobutanesulfonate (PFBS)	6.3	299	80 / 99 [1]	$^{13}C_4$-PFOS
Perfluorohexanesulfonate (PFHxS)	7.2	399	80 / 99 [1]	$^{13}C_4$-PFOS
Perfluorooctane sulfonate (PFOS)	8.2	499	80 / 99 [1]	$^{13}C_4$-PFOS
Perfluorooctane sulfonamide (PFOSA) [2]	9.9	498	78	$^{13}C_4$-PFOS
Surrogate Standard				
$^{13}C_4$-Heptafluorobutyric acid ($^{13}C_4$-PFBA)	5.0	217	172	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluorocaproic acid ($^{13}C_2$-PFHxA)	6.2	315	270	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluorooctanoic acid ($^{13}C_2$-PFOA)	7.0	415	370	$^{13}C_4$-PFOA
$^{13}C_5$-Heptadecafluorononanoic acid ($^{13}C_5$-PFNA)	7.4	470	423	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluorodecanoic acid ($^{13}C_2$-PFDA)	7.9	515	470	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluoro-n-(1,2)decanoic acid ($^{13}C_2$-PFDoA)	9.0	615	570	$^{13}C_2$-FOUEA
$^{13}C_4$–Perfluorooctanesulfonate ($^{13}C_4$-PFOS)	8.2	503	80 / 99[1]	$^{13}C_2$-FOUEA
d7-N-Me-Perfluoro-1-octanesulfonamidoethanol (d7-Me-FOSE)	~10.6	623	59	$^{13}C_2$-FOUEA
Recovery Standard				
$^{13}C_2$-2H-Perfluoro-2-decenoic acid ($^{13}C_2$-FOUEA)	7.3	459	394	-
$^{13}C_4$-Perfluorooctanoic acid ($^{13}C_4$-PFOA)	6.9	417	372	-

[1] Quantification is based on m/z 80 daughter, m/z 99 may be used as alternate if necessary to avoid interference.

[2] PFOSA quantified against d7-Me-FOSE if collected in separate fraction.

PERFLUORINATED ORGANIC ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-060

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG31212

Analysis WG30691

10 December 2009

PERFLUORINATED ORGANIC ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-060

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG31212
Analysis WG30691

Prepared for:
ORSANCO

Prepared by:
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CANADA

Contact: Candice Navaroli
Project Manager

10 December 2009

OHIO RIVER VALLEY WATER SANITATION COMMISSION
WATER SAMPLES **PERFLUORINATED ORGANIC ANALYSIS**
AXYS METHOD: MLA-060

Project: EMERGING CONTAMINANTS IN MAINSTEM OHIO

4562: L13811-1 and -2

11 December 2009

NARRATIVE

This narrative describes the analysis of two water samples for the determination of perfluorinated organic compounds using high performance liquid chromatography/tandem mass spectrometry (HPLC/MS-MS).

SAMPLE RECEIPT, STORAGE AND PREPARATION

The water samples were received at AXYS on the 22nd of October 2009. Details of sample conditions upon receipt are provided on the Sample Receiving Record forms included in the sample documentation section of this data package. The samples arrived at Axys at -2°C and were stored at 4°C prior to sample preparation and analysis.

SAMPLE EXTRACTION AND ANALYSIS

The samples were analyzed in one analysis batch named WG30691. Composition of the batch is shown on the Cover pages and the Batch List forms included in this data package.

Sample extraction, instrumental analysis and analyte quantification procedures were in accordance with AXYS Method MLA-060: ***Analytical Procedure for the Analysis of Perfluorinated Organic Compounds in Aqueous Samples by LC-MS/MS.***

The samples were filtered and accurately weighed. After spiked with ^{13}C-labelled quantification standards, the samples were extracted and cleaned up using SPE cartridges. The resulted extracts were instrumentally analyzed using liquid chromatography/mass spectrometry (LC-MS/MS). Analyte concentrations were determined by isotope dilution/internal standard quantification, comparing the area response of the quantification ion to that of the ^{13}C-labelled standards and correcting for response factors. Quadratic quantification equations with 1/X weighting fit were determined from a multi-point calibration series prepared alongside the samples.

The reporting limit (RL) was defined as the concentration equivalent to the lowest calibration standard analyzed in the initial calibration (CS0) or the sample specific detection limits, whichever was greater.

REPORTING CONVENTIONS

The AXYS contract number assigned for internal tracking was 4562. Samples were assigned a unique laboratory identifier of the form L13811-X, where X = numeral. All data reports reference these unique AXYS IDs plus the client's sample identifier. To assist with locating data, a table correlating AXYS ID with the client sample number is included in this data package. The report forms were generated using Laboratory Information Management System (LIMS).

The following laboratory qualifier flags were used in this data package:

U = identifies a compound that was not detected

Results are reported in concentration units of nanograms per Litre (ng/L). Concentration and detection limits are provided to three significant figures. Analysis results for each sample are provided on Analysis Report form 1A and form 2.

QA/QC NOTE

Samples and QC samples analyzed in one analysis batch were carried intact through the entire analytical process. The sample data were reviewed and evaluated in relation to the batch QC samples.

- Sample analyte concentrations are not blank corrected.
- By virtue of the isotope dilution/internal standard quantification procedures, data are recovery corrected for possible losses during extraction and cleanup.
- All linearity, CAL/VER, OPR, duplicate and labeled compound recovery specifications were met.

ANALYTICAL DISCUSSION

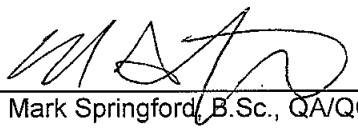
No analytical difficulties were encountered.

DATA PACKAGE

This data package is assigned a unique identifier, DPWG31212, shown on the cover page of the data package. Included in the data package after this narrative are the following documents:

- Sample 'Cover Page' and 'Correlation Table'
- Method Summary
- Sample receiving documentation
- Laboratory extraction logs for each sample
- Sample data reports (in order of AXYS Sample ID)
- Laboratory QC data reports
- Instrumental QC data reports (organized by analysis date)
- Sample raw data (in order of AXYS Sample ID)
- Laboratory QC raw data
- Instrumental QC raw data (organized by analysis date)

I certify that this data package is in compliance with the terms and conditions of the contract, both technically and for completeness, except for the conditions detailed above. In addition, I certify, that to the best of my knowledge and belief, the data as reported are true and accurate. The following signature, on behalf of AXYS Analytical Services Ltd, authorizes the release of the data contained in this data package.



Signed: Mark Springford, B.Sc., QA/QC Chemist

11 December 2009

Date Signed

AXYS Analytical Services Ltd.

Analytical Procedure for the Analysis of Perfluorinated Organic Compounds in Aqueous Samples by LC-MS/MS

Method MLA-060 describes the analysis of perfluorinated organic compounds (PFC) in aqueous samples. Typical quantification limits are in the range of 1 - 2 ng/L for a 0.5 L sample size.

EXTRACTION AND CLEANUP

Sample size may be up to 1000 mL. Samples are stored in HDPE (high density polyethylene) containers. Samples are filtered, adjusted to pH 7, spiked with surrogate standards and extracted by solid phase extraction (SPE) using weak anion exchange cartridges. Wash and elution procedures are chosen to meet various analysis requirements. The eluates are spiked with recovery standards and analyzed by HPLC-MS/MS. Calibration solutions are processed through SPE in the same way as the samples.

QUALITY ASSURANCE / QUALITY CONTROL

All samples are analyzed in batches. The composition of a batch is detailed on a batch sheet. Each batch has the following composition:

- Batch Size - Each batch consists of test samples and additional QC samples.
- Blanks – 5% of the samples within a batch are procedural blanks.
- Duplicates – 5% of the samples within a batch are analyzed in duplicate, provided there is sufficient sample.
- Matrix Spike/Matrix Spike Duplicate analyzed upon client request.
- OPR (Spiked Reference Sample) – 5% of the samples within a batch are spiked reference samples.

QC Specification: Procedural Blank Levels and OPR Recoveries

Analyte		Procedural Blank Level ng/sample [2]	OPR Recovery Range (%)[1]
Perfluorobutanoate	(PFBA)	<0.25	80 – 120 [1]
Perfluoropentanoate	(PFPeA)	<0.25	80 – 120 [1]
Perfluorohexanoate	(PFHxA)	<0.25	80 – 120 [1]
Perfluoroheptanoate	(PFHpA)	<0.25	80 – 120 [1]

Perfluorooctanoate	(PFOA)	<0.25	80 – 120 [1]
Perfluorononanoate	(PFNA)	<0.25	80 – 120 [1]
Perfluorodecanoate	(PFDA)	<0.25	80 – 120 [1]
Perfluoroundecanoate	(PFUnA)	<0.25	80 – 120 [1]
Perfluorododecanoate	(PFDoA)	<0.25	80 – 120 [1]
Perfluorobutanesulfonate	(PFBS)	<0.25	70 - 130
Perfluorohexanesulfonate	(PFHxS)	<0.25	70 – 130
Perfluorooctanesulfonate	(PFOS)	<0.25	70 – 130
Perfluorooctane sulfonamide	(PFOSA)	<0.25	70 – 130

1. Additional criteria– recovery for 2 compounds may be 75-125% and for one compound 70-130%
2. For results reported to higher reporting limits, the blank acceptance limit is equal to the reporting limit. Higher blank may be accepted where sample concentrations exceed blank levels by >x10.

QC Specification: Surrogate Standard Recoveries (Calibration Solutions and Samples)

Surrogate Standard	**Recovery Range [2]**
$^{13}C_4$-Heptafluorobutyric acid ($^{13}C_4$-PFBA)	20 - 150%
$^{13}C_2$-Perfluorocaproic acid ($^{13}C_2$-PFHxA)	40 - 150%
$^{13}C_2$- Perfluorooctanoic acid ($^{13}C_2$-PFOA)	40 - 150%
$^{13}C_5$-Heptadecafluorononanoic acid ($^{13}C_5$-PFNA)	40 - 150%
$^{13}C_2$- Perfluorodecanoic acid ($^{13}C_2$-PFDA)	40 - 150%
$^{13}C_2$-Perfluoro-n-(1,2)decanoic acid ($^{13}C_2$-PFDoA)	40 - 150%
$^{13}C_4$-Perfluorooctanesulfonate ($^{13}C_4$-PFOS)	40 - 150%
d7-N-Me-Perfluoro-1-octanesulfonamidoethanol (d7-MeFOSE)	40 - 150%

2. Lower recoveries may be accepted based on application and professional judgment

QC Specification Table: Other Parameters

QC Parameter	Specification
Instrument Sensitivity	Daily, S:N ≥ 3:1 for all analytes for lowest calibration standard.
Initial Calibration (native compounds)	Run initially, and as required to maintain calibration verification and instrument sensitivity. (1/x) weighted quadratic, exclude origin. Calculated conc. 75-125 % of actual (lowest cal may be 70-130%), R^2 > 0.990
Continuing Calibration Verification (native compounds)	Run every 20 samples or more frequently, quantify against I-CAL. Calculated conc. 70-130% actual for a maximum of three compounds with the remainder 80 –120 % of actual
Instrumental Carryover and Instrument Background	Every Initial Calibration, Cal/Ver, or SPM: < 0.3 % carryover and area response of analytes in instrument blank < 800 judged following two previous methanol blank injections.
Duplicate Samples or MS/MSD	If conc. > 5 times R.L., RPD < 40% If conc. < 5 times R.L., difference between pairs < R.L.

ANALYSIS BY LC-MS/MS

Analysis of sample extracts for perfluorinated organics by HPLC-MS/MS is performed on a high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer. The MS is run at unit mass resolution in the Multiple Reaction Monitoring (MRM) mode.

Instrument specifications:

Instrument	Waters 2690 or Waters 2795 HPLC, Micromass Quattro Ultima MS/MS
LC Column	Waters Xtera C18MS Reverse Phase C18, 10.0 cm, 2.1 mm i.d., 3.5 µm particle size (or equivalent)
Ionization	Negative Ion Electrospray
Acquisition	MRM mode, unit resolution
Injection Volume	15 µL

LC-MS/MS Operating Conditions:

LC Gradient Program				**General LC Conditions**	
Time (min)	**Flow mixture[1]**	**LC Flow Rate Program**	**Gradient Curve**	Column Temp (°C)	40
0.0	15% solvent A 85% solvent B	0.15 mL/min	1	Max Pressure (bar)	300.0
1.0	15% solvent A 85% solvent B	0.15 mL/min	1	**MS Conditions**	
5.0	70% solvent A 30% solvent B	0.20 mL/min	4	Source Temp (°C)	120
8.5	100% solvent A	0.20 mL/min	4	Desolvation Temp (°C)	300
11	100% solvent A	0.20 mL/min	4	Capillary Voltage	2.75
11.3 - 14.5	15% solvent A 85% solvent B	0.20 mL/min	2	Gases	~70L/hr cone ~300L/hr desolvation

[1] Eluent A = 90% CH_3CN (aqueous), Eluent B = 12.1 mM NH_4OAc in 0.1% AcOH (aqueous)

Initial calibration of the LC-MS/MS instrument is performed by the analysis of six or more calibration solutions. A mid-level calibration standard is analyzed to verify the initial calibration after every 20th sample (including QC samples) injected at a minimum. All calibration solutions go through the same SPE extraction/cleanup procedures as the samples.

ANALYTE IDENTIFICATION

Positive identification of target PFC, surrogate standard and recovery standards require:

- > 3:1 S:N for parent ion to daughter ion transition.
- Compound retention time falls within 0.4 minutes of the predicted retention times from the mean determined from the Initial Calibration. Natives with labelled surrogate standards must elute within 0.1 minutes of the associated labelled surrogates. Typical retention times are shown in Table 9.

QUANTIFICATION AND DATA REPORTING PROCEDURES

Target compounds are quantified using the internal standard method, comparing the area of the quantification ion to that of the ^{13}C-labelled standard and correcting for response factors.

Quadratic calibration equations are determined from a multi-point calibration series with 1/X weighing fit as described by the following general equation:

$Y = a + bX + cX^2$ (general quadratic equation)

Where Y = (area target/area surr) x weight surr
X = weight target
a,b,c are empirical constants

Concentrations in samples are determined as:

$$Sample\ Conc = \frac{-b \pm \sqrt{b^2 - 4c\left(a - \left(\frac{area\ of\ target}{area\ of\ sur}\ x\ weight\ sur\right)\right)}}{2c\ x\ sample\ size}$$

The recovery of the surrogate standard is calculated **(by internal standard quantification against the recovery standard using an average RRF)** and monitored as an indication of overall data quality. Final target concentrations are recovery corrected by this method of quantification.

Sample Specific Detection Limits (SDL) are determined .by converting the area equivalent of 3.0 times the estimated chromatographic noise height to a concentration in the same manner that target peak responses are converted to final concentrations. The SDL accounts for any effect of matrix on the detection system and for recovery achieved through the analytical work-up.

Results are reported to the greater of the SDL or the concentration equivalent to the lowest calibration standard analyzed.

Table 9. Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (minutes)	Parent Ion Mass	Daughter Ion Mass	Quantified Against
Target Analytes				
Perfluorobutanoate (PFBA)	5.0	213	169	$^{13}C_4$-PFBA
Perfluoropentanoate (PFPeA)	5.8	263	219	$^{13}C_2$-PFHxA
Perfluorohexanoate (PFHxA)	6.2	313	269	$^{13}C_2$-PFHxA
Perfluoroheptanoate (PFHpA)	6.6	363	319	$^{13}C_2$-PFHxA
Perfluorooctanoate (PFOA)	7.0	413	369 / 219	$^{13}C_2$-PFOA
Perfluorononanoate (PFNA)	7.4	463	419	$^{13}C_5$-PFNA
Perfluorodecanoate (PFDA)	7.9	513	469	$^{13}C_2$-PFDA
Perfluoroundecanoate (PFUnA)	8.5	563	519	$^{13}C_2$-PFDA
Perfluorododecanoate (PFDoA)	9.0	613	569	$^{13}C_2$-PFDoA
Perfluorobutanesulfonate (PFBS)	6.3	299	80 / 99 [1]	$^{13}C_4$-PFOS
Perfluorohexanesulfonate (PFHxS)	7.2	399	80 / 99 [1]	$^{13}C_4$-PFOS
Perfluorooctane sulfonate (PFOS)	8.2	499	80 / 99 [1]	$^{13}C_4$-PFOS
Perfluorooctane sulfonamide (PFOSA) [2]	9.9	498	78	$^{13}C_4$-PFOS
Surrogate Standard				
$^{13}C_4$-Heptafluorobutyric acid ($^{13}C_4$-PFBA)	5.0	217	172	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluorocaproic acid ($^{13}C_2$-PFHxA)	6.2	315	270	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluorooctanoic acid ($^{13}C_2$-PFOA)	7.0	415	370	$^{13}C_4$-PFOA
$^{13}C_5$-Heptadecafluorononanoic acid ($^{13}C_5$-PFNA)	7.4	470	423	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluorodecanoic acid ($^{13}C_2$-PFDA)	7.9	515	470	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluoro-n-(1,2)decanoic acid ($^{13}C_2$-PFDoA)	9.0	615	570	$^{13}C_2$-FOUEA
$^{13}C_4$–Perfluorooctanesulfonate ($^{13}C_4$-PFOS)	8.2	503	80 / 99[1]	$^{13}C_2$-FOUEA
d7-N-Me-Perfluoro-1-octanesulfonamidoethanol (d7-Me-FOSE)	~10.6	623	59	$^{13}C_2$-FOUEA
Recovery Standard				
$^{13}C_2$-2H-Perfluoro-2-decenoic acid ($^{13}C_2$-FOUEA)	7.3	459	394	-
$^{13}C_4$-Perfluorooctanoic acid ($^{13}C_4$-PFOA)	6.9	417	372	-

[1] Quantification is based on m/z 80 daughter, m/z 99 may be used as alternate if necessary to avoid interference.

[2] PFOSA quantified against d7-Me-FOSE if collected in separate fraction.

PERFLUORINATED ORGANIC ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-060

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG31213

Analysis WG30691

10 December 2009

PERFLUORINATED ORGANIC ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-060

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG31213
Analysis WG30691

Prepared for:
ORSANCO

Prepared by:
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Contact: Candice Navaroli
Project Manager

10 December 2009

OHIO RIVER VALLEY WATER SANITATION COMMISSION
WATER SAMPLES **PERFLUORINATED ORGANIC ANALYSIS**
AXYS METHOD: MLA-060

Project: EMERGING CONTAMINANTS IN MAINSTEM OHIO

4562: L13813-1 and -2

11 December 2009

NARRATIVE

This narrative describes the analysis of two water samples for the determination of perfluorinated organic compounds using high performance liquid chromatography/tandem mass spectrometry (HPLC/MS-MS).

SAMPLE RECEIPT, STORAGE AND PREPARATION

The water samples were received at AXYS on the 22nd and 23rd of October 2009. Details of sample conditions upon receipt are provided on the Sample Receiving Record forms included in the sample documentation section of this data package. Sample Site 11 (Axys ID: L13813-1) arrived at Axys at -2°C and sample Site 25 (Axys ID: L13813-2) arrived at 5°C and both were stored at 4°C prior to sample preparation and analysis. Despite the elevated temperature of the second sample analysis was allowed to proceed through communication with the client.

SAMPLE EXTRACTION AND ANALYSIS

The samples were analyzed in one analysis batch named WG30691. Composition of the batch is shown on the Cover pages and the Batch List forms included in this data package.

Sample extraction, instrumental analysis and analyte quantification procedures were in accordance with AXYS Method MLA-060: ***Analytical Procedure for the Analysis of Perfluorinated Organic Compounds in Aqueous Samples by LC-MS/MS.***

The samples were filtered and accurately weighed. After spiked with ^{13}C-labelled quantification standards, the samples were extracted and cleaned up using SPE cartridges. The resulted extracts were instrumentally analyzed using liquid chromatography/mass spectrometry (LC-MS/MS). Analyte concentrations were determined by isotope dilution/internal standard quantification, comparing the area response of the quantification ion to that of the ^{13}C-labelled standards and correcting for response factors. Quadratic quantification equations with 1/X weighting fit were determined from a multi-point calibration series prepared alongside the samples.

The reporting limit (RL) was defined as the concentration equivalent to the lowest calibration standard analyzed in the initial calibration (CS0) or the sample specific detection limits, whichever was greater.

REPORTING CONVENTIONS

The AXYS contract number assigned for internal tracking was 4562. Samples were assigned a unique laboratory identifier of the form L13813-X, where X = numeral. All data reports reference these unique AXYS IDs plus the client's sample identifier. To assist with locating data, a table correlating AXYS ID with the client sample number is included in this data package. The report forms were generated using Laboratory Information Management System (LIMS).

The following laboratory qualifier flags were used in this data package:

U = identifies a compound that was not detected

Results are reported in concentration units of nanograms per Litre (ng/L). Concentration and detection limits are provided to three significant figures. Analysis results for each sample are provided on Analysis Report form 1A and form 2.

QA/QC NOTE

Samples and QC samples analyzed in one analysis batch were carried intact through the entire analytical process. The sample data were reviewed and evaluated in relation to the batch QC samples.

- Sample analyte concentrations are not blank corrected.
- By virtue of the isotope dilution/internal standard quantification procedures, data are recovery corrected for possible losses during extraction and cleanup.
- All linearity, CAL/VER, OPR, duplicate and labeled compound recovery specifications were met.

ANALYTICAL DISCUSSION

No analytical difficulties were encountered.

DATA PACKAGE

This data package is assigned a unique identifier, DPWG31213, shown on the cover page of the data package. Included in the data package after this narrative are the following documents:

- Sample 'Cover Page' and 'Correlation Table'
- Method Summary
- Sample receiving documentation
- Laboratory extraction logs for each sample
- Sample data reports (in order of AXYS Sample ID)
- Laboratory QC data reports
- Instrumental QC data reports (organized by analysis date)
- Sample raw data (in order of AXYS Sample ID)
- Laboratory QC raw data
- Instrumental QC raw data (organized by analysis date)

I certify that this data package is in compliance with the terms and conditions of the contract, both technically and for completeness, except for the conditions detailed above. In addition, I certify, that to the best of my knowledge and belief, the data as reported are true and accurate. The following signature, on behalf of AXYS Analytical Services Ltd, authorizes the release of the data contained in this data package.

Signed: Mark Springford, B.Sc., QA/QC Chemist

11 December 2009
Date Signed

AXYS Analytical Services Ltd.

Analytical Procedure for the Analysis of Perfluorinated Organic Compounds in Aqueous Samples by LC-MS/MS

Method MLA-060 describes the analysis of perfluorinated organic compounds (PFC) in aqueous samples. Typical quantification limits are in the range of 1 - 2 ng/L for a 0.5 L sample size.

EXTRACTION AND CLEANUP

Sample size may be up to 1000 mL. Samples are stored in HDPE (high density polyethylene) containers. Samples are filtered, adjusted to pH 7, spiked with surrogate standards and extracted by solid phase extraction (SPE) using weak anion exchange cartridges. Wash and elution procedures are chosen to meet various analysis requirements. The eluates are spiked with recovery standards and analyzed by HPLC-MS/MS. Calibration solutions are processed through SPE in the same way as the samples.

QUALITY ASSURANCE / QUALITY CONTROL

All samples are analyzed in batches. The composition of a batch is detailed on a batch sheet. Each batch has the following composition:

- Batch Size - Each batch consists of test samples and additional QC samples.
- Blanks – 5% of the samples within a batch are procedural blanks.
- Duplicates – 5% of the samples within a batch are analyzed in duplicate, provided there is sufficient sample.
- Matrix Spike/Matrix Spike Duplicate analyzed upon client request.
- OPR (Spiked Reference Sample) – 5% of the samples within a batch are spiked reference samples.

QC Specification: Procedural Blank Levels and OPR Recoveries

Analyte		Procedural Blank Level ng/sample [2]	OPR Recovery Range (%)[1]
Perfluorobutanoate	(PFBA)	<0.25	80 – 120 [1]
Perfluoropentanoate	(PFPeA)	<0.25	80 – 120 [1]
Perfluorohexanoate	(PFHxA)	<0.25	80 – 120 [1]
Perfluoroheptanoate	(PFHpA)	<0.25	80 – 120 [1]

Perfluorooctanoate	(PFOA)	<0.25	80 – 120 [1]
Perfluorononanoate	(PFNA)	<0.25	80 – 120 [1]
Perfluorodecanoate	(PFDA)	<0.25	80 – 120 [1]
Perfluoroundecanoate	(PFUnA)	<0.25	80 – 120 [1]
Perfluorododecanoate	(PFDoA)	<0.25	80 – 120 [1]
Perfluorobutanesulfonate	(PFBS)	<0.25	70 - 130
Perfluorohexanesulfonate	(PFHxS)	<0.25	70 – 130
Perfluorooctanesulfonate	(PFOS)	<0.25	70 – 130
Perfluorooctane sulfonamide	(PFOSA)	<0.25	70 – 130

1. Additional criteria– recovery for 2 compounds may be 75-125% and for one compound 70-130%
2. For results reported to higher reporting limits, the blank acceptance limit is equal to the reporting limit. Higher blank may be accepted where sample concentrations exceed blank levels by >x10.

QC Specification: Surrogate Standard Recoveries (Calibration Solutions and Samples)

Surrogate Standard	**Recovery Range [2]**
$^{13}C_4$-Heptafluorobutyric acid ($^{13}C_4$-PFBA)	20 - 150%
$^{13}C_2$-Perfluorocaproic acid ($^{13}C_2$-PFHxA)	40 - 150%
$^{13}C_2$- Perfluorooctanoic acid ($^{13}C_2$-PFOA)	40 - 150%
$^{13}C_5$-Heptadecafluorononanoic acid ($^{13}C_5$-PFNA)	40 - 150%
$^{13}C_2$- Perfluorodecanoic acid ($^{13}C_2$-PFDA)	40 - 150%
$^{13}C_2$-Perfluoro-n-(1,2)decanoic acid ($^{13}C_2$-PFDoA)	40 - 150%
$^{13}C_4$-Perfluorooctanesulfonate ($^{13}C_4$-PFOS)	40 - 150%
d7-N-Me-Perfluoro-1-octanesulfonamidoethanol (d7-MeFOSE)	40 - 150%

2. Lower recoveries may be accepted based on application and professional judgment

QC Specification Table: Other Parameters

QC Parameter	Specification
Instrument Sensitivity	Daily, S:N ≥ 3:1 for all analytes for lowest calibration standard.
Initial Calibration (native compounds)	Run initially, and as required to maintain calibration verification and instrument sensitivity. (1/x) weighted quadratic, exclude origin. Calculated conc. 75-125 % of actual (lowest cal may be 70-130%), R^2 > 0.990
Continuing Calibration Verification (native compounds)	Run every 20 samples or more frequently, quantify against I-CAL. Calculated conc. 70-130% actual for a maximum of three compounds with the remainder 80 –120 % of actual
Instrumental Carryover and Instrument Background	Every Initial Calibration, Cal/Ver, or SPM: < 0.3 % carryover and area response of analytes in instrument blank < 800 judged following two previous methanol blank injections.
Duplicate Samples or MS/MSD	If conc. > 5 times R.L., RPD < 40% If conc. < 5 times R.L., difference between pairs < R.L.

ANALYSIS BY LC-MS/MS

Analysis of sample extracts for perfluorinated organics by HPLC-MS/MS is performed on a high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer. The MS is run at unit mass resolution in the Multiple Reaction Monitoring (MRM) mode.

Instrument specifications:

Instrument	Waters 2690 or Waters 2795 HPLC, Micromass Quattro Ultima MS/MS
LC Column	Waters Xtera C18MS Reverse Phase C18, 10.0 cm, 2.1 mm i.d., 3.5 µm particle size (or equivalent)
Ionization	Negative Ion Electrospray
Acquisition	MRM mode, unit resolution
Injection Volume	15 µL

LC-MS/MS Operating Conditions:

LC Gradient Program				**General LC Conditions**	
Time (min)	**Flow mixture[1]**	**LC Flow Rate Program**	**Gradient Curve**	Column Temp (°C)	40
0.0	15% solvent A 85% solvent B	0.15 mL/min	1	Max Pressure (bar)	300.0
1.0	15% solvent A 85% solvent B	0.15 mL/min	1	**MS Conditions**	
5.0	70% solvent A 30% solvent B	0.20 mL/min	4	Source Temp (°C)	120
8.5	100% solvent A	0.20 mL/min	4	Desolvation Temp (°C)	300
11	100% solvent A	0.20 mL/min	4	Capillary Voltage	2.75
11.3 - 14.5	15% solvent A 85% solvent B	0.20 mL/min	2	Gases	~70L/hr cone ~300L/hr desolvation

[1] Eluent A = 90% CH_3CN (aqueous), Eluent B = 12.1 mM NH_4OAc in 0.1% AcOH (aqueous)

Initial calibration of the LC-MS/MS instrument is performed by the analysis of six or more calibration solutions. A mid-level calibration standard is analyzed to verify the initial calibration after every 20th sample (including QC samples) injected at a minimum. All calibration solutions go through the same SPE extraction/cleanup procedures as the samples.

ANALYTE IDENTIFICATION

Positive identification of target PFC, surrogate standard and recovery standards require:

- > 3:1 S:N for parent ion to daughter ion transition.
- Compound retention time falls within 0.4 minutes of the predicted retention times from the mean determined from the Initial Calibration. Natives with labelled surrogate standards must elute within 0.1 minutes of the associated labelled surrogates. Typical retention times are shown in Table 9.

QUANTIFICATION AND DATA REPORTING PROCEDURES

Target compounds are quantified using the internal standard method, comparing the area of the quantification ion to that of the ^{13}C-labelled standard and correcting for response factors.

Quadratic calibration equations are determined from a multi-point calibration series with 1/X weighing fit as described by the following general equation:

$Y = a + bX + cX^2$ (general quadratic equation)

Where Y = (area target/area surr) x weight surr
X = weight target
a,b,c are empirical constants

Concentrations in samples are determined as:

$$Sample\ Conc = \frac{-b \pm \sqrt{b^2 - 4c\left(a - \left(\frac{area\ of\ target}{area\ of\ sur}\ x\ weight\ sur\right)\right)}}{2c\ x\ sample\ size}$$

The recovery of the surrogate standard is calculated **(by internal standard quantification against the recovery standard using an average RRF)** and monitored as an indication of overall data quality. Final target concentrations are recovery corrected by this method of quantification.

Sample Specific Detection Limits (SDL) are determined .by converting the area equivalent of 3.0 times the estimated chromatographic noise height to a concentration in the same manner that target peak responses are converted to final concentrations. The SDL accounts for any effect of matrix on the detection system and for recovery achieved through the analytical work-up.

Results are reported to the greater of the SDL or the concentration equivalent to the lowest calibration standard analyzed.

Table 9. Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (minutes)	Parent Ion Mass	Daughter Ion Mass	Quantified Against
Target Analytes				
Perfluorobutanoate (PFBA)	5.0	213	169	$^{13}C_4$-PFBA
Perfluoropentanoate (PFPeA)	5.8	263	219	$^{13}C_2$-PFHxA
Perfluorohexanoate (PFHxA)	6.2	313	269	$^{13}C_2$-PFHxA
Perfluoroheptanoate (PFHpA)	6.6	363	319	$^{13}C_2$-PFHxA
Perfluorooctanoate (PFOA)	7.0	413	369 / 219	$^{13}C_2$-PFOA
Perfluorononanoate (PFNA)	7.4	463	419	$^{13}C_5$-PFNA
Perfluorodecanoate (PFDA)	7.9	513	469	$^{13}C_2$-PFDA
Perfluoroundecanoate (PFUnA)	8.5	563	519	$^{13}C_2$-PFDA
Perfluorododecanoate (PFDoA)	9.0	613	569	$^{13}C_2$-PFDoA
Perfluorobutanesulfonate (PFBS)	6.3	299	80 / 99 [1]	$^{13}C_4$-PFOS
Perfluorohexanesulfonate (PFHxS)	7.2	399	80 / 99 [1]	$^{13}C_4$-PFOS
Perfluorooctane sulfonate (PFOS)	8.2	499	80 / 99 [1]	$^{13}C_4$-PFOS
Perfluorooctane sulfonamide (PFOSA) [2]	9.9	498	78	$^{13}C_4$-PFOS
Surrogate Standard				
$^{13}C_4$-Heptafluorobutyric acid ($^{13}C_4$-PFBA)	5.0	217	172	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluorocaproic acid ($^{13}C_2$-PFHxA)	6.2	315	270	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluorooctanoic acid ($^{13}C_2$-PFOA)	7.0	415	370	$^{13}C_4$-PFOA
$^{13}C_5$-Heptadecafluorononanoic acid ($^{13}C_5$-PFNA)	7.4	470	423	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluorodecanoic acid ($^{13}C_2$-PFDA)	7.9	515	470	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluoro-n-(1,2)decanoic acid ($^{13}C_2$-PFDoA)	9.0	615	570	$^{13}C_2$-FOUEA
$^{13}C_4$–Perfluorooctanesulfonate ($^{13}C_4$-PFOS)	8.2	503	80 / 99[1]	$^{13}C_2$-FOUEA
d7-N-Me-Perfluoro-1-octanesulfonamidoethanol (d7-Me-FOSE)	~10.6	623	59	$^{13}C_2$-FOUEA
Recovery Standard				
$^{13}C_2$-2H-Perfluoro-2-decenoic acid ($^{13}C_2$-FOUEA)	7.3	459	394	-
$^{13}C_4$-Perfluorooctanoic acid ($^{13}C_4$-PFOA)	6.9	417	372	-

[1] Quantification is based on m/z 80 daughter, m/z 99 may be used as alternate if necessary to avoid interference.

[2] PFOSA quantified against d7-Me-FOSE if collected in separate fraction.

PERFLUORINATED ORGANIC ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-060

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG30758

Analysis WG30317

3 November 2009

PERFLUORINATED ORGANIC ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-060

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG30758
Analysis WG30317

Prepared for:
ORSANCO

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3 November 2009

OHIO RIVER VALLEY WATER SANITATION COMMISSION
WATER SAMPLES **PERFLUORINATED ORGANIC ANALYSIS**
AXYS METHOD: MLA-060

Project: EMERGING CONTAMINANTS IN MAINSTEM OHIO

4562: L13550-1 to -15

4 November 2009

NARRATIVE

This narrative describes the analysis of fifteen water samples for the determination of perfluorinated organic compounds using high performance liquid chromatography/tandem mass spectrometry (HPLC/MS-MS).

SAMPLE RECEIPT, STORAGE AND PREPARATION

Water samples were received at AXYS on the 17th of September 2009. Details of sample conditions upon receipt are provided on the Sample Receiving Record forms included in the sample documentation section of this data package. The samples were stored at 4°C prior to sample preparation and analysis.

SAMPLE EXTRACTION AND ANALYSIS

The samples were analyzed in one analysis batch named WG30317. Composition of the batch is shown on the Cover pages and the Batch List forms included in this data package.

Sample extraction, instrumental analysis and analyte quantification procedures were in accordance with AXYS Method MLA-060: ***Analytical Procedure for the Analysis of Perfluorinated Organic Compounds in Aqueous Samples by LC-MS/MS.***

The samples were filtered and accurately weighed. After spiked with ^{13}C-labelled quantification standards, the samples were extracted and cleaned up using SPE cartridges. The resulted extracts were instrumentally analyzed using liquid chromatography/mass spectrometry (LC-MS/MS). Analyte concentrations were determined by isotope dilution/internal standard quantification, comparing the area response of the quantification ion to that of the ^{13}C-labelled standards and correcting for response factors. Quadratic quantification equations with 1/X weighting fit were determined from a multi-point calibration series prepared alongside the samples.

The reporting limit (RL) was defined as the concentration equivalent to the lowest calibration standard analyzed in the initial calibration (CS0) or the sample specific detection limits, whichever was greater.

REPORTING CONVENTIONS

The AXYS contract number assigned for internal tracking was 4562. Samples were assigned a unique laboratory identifier of the form L13550-XX, where X = numeral. All data reports reference these unique AXYS IDs plus the client's sample identifier. To assist with locating data, a table correlating AXYS ID with the client sample number is included in this data package. The report forms were generated using Laboratory Information Management System (LIMS).

The following laboratory qualifier flags were used in this data package:

U = identifies a compound that was not detected

Results are reported in concentration units of nanograms per Litre (ng/L). Concentration and detection limits are provided to three significant figures. Analysis results for each sample are provided on Analysis Report form 1A and form 2.

QA/QC NOTE

Samples and QC samples analyzed in one analysis batch were carried intact through the entire analytical process. The sample data were reviewed and evaluated in relation to the batch QC samples.

- Sample analyte concentrations are not blank corrected.
- By virtue of the isotope dilution/internal standard quantification procedures, data are recovery corrected for possible losses during extraction and cleanup.
- All linearity, CAL/VER, OPR, duplicate and labeled compound recovery specifications were met except the following:

PFDoA and $^{13}C_2$-PFDoA were excluded from the highest level CS7 (data filename FC9G_457 S:11) of the initial calibrations. However, a minimum of 7 calibration standard points were used to construct the regression equations for quantification of target analytes or calculate response factor (RF) for quantification of labeled surrogates. As multi-point calibrations were used, sample data are deemed not to be significantly affected.

ANALYTICAL DISCUSSION

No analytical difficulties were encountered.

DATA PACKAGE

This data package is assigned a unique identifier, DPWG30758, shown on the cover page of the data package. Included in the data package after this narrative are the following documents:

- Sample 'Cover Page' and 'Correlation Table'
- Method Summary
- Sample receiving documentation
- Laboratory extraction logs for each sample
- Sample data reports (in order of AXYS Sample ID)
- Laboratory QC data reports
- Instrumental QC data reports (organized by analysis date)
- Sample raw data (in order of AXYS Sample ID)
- Laboratory QC raw data
- Instrumental QC raw data (organized by analysis date)

I certify that this data package is in compliance with the terms and conditions of the contract, both technically and for completeness, except for the conditions detailed above. In addition, I certify, that to the best of my knowledge and belief, the data as reported are true and accurate. The following signature, on behalf of AXYS Analytical Services Ltd, authorizes the release of the data contained in this data package.

Signed: Mark Springford, B.Sc., QA/QC Chemist

4 November 2009

Date Signed

AXYS Analytical Services Ltd.

Analytical Procedure for the Analysis of Perfluorinated Organic Compounds in Aqueous Samples by LC-MS/MS

Method MLA-060 describes the analysis of perfluorinated organic compounds (PFC) in aqueous samples. Typical quantification limits are in the range of 1 - 2 ng/L for a 0.5 L sample size.

EXTRACTION AND CLEANUP

Sample size may be up to 1000 mL. Samples are stored in HDPE (high density polyethylene) containers. Samples are filtered, adjusted to pH 7, spiked with surrogate standards and extracted by solid phase extraction (SPE) using weak anion exchange cartridges. Wash and elution procedures are chosen to meet various analysis requirements. The eluates are spiked with recovery standards and analyzed by HPLC-MS/MS. Calibration solutions are processed through SPE in the same way as the samples.

QUALITY ASSURANCE / QUALITY CONTROL

All samples are analyzed in batches. The composition of a batch is detailed on a batch sheet. Each batch has the following composition:

- Batch Size - Each batch consists of test samples and additional QC samples.
- Blanks – 5% of the samples within a batch are procedural blanks.
- Duplicates – 5% of the samples within a batch are analyzed in duplicate, provided there is sufficient sample.
- Matrix Spike/Matrix Spike Duplicate analyzed upon client request.
- OPR (Spiked Reference Sample) – 5% of the samples within a batch are spiked reference samples.

QC Specification: Procedural Blank Levels and OPR Recoveries

Analyte		Procedural Blank Level ng/sample [2]	OPR Recovery Range (%)[1]
Perfluorobutanoate	(PFBA)	<0.25	80 – 120 [1]
Perfluoropentanoate	(PFPeA)	<0.25	80 – 120 [1]
Perfluorohexanoate	(PFHxA)	<0.25	80 – 120 [1]
Perfluoroheptanoate	(PFHpA)	<0.25	80 – 120 [1]

Perfluorooctanoate	(PFOA)	<0.25	80 – 120 [1]
Perfluorononanoate	(PFNA)	<0.25	80 – 120 [1]
Perfluorodecanoate	(PFDA)	<0.25	80 – 120 [1]
Perfluoroundecanoate	(PFUnA)	<0.25	80 – 120 [1]
Perfluorododecanoate	(PFDoA)	<0.25	80 – 120 [1]
Perfluorobutanesulfonate	(PFBS)	<0.25	70 - 130
Perfluorohexanesulfonate	(PFHxS)	<0.25	70 – 130
Perfluorooctanesulfonate	(PFOS)	<0.25	70 – 130
Perfluorooctane sulfonamide	(PFOSA)	<0.25	70 – 130

1. Additional criteria– recovery for 2 compounds may be 75-125% and for one compound 70-130%
2. For results reported to higher reporting limits, the blank acceptance limit is equal to the reporting limit. Higher blank may be accepted where sample concentrations exceed blank levels by >x10.

QC Specification: Surrogate Standard Recoveries (Calibration Solutions and Samples)

Surrogate Standard	Recovery Range [2]
$^{13}C_4$-Heptafluorobutyric acid ($^{13}C_4$-PFBA)	20 - 150%
$^{13}C_2$-Perfluorocaproic acid ($^{13}C_2$-PFHxA)	40 - 150%
$^{13}C_2$- Perfluorooctanoic acid ($^{13}C_2$-PFOA)	40 - 150%
$^{13}C_5$-Heptadecafluorononanoic acid ($^{13}C_5$-PFNA)	40 - 150%
$^{13}C_2$- Perfluorodecanoic acid ($^{13}C_2$-PFDA)	40 - 150%
$^{13}C_2$-Perfluoro-n-(1,2)decanoic acid ($^{13}C_2$-PFDoA)	40 - 150%
$^{13}C_4$-Perfluorooctanesulfonate ($^{13}C_4$-PFOS)	40 - 150%
d7-N-Me-Perfluoro-1-octanesulfonamidoethanol (d7-MeFOSE)	40 - 150%

2. Lower recoveries may be accepted based on application and professional judgment

QC Specification Table: Other Parameters

QC Parameter	Specification
Instrument Sensitivity	Daily, S:N ≥ 3:1 for all analytes for lowest calibration standard.
Initial Calibration (native compounds)	Run initially, and as required to maintain calibration verification and instrument sensitivity. (1/x) weighted quadratic, exclude origin. Calculated conc. 75-125 % of actual (lowest cal may be 70-130%), R^2 > 0.990
Continuing Calibration Verification (native compounds)	Run every 20 samples or more frequently, quantify against I-CAL. Calculated conc. 70-130% actual for a maximum of three compounds with the remainder 80 –120 % of actual
Instrumental Carryover and Instrument Background	Every Initial Calibration, Cal/Ver, or SPM: < 0.3 % carryover and area response of analytes in instrument blank < 800 judged following two previous methanol blank injections.
Duplicate Samples or MS/MSD	If conc. > 5 times R.L., RPD < 40% If conc. < 5 times R.L., difference between pairs < R.L.

ANALYSIS BY LC-MS/MS

Analysis of sample extracts for perfluorinated organics by HPLC-MS/MS is performed on a high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer. The MS is run at unit mass resolution in the Multiple Reaction Monitoring (MRM) mode.

Instrument specifications:

Instrument	Waters 2690 or Waters 2795 HPLC, Micromass Quattro Ultima MS/MS
LC Column	Waters Xtera C18MS Reverse Phase C18, 10.0 cm, 2.1 mm i.d., 3.5 µm particle size (or equivalent)
Ionization	Negative Ion Electrospray
Acquisition	MRM mode, unit resolution
Injection Volume	15 µL

LC-MS/MS Operating Conditions:

LC Gradient Program				**General LC Conditions**	
Time (min)	**Flow mixture[1]**	**LC Flow Rate Program**	**Gradient Curve**	Column Temp (°C)	40
0.0	15% solvent A 85% solvent B	0.15 mL/min	1	Max Pressure (bar)	300.0
1.0	15% solvent A 85% solvent B	0.15 mL/min	1	**MS Conditions**	
5.0	70% solvent A 30% solvent B	0.20 mL/min	4	Source Temp (°C)	120
8.5	100% solvent A	0.20 mL/min	4	Desolvation Temp (°C)	300
11	100% solvent A	0.20 mL/min	4	Capillary Voltage	2.75
11.3 - 14.5	15% solvent A 85% solvent B	0.20 mL/min	2	Gases	~70L/hr cone ~300L/hr desolvation

[1] Eluent A = 90% CH_3CN (aqueous), Eluent B = 12.1 mM NH_4OAc in 0.1% AcOH (aqueous)

Initial calibration of the LC-MS/MS instrument is performed by the analysis of six or more calibration solutions. A mid-level calibration standard is analyzed to verify the initial calibration after every 20th sample (including QC samples) injected at a minimum. All calibration solutions go through the same SPE extraction/cleanup procedures as the samples.

ANALYTE IDENTIFICATION

Positive identification of target PFC, surrogate standard and recovery standards require:

- > 3:1 S:N for parent ion to daughter ion transition.
- Compound retention time falls within 0.4 minutes of the predicted retention times from the mean determined from the Initial Calibration. Natives with labelled surrogate standards must elute within 0.1 minutes of the associated labelled surrogates. Typical retention times are shown in Table 9.

QUANTIFICATION AND DATA REPORTING PROCEDURES

Target compounds are quantified using the internal standard method, comparing the area of the quantification ion to that of the ^{13}C-labelled standard and correcting for response factors.

Quadratic calibration equations are determined from a multi-point calibration series with 1/X weighing fit as described by the following general equation:

$Y = a + bX + cX^2$ (general quadratic equation)

Where Y = (area target/area surr) x weight surr
X = weight target
a,b,c are empirical constants

Concentrations in samples are determined as:

$$Sample\ Conc = \frac{-b \pm \sqrt{b^2 - 4c\left(a - \left(\frac{area\ of\ target}{area\ of\ sur}\ x\ weight\ sur\right)\right)}}{2c\ x\ sample\ size}$$

The recovery of the surrogate standard is calculated **(by internal standard quantification against the recovery standard using an average RRF)** and monitored as an indication of overall data quality. Final target concentrations are recovery corrected by this method of quantification.

Sample Specific Detection Limits (SDL) are determined .by converting the area equivalent of 3.0 times the estimated chromatographic noise height to a concentration in the same manner that target peak responses are converted to final concentrations. The SDL accounts for any effect of matrix on the detection system and for recovery achieved through the analytical work-up.

Results are reported to the greater of the SDL or the concentration equivalent to the lowest calibration standard analyzed.

Table 9. Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (minutes)	Parent Ion Mass	Daughter Ion Mass	Quantified Against
Target Analytes				
Perfluorobutanoate (PFBA)	5.0	213	169	$^{13}C_4$-PFBA
Perfluoropentanoate (PFPeA)	5.8	263	219	$^{13}C_2$-PFHxA
Perfluorohexanoate (PFHxA)	6.2	313	269	$^{13}C_2$-PFHxA
Perfluoroheptanoate (PFHpA)	6.6	363	319	$^{13}C_2$-PFHxA
Perfluorooctanoate (PFOA)	7.0	413	369 / 219	$^{13}C_2$-PFOA
Perfluorononanoate (PFNA)	7.4	463	419	$^{13}C_5$-PFNA
Perfluorodecanoate (PFDA)	7.9	513	469	$^{13}C_2$-PFDA
Perfluoroundecanoate (PFUnA)	8.5	563	519	$^{13}C_2$-PFDA
Perfluorododecanoate (PFDoA)	9.0	613	569	$^{13}C_2$-PFDoA
Perfluorobutanesulfonate (PFBS)	6.3	299	80 / 99 [1]	$^{13}C_4$-PFOS
Perfluorohexanesulfonate (PFHxS)	7.2	399	80 / 99 [1]	$^{13}C_4$-PFOS
Perfluorooctane sulfonate (PFOS)	8.2	499	80 / 99 [1]	$^{13}C_4$-PFOS
Perfluorooctane sulfonamide (PFOSA) [2]	9.9	498	78	$^{13}C_4$-PFOS
Surrogate Standard				
$^{13}C_4$-Heptafluorobutyric acid ($^{13}C_4$-PFBA)	5.0	217	172	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluorocaproic acid ($^{13}C_2$-PFHxA)	6.2	315	270	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluorooctanoic acid ($^{13}C_2$-PFOA)	7.0	415	370	$^{13}C_4$-PFOA
$^{13}C_5$-Heptadecafluorononanoic acid ($^{13}C_5$-PFNA)	7.4	470	423	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluorodecanoic acid ($^{13}C_2$-PFDA)	7.9	515	470	$^{13}C_2$-FOUEA
$^{13}C_2$-Perfluoro-n-(1,2)decanoic acid ($^{13}C_2$-PFDoA)	9.0	615	570	$^{13}C_2$-FOUEA
$^{13}C_4$–Perfluorooctanesulfonate ($^{13}C_4$-PFOS)	8.2	503	80 / 99[1]	$^{13}C_2$-FOUEA
d7-N-Me-Perfluoro-1-octanesulfonamidoethanol (d7-Me-FOSE)	~10.6	623	59	$^{13}C_2$-FOUEA
Recovery Standard				
$^{13}C_2$-2H-Perfluoro-2-decenoic acid ($^{13}C_2$-FOUEA)	7.3	459	394	-
$^{13}C_4$-Perfluorooctanoic acid ($^{13}C_4$-PFOA)	6.9	417	372	-

[1] Quantification is based on m/z 80 daughter, m/z 99 may be used as alternate if necessary to avoid interference.

[2] PFOSA quantified against d7-Me-FOSE if collected in separate fraction.

PHARMACEUTICAL AND PERSONAL CARE PRODUCT ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-075

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG30909

Analysis WG30313, WG30338

16 November 2009

PHARMACEUTICAL AND PERSONAL CARE PRODUCT ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-075

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG30909
Analysis WG30313, WG30338

Prepared for:
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16 November 2009

ORSANCO
AQUEOUS SAMPLES

PHARMACEUTICAL AND PERSONAL-CARE PRODUCTS ANALYSIS
AXYS METHOD: MLA-075
4562: L13603-1 to -8

PROJECT NAME: EMERGING CONTAMINANTS IN MAINSTEM OHIO

17 November 2009

NARRATIVE

This narrative describes the analysis of eight aqueous samples for the determination of Pharmaceutical and Personal-Care Products using High Performance Liquid Chromatography coupled with tandem Mass Spectrometry (LC- MS/MS).

SAMPLE RECEIPT AND STORAGE

The samples were received on the 23rd of September 2009. Details of sample conditions upon receipt are provided in the Sample Receiving Record form included in the sample documentation section of this data package. The temperature of the samples upon receipt was between -1 to 6 °C, exceeding the requirement of 4 °C for the samples Site 23 and Site 24 (AXYS ID: L13603-7 and -8, respectively). The analysis for these two samples was allowed to proceed through communicating with the client. The samples were stored at 4 °C prior to sample preparation and analysis.

SAMPLE PREPARATION AND ANALYSIS

Samples and QC samples (a procedural blank and a lab-generated reference sample known as the Ongoing Precision and Recovery (OPR)) were analyzed in two analysis batches named WG30313 and WG30338. Composition of the analysis batches is shown on the Cover Page and Correlation Table, and on the Batch Lists that accompany he extraction workup sheets.

Extraction and analysis procedures were in accordance with AXYS Method MLA-075: ***Analytical Procedure for the Analysis of Pharmaceutical and Personal Care Products in Solid and Aqueous Samples by LC-MS/MS.*** A method summary (MSU-075) for the AXYS Method MLA-075 is included in this data package.

Two aliquots of accurately weighed sub-sample for each sample (approximately 1 liter) were spiked with labeled quantification standards and extracted with acetonitrile using sonication at pH 2 (in analysis batch WG30338) and pH 10 (in analysis batch WG30313), respectively. The resulting extracts were reduced in volume, reconstituted in water and cleaned up on Waters Oasis HLB SPE cartridges. The final extract was reduced in volume and spiked with labeled recovery (internal) standards prior to instrumental analysis. Analysis was performed on Waters 2690 or 2795 HPLC equipped with Micromass Quattro Ultima MS/MS using five instrument and LC conditions as shown in the table below.

Target Group	LC Column	Ionization	Acquisition	LC Conditions
List 1	Waters Xtera C18 (10.0 cm, 2.1 mm i.d., 3.5 µm particle size)	Positive Ion Electrospray	MRM mode, unit resolution	1
List 2	Waters Xtera C18 (10.0 cm, 2.1 mm i.d., 3.5 µm particle size)	Positive Ion Electrospray	MRM mode, unit resolution	2
List 3	Waters Xtera C18 (10.0 cm, 2.1 mm i.d., 3.5 µm particle size)	Negative Ion Electrospray	MRM mode, unit resolution	3
List 4	Waters Atlantis HILIC (10.0 cm, 2.1 mm i.d., 3.0 µm particle size)	Positive Ion Electrospray	MRM mode, unit resolution	4
List 5	Waters Xtera C18 (10.0 cm, 2.1 mm i.d., 3.5 µm particle size)	Positive Ion Electrospray	MRM mode, unit resolution	5

CALCULATION

Target analyte concentrations were determined by isotope dilution or internal standard quantification procedures using MassLynx software. Quantification was conducted by comparing the area of the quantification ion to that of the quantification standard (surrogate) and correcting for response factors.

For all target compounds, linear equations were determined from a multi-point calibration series prepared alongside the samples with 1/X weighting fit. Formulae used in the conversion of the raw chromatograms to concentration are provided in the method summary document.

Sample specific detection limits (SDLs) were calculated for each target analyte and used as the detection qualifier. If the MassLynx software selected an unrepresentative area for the detection limit calculation, the data interpretation chemist or the QA chemist made corrections. These corrections are hand noted on the quantification report pages attached to the chromatograms.

The lower reporting limit for each target compound is defined as the concentration equivalent to the lowest calibration standard analyzed, prorated for the extract volume and sample size, or the SDL, whichever is greater.

REPORTING CONVENTIONS

The AXYS contract number assigned for internal tracking was 4562. The samples were assigned a unique laboratory identifier of the form L13603-X, where X is a numeral. All data reports reference the unique AXYS IDs plus the client sample identifiers.

Any extra work required and performed after the initial instrumental analysis of the sample's extract is given an extra "test suffix" code. The single letter code per extra work performed is added to the AXYS sample ID as a suffix, and is combined with any other applicable test suffix codes. The extra work codes used to report data in this package include:

i	=	instrumental re-analysis performed on the sample extract

The following laboratory qualifier flags were used in this data package:

U	=	identifies a compound that was not detected
V	=	surrogate recovery is not within method/contract control limit

Results are reported in concentration units of nanograms per litre (ng/L). Concentration and reporting limits are provided to three significant figures. Analysis results for each sample are provided on Analysis Report form 1A/2.

QA/QC NOTES

The samples and associated QC samples analyzed in an analysis batch were carried intact through the entire analytical process. The sample data were reviewed and evaluated in relation to the batch QC samples.

- Sample analyte concentrations are not blank corrected. The data should be evaluated with consideration to the procedural blank results.
- By virtue of the isotope dilution/internal standard quantification procedures, data are recovery corrected for possible losses during extraction and cleanup.
- The Signal/Noise (S/N) ratios were measured as '0' for some compounds in the QC samples and sample data. This has been determined to be a limitation of the software and would not affect the data.

- All linearity, calibration verification, OPR and labeled compound recovery specifications were met with the following exceptions:

List 1 Compounds (WG30338)

At least 6 calibration points were used in quantification of the initial calibration (QA9J_185 S: 5 to S: 11) for all the analytes except for Caffeine, Carbadox, Digoxin, Enrofloxacin, Ormetoprim, Penicillin G, Sulfamethizole, Sulfathiazole, Thiabendazole, Virginiamycin and 1,7-Dimethylxanthine which was quantified using 5 calibration points. The lowest level calibration standard CS0 for Virginiamycin was excluded from the initial calibration since it was not detected in the standard. As a result, the CS1 level calibration was used as detection qualifier for these analytes in samples. Given that Virginiamycin in all client samples was not detected, sample data were not impacted by the variance.

Percent recovery of Sulfadimethoxine in the lowest level calibration (CS0) in the initial calibration was slightly above the method limit of 130%. Since the continuing calibration (QA9J_185 S: 14) and the OPR (AXYS ID: WG30338-102) met method criteria data are not considered significantly affected by this variance.

Percent recovery of surrogate $^{13}C_3$-Caffeine in the sample Site 22 (AXYS ID: L13603-6) was above the method upper limit of 140% and the surrogate has been flagged with a 'V' on the report forms. Since the isotope dilution method of quantification produces data that are recovery corrected, the slight variance from the method acceptance criteria are deemed not to affect the quantification of these analytes. Percent surrogate recoveries are used as a general method performance indicator only.

List 2 Compounds (WG30338)

Due to ion suppression that caused significant drop of responses and/or non-linearity, some high-level calibration standards in the initial calibration (filename QB9K_200 S: 9 to S: 15) were excluded. However, a minimum of 6 calibration standard points was used to construct the linear equations for quantification of target analytes or to calculate response factor (RF) for quantification of labeled surrogates. As multi-point calibrations were used, sample data are deemed not to be significantly affected.

List 3 Compounds (WG30338)

At least 6 calibration points were used in quantification of the initial calibration (Data filename: QF9K_202 S: 7 to S: 13) for all the analytes except for Hydrochlorothiazide, which was quantified using 5 calibration points (CS0 to CS4). Since multiple calibrations were used, sample data are deemed not be significantly affected.

List 4 Compounds (WG30313)

Due to the analytical cross-interference between Hydrocodone and Codeine, a correction has been applied to these compounds on the report forms. Details of the Hydrocodone/Codeine correction are provided in this data package.

Due to ion suppression that caused significant drop of responses, the highest level calibration standard (data filename QG9K_196 S: 16) in the initial calibration was excluded for Clonidine, Metformin and Triamterene. However, a minimum of 5 calibration standard points was used to construct the linear equations for quantification of target analytes. Since multiple calibrations were used, sample data were deemed not be significantly affected.

In the initial CS0 calibration standard (data filename QG9K_196 S: 10), responses or signal to noise ratios for Atorvastatin and Clonidine did not meet the method requirements. As a result, CS0 calibration was not included in the initial calibration for the two compounds and CS1 calibration standard concentrations were used as detection qualifier for the two compounds.

Percent recoveries of D_3-Cotinine in samples Site 19 and Site 20 (AXYS ID L13603-3 and -4 respectively), d5-Enalapril in sample Site 21 (AXYS ID L13603-5), D_5-Amphetamine in sample Site 24 (AXYS ID L13603-8), and D_3-Hydrocodone in the OPR (AXYS ID WG30313-102) were below the method nominal lower control limits. Percent recoveries of D_4-Clonidine and D_7-Atenolol in sample Site 24 were above the method nominal upper control limits. These labeled surrogates are flagged with a 'V' on the report form. Since the isotope dilution method of quantification produces data that are recovery corrected, the variances from the method acceptance criteria are deemed not to affect the quantification of the analytes. This is also indicated by the fact that the percent recovery of target analyte Hydrocodone in the OPR was within the method acceptance criteria though the recovery of its labeled surrogate D_3-Hydrocodone was below the method lower control limits. Percent surrogate recoveries are used as general method performance indicator only.

List 5 Compounds (WG30338)

At least 6 calibration points were used in quantification of the initial calibration (QE9J_201 S: 4 to S: 10) for all the analytes. Since multiple calibrations were used, sample data were deemed not be significantly affected.

ANALYTICAL DISCUSSION

List 1, 2, 3 Compounds (WG30338)

No analytical difficulties were encountered.

List 4 Compounds (WG30313)

The sample Site 22 required 2 SPE cartridges eluted with 6 mL methanol and 9 mL 2 % formic acid, extracts were combined, concentrated to 4 mL, and then spiked with recovery standard. Since the percent recovery of the surrogates in the sample met method criteria, data are not considered to be significantly affected by this variance.

List 5 Compounds (WG30338)

The initial instrumental analysis results of the samples and QC samples did not meet all method specifications. These sample extracts were instrumentally re-analyzed and method specifications were met. Sample concentrations are reported from the re-injection data (indicated by suffix '**i**' on AXYS IDs).

DATA PACKAGE

This data package has been assigned a unique identifier, DPWG30909, shown on the cover page. Included in this data package following the narrative is the following documentation:

- Method summary (MSU-075)
- Hydrocodone/Codeine correction summary
- Narrative Appendix
- Sample 'Cover Page' and 'Correlation Table'
- Sample Receiving Documentation
- Laboratory extraction worksheets
- Sample data reports (in order of AXYS Sample ID)
- Laboratory QC data reports
- Instrument run (injection) log
- Instrumental QC data reports (organized by analysis date)
- Sample raw data (in order of AXYS Sample ID)

- Laboratory QC raw data
- Instrumental QC raw data (organized by analysis date)

I certify that this data package is in compliance with the terms and conditions of the contract, both technically and for completeness, except for the conditions detailed above. In addition, I certify, that to the best of my knowledge and belief, the data as reported are true and accurate. The following signature, on behalf of AXYS Analytical Services Ltd, authorizes the release of the data contained in this data package.

Signed: Bryan Alonzo, B.Sc., QA/QC Chemist

17-Nov-09
Date Signed

AXYS Analytical Services Ltd.

SUMMARY OF AXYS METHOD MLA-075:

ANALYTICAL PROCEDURES FOR THE ANALYSIS OF PHARMACEUTICAL AND PERSONAL CARE COMPOUNDS IN SOLID AND AQUEOUS SAMPLES BY LC-MS/MS

ANALYTE LISTS

List 1 (Acid extraction, positive ESI)	
Acetaminophen	Norfloxacin
Ampicillin [1]	Norgestimate
Azithromycin	Ofloxacin
Caffeine	Ormetoprim
Carbadox	Oxacillin
Carbamazepine	Oxolinic acid
Cefotaxime	Penicillin G
Ciprofloxacin	Penicillin V
Clarithromycin	Roxithromycin
Clinafloxacin	Sarafloxacin
Cloxacillin	Sulfachloropyridazine
Dehydronifedipine	Sulfadiazine
Digoxigenin	Sulfadimethoxine
Digoxin	Sulfamerazine
Diltiazem	Sulfamethazine
1,7-Dimethylxanthine	Sulfamethizole
Diphenhydramine	Sulfamethoxazole
Enrofloxacin	Sulfanilamide
Erythromycin	Sulfathiazole
Flumequine	Thiabendazole
Fluoxetine	Trimethoprim
Lincomycin	Tylosin
Lomefloxacin	Virginiamycin
Miconazole	
List 2 (Tetracyclines, positive ESI)	
Anhydrochlortetracycline (ACTC)	4-Epichlortetracycline (ECTC)
Anhydrotetracycline (ATC)	4-Epioxytetracycline (EOTC)
Chlortetracycline (CTC)	4-Epitetracycline (ETC)
Demeclocycline	Isochlortetracycline (ICTC)
Doxycycline	Minocycline
4-Epianhydrochlortetracycline (EACTC)	Oxytetracycline (OTC)
4-Epianhydrotetracycline (EATC)	Tetracycline (TC)

List 3 (Acid extraction, negative ESI)	
Bisphenol A	2-hydroxy-ibuprofen
Furosemide	Ibuprofen
Gemfibrozil	Naproxen
Glipizide	Triclocarban
Glyburide	Triclosan
Hydrochlorothiazide	Warfarin
List 4 (Base extraction, positive ESI)	
Albuterol	Cotinine
Amphetamine	Enalapril
Atenolol	Hydrocodone
Atorvastatin	Metformin
Cimetidine	Oxycodone
Clonidine	Ranitidine
Codeine	Triamterene
List 5 (Acid Extraction, positive ESI)	
Alprazolam	Metoprolol
Amitriptyline	Norfluoxetine
Amlodipine	Norverapamil
Benzoylecgonine	Paroxetine
Benztropine	Prednisolone
Betamethasone	Prednisone
Cocaine	Promethazine
DEET (N,N-diethyl-m-toluamide)	Propoxyphene
Desmethyldiltiazem	Propranolol
Diazepam	Sertraline
Fluocinonide	Simvastatin
Fluticasone propionate	Theophylline
Hydrocortisone	Trenbolone
10-hydroxy-amitriptyline	Trenbolone acetate
Meprobamate	Valsartan
Methylprednisolone	Verapamil

[1] Due to instability accuracy of Ampicillin data is unknown.

EXTRACTION AND CLEANUP PROCEDURES

The analysis requires extraction at two different pH conditions: At pH 10 for analysis of fourteen analytes (List 4); and at pH 2.0 for the analysis of the other analytes (Lists 1, 2, 3, and 5). Prior to extraction and/or clean-up samples are adjusted to the required pH and spiked with surrogates.

Solid samples are repeatedly extracted by sonication with aqueous buffered acetonitrile and pure acetonitrile, concentrated by rotary evaporation, and diluted with ultra pure water to 200 mL. The acidic extract is treated with EDTA. The extracts are cleaned up by solid phase extraction (SPE), filtered, and analyzed by LC/ESI-MS/MS in positive and negative ionization modes requiring a total of five runs to analyze the complete list of analytes.

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All aqueous samples are filtered and the aqueous portion is cleaned up by solid phase extraction before analysis by LC/ESI-MS/MS.

Aqueous samples with no or limited visible particulate (e.g. surface water, ground water, wastewater treatment final effluent, typically with < 100 mg/L TSS) normally can be processed with up to 1L samples sizes. The sample is filtered and routinely only the aqueous phase is analyzed. However, upon specific agreement a separate extraction may be performed on the solids phase. The solids extract may in this case either be carried through the analysis individually as a separate sample that is reported separately, or the aqueous extract and the solids extract may be combined just prior to clean-up and reported as a combined aqueous/solids phase result.

For mixed phase aqueous/solids samples with significant solids and distinct aqueous and solids phases such as wastewater influent or process streams the sample may either be analyzed as an aqueous phase only or as two separate samples, one aqueous and one solid.

ANALYSIS BY LC-MS/MS

The analysis is performed on a high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer. Instrument calibration is performed using a series of calibration solutions (7 points) covering the working concentration range of the instrument specific for the individual compounds of interest. The LC/MS/MS is run in MRM (Multiple Reaction Monitoring) mode and quantification is performed by recording the peak areas of the applicable parent ion/daughter ion transitions. Some analytes are analyzed in the ESI positive mode and some are analyzed in the ESI negative mode.

List 1 – Acid Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Sulfanilamide	2.5	190.0	155.8	$^{13}C_6$-Sulfamethazine
Acetaminophen	4.6	152.2	110.0	$^{13}C_2$-^{15}N-Acetaminophen
Sulfadiazine	6.0	251.2	156.1	$^{13}C_6$-Sulfamethazine
1,7-Dimethylxanthine	6.9	181.2	124.0	$^{13}C_3$-Caffeine
Sulfathiazole	7.7	256.3	156.0	$^{13}C_6$-Sulfamethoxazole
Sulfamerazine	8.7	265.0	156.0	$^{13}C_6$-Sulfamethazine
Caffeine	9.3	195.0	138.0	$^{13}C_3$-Caffeine
Lincomycin	9.3	407.5	126.0	$^{13}C_3$-Trimethoprim
Sulfamethizole	10.0	271.0	156.0	$^{13}C_6$-Sulfamethoxazole
Thiabendazole	10.0	202.1	175.1	d_6-Thiabendazole
Trimethoprim	10.0	291.0	230.0	$^{13}C_3$-Trimethoprim
Sulfamethazine	10.1	279.0	156.0	$^{13}C_6$-Sulfamethazine
Cefotaxime	10.2	456.4	396.1	$^{13}C_3$-Trimethoprim
Carbadox	10.5	263.2	231.2	$^{13}C_3$-Trimethoprim
Ormetoprim	10.5	275.3	259.1	$^{13}C_3$-Trimethoprim
Norfloxacin	10.7	320.0	302.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Ofloxacin	10.8	362.2	318.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sulfachloropyridazine	10.8	285.0	156.0	$^{13}C_6$-Sulfamethazine
Ciprofloxacin	10.9	332.2	314.2	$^{13}C_3$, ^{15}N-Ciprofloxacin
Lomefloxacin	11.2	352.2	308.1	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sulfamethoxazole	11.2	254.0	156.0	$^{13}C_6$-Sulfamethoxazole

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Enrofloxacin	11.5	360.0	316.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sarafloxacin	11.9	386.0	299.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Clinafloxacin	12.1	366.3	348.1	$^{13}C_3$, ^{15}N-Ciprofloxacin
Digoxigenin	12.6	391.2	355.2	$^{13}C_3$-Trimethoprim
Oxolinic Acid	13.1	261.8	243.8	$^{13}C_3$-Trimethoprim
Sulfadimethoxine	13.2	311.0	156.0	$^{13}C_6$-Sulfamethoxazole
Diphenhydramine	14.5	256.8	168.1	$^{13}C_3$-Trimethoprim
Penicillin G	14.6	367.5	160.2	$^{13}C_3$-Trimethoprim
Azithromycin	14.8	749.9	591.6	$^{13}C_3$-Trimethoprim
Flumequine	15.2	262.0	173.7	$^{13}C_3$-Trimethoprim
Ampicillin	15.3	350.3	160.2	$^{13}C_3$-Trimethoprim
Carbamazepine	15.3	237.4	194.2	$^{13}C_3$-Trimethoprim
Diltiazem	15.3	415.5	178.0	$^{13}C_3$-Trimethoprim
Penicillin V	15.4	383.4	160.2	$^{13}C_3$-Trimethoprim
Erythromycin [1]	15.9	734.4	158	not quantified
Tylosin	16.3	916.0	772.0	$^{13}C_6$-Sulfamethazine
Oxacillin	16.4	434.3	160.1	$^{13}C_3$-Trimethoprim
Dehydronifedipine	16.5	345.5	284.1	$^{13}C_3$-Trimethoprim
Digoxin	16.6	803.1	283.0	$^{13}C_3$-Trimethoprim
Cloxacillin	16.9	469.1	160.1	$^{13}C_3$-Trimethoprim
Fluoxetine	16.9	310.3	148.0	d_5-Fluoxetine
Virginiamycin	17.3	508.0	355.0	$^{13}C_3$-Trimethoprim
Clarithromycin	17.5	748.9	158.2	$^{13}C_6$-Sulfamethazine
Erythromycin - H_2O [1]	17.7	716.4	158	$^{13}C_2$-Erythromycin - H_2O
Roxithromycin	17.8	837.0	679.0	$^{13}C_6$-Sulfamethazine
Miconazole	20.1	417.0	161.0	$^{13}C_3$-Trimethoprim
Norgestimate	21.7	370.5	124.0	$^{13}C_3$-Trimethoprim
Surrogate Standard				
$^{13}C_2$-^{15}N-Acetaminophen	4.5	155.2	111.0	$^{13}C_3$-Atrazine
$^{13}C_3$-Caffeine	9.3	198.0	140.0	$^{13}C_3$-Atrazine
d_6-Thiabendazole	9.8	208.1	180.1	$^{13}C_3$-Atrazine
$^{13}C_3$-Trimethoprim	10.0	294.0	233.0	$^{13}C_3$-Atrazine
$^{13}C_6$-Sulfamethazine	10.1	285.1	162.1	$^{13}C_3$-Atrazine
$^{13}C_3$, ^{15}N-Ciprofloxacin	10.9	336.1	318.2	$^{13}C_3$-Atrazine
$^{13}C_6$-Sulfamethoxazole	11.2	260.0	162.0	$^{13}C_3$-Atrazine
$^{13}C_2$-Erythromycin [1]	15.9	736.4	160.0	monitor for less than 5%
d_5-Fluoxetine	16.8	315.3	153.0	$^{13}C_3$-Atrazine
$^{13}C_2$-Erythromycin - H_2O [1]	17.7	718.4	160.0	$^{13}C_3$-Atrazine
Recovery Standard				
$^{13}C_3$-Atrazine	15.9	219.5	176.9 (134.0)	External Standard

[1] Because of intramolecular dehydration during the analytical procedure erythromycin is quantified as the dehydration product “erythromycin – H_2O” [5]. The peak area of the $^{13}C_2$-Erythromycin is monitored and must be less than 5% of the $^{13}C_2$-Erythromycin - H_2O peak area. If it is greater, the Erythromycin - H_2O result is flagged as ‘accuracy unknown’.

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List 2 – Acid Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Minocycline	5.1	458.0	441.0	d_6-Thiabendazole
Epitetracycline (ETC)	8.1	445.2	410.2	d_6-Thiabendazole
Epioxytetracycline (EOTC)	8.6	461.2	426.2	d_6-Thiabendazole
Oxytetracycline (OTC)	9.4	461.2	426.2	d_6-Thiabendazole
Tetracycline (TC)	9.9	445.2	410.2	d_6-Thiabendazole
Demeclocycline	11.7	465.0	430.0	d_6-Thiabendazole
Isochlortetracycline (ICTC) [1]	11.9	479.0	462.0	d_6-Thiabendazole
Epichlortetracycline (ECTC)	12.0	479.0	444.0	d_6-Thiabendazole
Chlortetracycline (CTC)	14.1	479.0	444.0	d_6-Thiabendazole
Doxycycline	16.7	445.2	428.2	d_6-Thiabendazole
Epianhydrotetracycline (EATC)	17.0	426.8	409.8	d_6-Thiabendazole
Anhydrotetracycline (ATC)	18.8	426.8	409.8	d_6-Thiabendazole
Epianhydrochlortetracycline (EACTC)	20.7	461.2	444.0	d_6-Thiabendazole
Anhydrochlortetracycline (ACTC)	22.1	461.2	444.0	d_6-Thiabendazole
Surrogate Standard				
d_6-Thiabendazole	7.1	208.1	180.1	$^{13}C_3$-Atrazine
Recovery Standard				
$^{13}C_3$-Atrazine	21.2	219.5	176.9	External Standard

[1] Isochlortetracycline (ICTC) is reported as the sum ICTC + ECTC due to a common transition ion.

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List 3 – Acid Extraction, Negative Electrospray Ionization (-)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Hydrochlorathiazide	2.3	296.0	268.0	^{13}C-d_3-Naproxen
Hydrochlorathiazide*	2.3	296.0	204.8	^{13}C-d_3-Naproxen
Furosemide	3.4	329.0	284.8	^{13}C-d_3-Naproxen
Furosemide*	3.4	329.0	204.7	^{13}C-d_3-Naproxen
2-hydroxy-ibuprofen	4.2	221.1	176.8	$^{13}C_3$-Ibuprofen
Bisphenol A	6.5	227.0	211.9	d6-Bisphenol A
Bisphenol A*	6.5	227.0	132.9	d6-Bisphenol A
Glipizide	6.9	444.2	319.0	d11-Glipizide
Glipizide*	6.9	444.2	169.8	d11-Glipizide
Naproxen	7.0	228.9	168.6	^{13}C-d_3-Naproxen
Warfarin	7.4	307.0	161.0	d_5-Warfarin
Glyburide	8.8	492.1	169.8	d3-Glyburide
Glyburide*	8.8	492.1	367.0	d3-Glyburide
Ibuprofen	8.8	205.1	161.1	$^{13}C_3$-Ibuprofen
Gemfibrozil	9.9	249.0	121.0	d_6-Gemfibrozil
Triclocarban	10.1	312.9	159.7	$^{13}C_6$-Triclocarban
Triclosan	10.2	286.8	35.0	$^{13}C_{12}$-Triclosan
Surrogate Standard				
d_6-Bisphenol A	6.5	233.0	214.8	$^{13}C_6$-2,4,5-T
d_6-Bisphenol A*	6.5	233.0	137.8	$^{13}C_6$-2,4,5-T
d_{11}-Glipizide	6.8	455.0	319.0	$^{13}C_6$-2,4,5-T
d_{11}-Glipizide*	6.8	455.0	169.8	$^{13}C_6$-2,4,5-T
^{13}C-d_3-Naproxen	7.0	232.9	168.6	$^{13}C_6$-2,4,5-T
d_5-Warfarin	7.4	312	161.0	$^{13}C_6$-2,4,5-T
d_3-Glyburide	8.7	495.0	169.9	$^{13}C_6$-2,4,5-T
d_3-Glyburide*	8.7	495.0	370.1	$^{13}C_6$-2,4,5-T
$^{13}C_3$-Ibuprofen	8.8	208.2	163.1	$^{13}C_6$-2,4,5-T
d_6-Gemfibrozil	9.9	255	121	$^{13}C_6$-2,4,5-T
$^{13}C_6$-Triclocarban	10.1	318.9	159.7	$^{13}C_6$-2,4,5-T
$^{13}C_{12}$-Triclosan	10.2	298.8	35	$^{13}C_6$-2,4,5-T
Recovery Standard				
$^{13}C_6$-2,4,5-Trichlorophen-oxyacetic acid ($^{13}C_6$-2,4,5-T)	4.9	258.8	200.7	External Standard

* Indicates secondary transition for possible diagnostic use.

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List 4 – Base Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Cotinine	4.0	177.0	98.0	d3-Cotinine
Cimetidine	4.7	253.1	159.0	d3-Cimetidine
Triamterene	5.4	254.1	236.9	d4-Clonidine
Triamterene*	5.4	254.1	103.7	d4-Clonidine
Enalapril	6.5	377.2	233.9	d5-Enalapril
Enalapril*	6.5	377.2	159.8	d5-Enalapril
Oxycodone	6.7	316.2	240.9	d6-Oxycodone
Oxycodone*	6.7	316.2	298.0	d6-Oxycodone
Clonidine	6.8	230.0	212.5	d4-Clonidine
Clonidine*	6.8	230.0	43.9	d4-Clonidine
Amphetamine	8.1	136.1	90.8	d5-Amphetamine
Amphetamine*	8.1	136.1	118.9	d5-Amphetamine
Albuterol	8.3	240.0	148.0	d_3-Albuterol
Codeine	8.4	300.0	152.0	d6-Codeine
Hydrocodone	8.6	300.2	198.8	d3-Hydrocodone
Hydrocodone*	8.6	300.2	170.6	d3-Hydrocodone
Atorvastatin	8.9	559.3	440.0	d5-Enalapril
Atorvastatin*	8.9	559.3	466.0	d5-Enalapril
Atenolol	9.0	267.2	144.7	d7-Atenolol
Atenolol*	9.0	267.2	189.7	d7-Atenolol
Metformin	9.5	131.1	60.1	d_6-Metformin
Ranitidine	18.8	315.0	175.9	d_3-Albuterol
Surrogate Standards				
d_3-Cotinine	4.0	180.0	79.9	d3-Amitriptyline
d_3-Cotinine*	4.0	180.0	101.0	d3-Amitriptyline
d_3-Cimetidine	4.7	256.0	161.8	d3-Amitriptyline
d_3-Cimetidine*	4.7	256.0	94.8	d3-Amitriptyline
d_5-Enalapril	6.5	382.0	238.8	d3-Amitriptyline
d_5-Enalapril*	6.5	382.0	164.8	d3-Amitriptyline
d_6-Oxycodone	6.7	322.1	262.0	d3-Amitriptyline
d_6-Oxycodone*	6.7	322.1	304.1	d3-Amitriptyline
d_4-Clonidine	6.8	234.0	216.7	d3-Amitriptyline
d_4-Clonidine*	6.8	234.0	47.9	d3-Amitriptyline
d_5-Amphetamine	8.1	141.1	92.9	d3-Amitriptyline
d_5-Amphetamine*	8.1	141.1	123.9	d3-Amitriptyline
d_3-Albuterol	8.3	243.0	151.0	d3-Amitriptyline
d_6-Codeine	8.4	306.0	151.8	d3-Amitriptyline
d_6-Codeine*	8.4	306.0	217.9	d3-Amitriptyline
d_3-Hydrocodone	8.6	303.1	198.9	d3-Amitriptyline
d_3-Hydrocodone*	8.6	303.1	170.8	d3-Amitriptyline
d_7-Atenolol	9.0	274.0	144.7	d3-Amitriptyline
d_7-Atenolol*	9.0	274.0	189.7	d3-Amitriptyline
d_6-Metformin	9.5	137.1	60.1	d3-Amitriptyline
Recovery Standards				

d_3-Amitriptyline	7.9	281.0	232.7	External Standard
d_3-Amitriptyline*	7.9	281.0	90.7	External Standard

* Indicates secondary transition for possible diagnostic use.

List 5 – Acid Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention	Parent Ion Mass	Daughter Ion Mass	Quantified against
Theophylline	2.7	181.1	123.8	13C-15N2-Theophylline
Theophylline*	2.7	181.1	95.8	13C-15N2-Theophylline
Benzoylecgonine	5.7	290.1	167.8	d8-Benzoylecgonine
Benzoylecgonine*	5.7	290.1	104.8	d8-Benzoylecgonine
Metoprolol	8.4	268.2	190.7	d7-Metoprolol
Metoprolol*	8.4	268.2	115.7	d7-Metoprolol
Cocaine	9.2	304.1	181.8	d3-Cocaine
Cocaine*	9.2	304.1	81.9	d3-Cocaine
Meprobamate	11.1	219.0	157.8	d7-Metoprolol
Meprobamate*	11.1	219.0	96.9	d7-Metoprolol
10-hydroxy-amitriptyline	12.0	294.2	215.0	d7-Propranolol
10-hydroxy-amitriptyline*	12.0	294.2	276.0	d7-Propranolol
Propranolol	14.6	260.2	115.8	d7-Propranolol
Propranolol*	14.6	260.2	182.7	d7-Propranolol
Prednisone	16.6	359.2	341.0	d7-Propranolol
Prednisone*	16.6	359.2	146.7	d7-Propranolol
Prednisolone	17.5	361.2	343.0	d7-Propranolol
Prednisolone*	17.5	361.2	324.7	d7-Propranolol
Hydrocortisone	17.6	363.2	120.7	d4-Hydrocortisone
Hydrocortisone*	17.6	363.2	326.7	d4-Hydrocortisone
Desmethyldiltiazem	18.8	401.2	177.8	d4-Promethazine
Desmethyldiltiazem*	18.8	401.2	149.5	d4-Promethazine
Promethazine	18.8	285.1	197.8	d4-Promethazine
Promethazine*	18.8	285.1	85.7	d4-Promethazine
DEET	20.6	192.0	118.6	d7-DEET
DEET	20.6	192.0	90.7	d7-DEET
Paroxetine	20.6	330.2	191.8	d6-Paroxetine
Paroxetine*	20.6	330.2	69.8	d6-Paroxetine
Norverapamil	21.0	441.3	164.7	d7-Propranolol
Norverapamil*	21.0	441.3	149.7	d7-Propranolol
Methylprednisolone	21.4	375.2	357.0	d2-Methylprednisolone
Methylprednisolone*	21.4	375.2	339.0	d2-Methylprednisolone
Verapamil	21.4	455.3	164.8	d6-Amitriptyline
Verapamil*	21.4	455.3	149.8	d6-Amitriptyline
Betamethasone	21.7	393.2	355.1	d6-Amitriptyline
Betamethasone*	21.7	393.2	373.0	d6-Amitriptyline
Propoxyphene	21.8	340.2	57.9	d5-Propoxyphene
Propoxyphene*	21.8	340.2	266.1	d5-Propoxyphene
Amitriptyline	22.4	278.2	232.8	d6-Amitriptyline
Amitriptyline*	22.4	278.2	90.7	d6-Amitriptyline
Trenbolone	22.5	271.2	198.7	d5-Alprazolam

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Trenbolone*	22.5	271.2	252.8	d5-Alprazolam
Benztropine	22.9	308.2	166.7	d3-Benztropine
Benztropine*	22.9	308.2	151.7	d3-Benztropine
Alprazolam	23.3	309.1	280.9	d5-Alprazolam
Alprazolam*	23.3	309.1	204.9	d5-Alprazolam
Amlodipine	23.8	409.1	237.8	d5-Norfluoxetine
Amlodipine*	23.8	409.1	293.8	d5-Norfluoxetine
Norfluoxetine	24.7	296.1	133.7	d5-Norfluoxetine
Sertraline	26.4	306.1	274.8	d7-Propranolol
Sertraline*	26.4	306.1	158.7	d7-Propranolol
Diazepam	29.1	285.1	192.8	d5-Diazepam
Diazepam*	29.1	285.1	153.8	d5-Diazepam
Valsartan	31.8	436.2	235.0	d5-Propoxyphene
Valsartan*	31.8	436.2	291.0	d5-Propoxyphene
Fluocinonide	34.8	495.2	337.0	d5-Alprazolam
Fluocinonide*	34.8	495.2	475.0	d5-Alprazolam
Trenbolone acetate	37.8	313.2	253.0	d5-Alprazolam
Trenbolone acetate*	37.8	313.2	271.0	d5-Alprazolam
Fluticasone propionate	37.9	501.2	293.0	d7-Metoprolol
Fluticasone propionate*	37.9	501.2	313.0	d7-Metoprolol
Simvastatin	40.1	419.3	285.0	d5-Propoxyphene
Simvastatin*	40.1	419.3	198.9	d5-Propoxyphene
Surrogate Standards				
^{13}C, $^{15}N_2$-Theophylline	2.7	184.0	124.7	$^{13}C_3$-Atrazine
^{13}C, $^{15}N_2$-Theophylline*	2.7	184.0	96.8	$^{13}C_3$-Atrazine
d_8-Benzoylecgonine	5.6	298.1	170.9	$^{13}C_3$-Atrazine
d_8-Benzoylecgonine*	5.6	298.1	109.8	$^{13}C_3$-Atrazine
d_7-Metoprolol	8.3	275.0	190.8	$^{13}C_3$-Atrazine
d_7-Metoprolol*	8.3	275.0	122.7	$^{13}C_3$-Atrazine
d_3-Cocaine	9.2	307.1	184.9	$^{13}C_3$-Atrazine
d_3-Cocaine*	9.2	30.7.1	84.8	$^{13}C_3$-Atrazine
d_7-Propranolol	14.4	267.0	116.0	$^{13}C_3$-Atrazine
d_7-Propranolol*	14.4	267.0	188.7	$^{13}C_3$-Atrazine
d_4-Hydrocortisone	17.6	367.0	120.8	$^{13}C_3$-Atrazine
d_4-Hydrocortisone*	17.6	367.0	331.0	$^{13}C_3$-Atrazine
d_4-Promethazine	18.6	289.0	201.8	$^{13}C_3$-Atrazine
d_4-Promethazine*	18.6	289.0	86.0	$^{13}C_3$-Atrazine
d_7-DEET	20.6	199.1	125.8	$^{13}C_3$-Atrazine
d_7-DEET*	20.6	199.1	97.8	$^{13}C_3$-Atrazine
d_6-Paroxetine	20.6	336.0	197.8	$^{13}C_3$-Atrazine
d_6-Paroxetine*	20.6	336.0	75.8	$^{13}C_3$-Atrazine
d_2-Methylprednisolone	21.4	377.0	359.0	$^{13}C_3$-Atrazine
d_2-Methylprednisolone*	21.4	377.0	341.0	$^{13}C_3$-Atrazine
d_5-Propoxyphene	21.8	245.2	266.1	$^{13}C_3$-Atrazine
d_5-Propoxyphene*	21.8	345.2	57.9	$^{13}C_3$-Atrazine
d_6-Amitriptyline	22.4	284.0	233.0	$^{13}C_3$-Atrazine
d_6-Amitriptyline*	22.4	284.0	90.8	$^{13}C_3$-Atrazine
d_3-Benztropine	22.9	311.0	166.7	$^{13}C_3$-Atrazine
d_3-Benztropine*	22.9	311.0	151.7	$^{13}C_3$-Atrazine
d_5-Alprazolam	23.1	314.1	285.9	$^{13}C_3$-Atrazine
d_5-Alprazolam*	23.1	314.1	209.9	$^{13}C_3$-Atrazine

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d_5-Norfluoxetine	24.7	301.0	138.7	$^{13}C_3$-Atrazine
d_5-Diazepam	29.1	290.1	197.9	$^{13}C_3$-Atrazine
d_5-Diazepam*	29.1	290.1	153.8	$^{13}C_3$-Atrazine
Recovery Standards				
$^{13}C_3$-Atrazine	18.8	219.5	176.9	External Standard
$^{13}C_3$-Atrazine *	18.8	219.5	134.0	External Standard

* Indicates secondary transition for possible diagnostic use.

QUALITY ACCEPTANCE CRITERIA

QC Acceptance Limits

	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
List 1 Compounds (APOS)						
Acetaminophen	70	140	70	140	30	≤60
Ampicillin [2]						
Azithromycin	10	130	10	130	130	≤1.5
Caffeine	25	160	35	150	60	≤15
Carbadox	25	180	35	180	40	≤1.5
Carbamazepine	25	200	35	200	40	≤1.5
Cefotaxime	10	300	10	300	60	≤6
Ciprofloxacin	25	180	35	180	40	≤6
Clarithromycin	50	160	50	160	30	≤1.5
Clinafloxacin	25	300	35	300	70	≤6
Cloxacillin	35	160	40	150	50	≤3
Dehydronifedipine	35	160	40	160	30	≤0.6
Digoxigenin	50	150	60	140	30	≤6
Digoxin	10	300	10	300	30	≤15
Diltiazem	20	160	25	160	50	≤0.3
1,7-Dimethylxanthine	30	300	40	300	60	≤150
Diphenhydramine	30	200	35	180	50	≤0.6
Enrofloxacin	30	220	40	220	40	≤3
Erythromycin - H_2O	70	130	70	130	30	≤0.3
Flumequine	40	160	50	160	30	≤1.5
Fluoxetine	60	150	70	140	30	≤1.5
Lincomycin	10	300	10	300	70	≤3
Lomefloxacin	50	250	60	250	30	≤3
Miconazole	35	130	40	130	30	≤1.5
Norfloxacin	10	250	25	220	40	≤15
Norgestimate	35	130	40	130	30	≤3
Ofloxacin	60	250	70	250	30	≤1.5
Ormetoprim	70	150	70	150	30	≤0.6
Oxacillin	20	130	20	130	40	≤3
Oxolinic Acid	60	150	70	150	30	≤0.6
Penicillin G	10	130	10	130	40	≤3
Penicillin V	40	140	50	140	30	≤3
Roxithromycin	50	140	50	140	30	≤0.3

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Sarafloxacin	50	200	60	180	30	≤15
Sulfachloropyridazine	60	160	70	160	30	≤1.5
Sulfadiazine	70	130	70	130	30	≤1.5
Sulfadimethoxine	35	160	40	160	30	≤0.3
Sulfamerazine	60	140	60	140	30	≤0.6
Sulfamethazine	70	130	70	130	30	≤0.6
Sulfamethizole	30	140	35	140	30	≤0.6
Sulfamethoxazole	70	130	70	130	30	≤0.6
Sulfanilamide	2	160	3	150	150	≤15
Sulfathiazole	30	180	30	160	50	≤1.5
Thiabendazole	60	150	60	150	30	≤1.5
Trimethoprim	50	150	60	150	30	≤1.5
Tylosin	10	180	20	180	40	≤6
Virginiamycin	15	300	15	250	90	≤3
Surrogate Standard						
$^{13}C_2$, ^{15}N-Acetaminophen	30	160	40	150	30	
$^{13}C_3$-Caffeine	40	140	50	140	30	
d_{10}-Carbamazepine-10,11-epoxide						
$^{13}C_3$, ^{15}N-Ciprofloxacin	7	150	9	140	70	
$^{13}C_2$-Erythromycin - H_2O	35	130	35	130	30	
d_5-Fluoxetine	40	130	50	130	30	
$^{13}C_6$-Sulfamethazine	30	160	35	150	40	
$^{13}C_6$-Sulfamethoxazole	30	140	40	130	30	
d_6-Thiabendazole	25	180	30	160	50	
$^{13}C_3$-Trimethoprim	30	140	40	130	30	
Recovery Standard						
$^{13}C_3$-Atrazine						
List 2 Compounds (TCYS)						
Anhydrochlortetracycline (ACTC)	15	200	20	180	70	≤15
Anhydrotetracycline (ATC)	20	160	20	150	50	≤15
Chlortetracycline (CTC)	30	250	35	250	60	≤6
Demeclocycline	35	180	35	160	50	≤15
Doxycycline	35	180	40	180	40	≤6
Epianhydrochlortetracycline (EACTC)	6	130	7	130	70	≤60
Epianhydrotetracycline (EATC)	15	200	20	200	60	≤15
Epichlortetracycline (ECTC)	25	180	30	160	50	≤15
Epioxytetracycline (EOTC)	25	180	35	160	40	≤6
Epitetracycline (ETC)	35	200	40	180	40	≤6
Isochlortetracycline (ICTC)	25	180	35	160	40	≤6
Minocycline	1	250	2	200	110	≤60
Oxytetracycline (OTC)	20	200	30	200	40	≤6
Tetracycline (TC)	20	200	30	180	40	≤6
Surrogate Standard						
d_6-Thiabendazole	25	140	25	130	50	

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Recovery Standard						
$^{13}C_3$-Atrazine						
	OPR Recovery and surrogate recovery in sample		**IPR**			**Blank Level (ng)**
			Average Recovery (%)		**RSD (%)**	
	Low	**High**	**Low**	**High**		
List 3 Compounds (ANEG)						
Bisphenol A	70	130	70	130	30	≤2500
Furosemide	65	130	70	130	30	≤40
Gemfibrozil	60	140	70	130	30	≤1.5
Glipizide	55	170	60	160	30	≤6
Glyburide	50	180	55	170	30	≤3
Hydroxychlorothiazide	70	200	70	180	30	≤20
2-hydroxy-ibuprofen	70	130	70	130	30	≤80
Ibuprofen	70	130	70	130	30	≤15
Naproxen	50	150	60	150	30	≤3
Triclocarban	60	140	70	130	30	≤3
Triclosan	70	130	70	130	30	≤60
Warfarin	70	140	70	140	30	≤1.5
Surrogate Standards						
d_6-Bisphenol A	50	170	60	160	30	
d_6-Gemfibrozil	50	150	55	140	30	
d_3-Glyburide	20	160	25	150	40	
d_{11}-Glipizide	30	180	35	170	50	
$^{13}C_3$-Ibuprofen	50	140	55	140	30	
^{13}C-d_3-Naproxen	30	150	35	140	30	
$^{13}C_6$-Triclocarban	20	160	25	150	50	
$^{13}C_{12}$-Triclosan	20	160	30	150	40	
d_5-Warfarin	35	250	50	250	30	
Recovery Standard						
$^{13}C_6$-2,4,5-Trichloro-phenoxyacetic acid						
List 4 Compounds (BPOS)						
Albuterol	50	160	50	160	30	≤0.3
Amphetamine	50	160	60	150	30	≤1.5
Atenolol	70	130	70	130	30	≤0.6
Atorvastatin	20	130	25	130	40	≤1.5
Cimetidine	15	130	20	130	50	≤0.6
Clonidine	70	130	70	130	30	≤1.5
Codeine	70	130	70	130	30	≤3
Cotinine	70	130	70	130	30	≤1.5
Enalapril	70	130	70	130	30	≤0.3
Hydrocodone	70	130	70	130	30	≤1.5
Metformin	70	160	70	160	30	≤30
Oxycodone	65	130	70	130	30	≤0.6
Ranitidine	25	140	30	140	50	≤0.6

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Triamterene	70	140	70	140	30	≤0.3
Surrogate Standards						
d_3-Albuterol	20	140	30	130	30	
d_5-Amphetamine	20	130	25	130	40	
d_7-Atenolol	70	130	70	130	30	
d_3-Cimetidine	15	130	15	130	50	
d_4-Clonidine	70	130	70	130	30	
d_6-Codeine	70	130	70	130	30	
d_3-Cotinine	70	140	70	135	30	
d_5-Enalapril	65	130	70	130	30	
d_3-Hydrocodone	70	130	70	130	30	
d_6-Metformin	3	130	4	130	130	
d_6-Oxycodone	50	150	60	140	30	
Recovery Standards						
d_3-Amitriptyline						
List 5 Compounds (APOS)						
Alprazolam	70	130	70	130	30	≤0.3
Amitriptyline	70	130	70	130	30	≤0.3
Amlodipine	45	130	50	130	30	≤1.5
Benzoylecgonine	70	130	70	130	30	≤0.3
Benztropine	70	130	70	130	30	≤0.3
Betamethasone	20	240	30	220	40	≤1.5
Cocaine	70	130	70	130	30	≤0.15
DEET	70	130	70	130	30	≤0.15
Desmethyldiltiazem	3	350	5	320	80	≤0.15
Diazepam	70	130	70	130	30	≤0.3
Fluocinonide	7	230	9	220	70	≤6
Fluticasone propionate	20	160	25	150	50	≤2
Hydrocortisone	15	220	20	200	80	≤60
10-hydroxy-amitriptyline	70	130	70	130	30	≤0.15
Meprobamate	65	150	70	140	30	≤4
Methylprednisolone	35	240	40	220	50	≤4
Metoprolol	70	130	70	130	30	≤1.5
Norfluoxetine	70	130	70	130	30	≤1.5
Norverapamil	55	130	60	130	30	≤0.15
Paroxetine	70	130	70	130	30	≤4
Prednisolone	35	240	40	220	50	≤6
Prednisone	50	180	60	170	30	≤20
Promethazine	70	130	70	130	30	≤0.4
Propoxyphene	70	130	70	130	30	≤0.3
Propranolol	70	150	70	150	30	≤2
Sertraline	50	130	55	130	30	≤0.4
Simvastatin	1	150	1	140	100	≤20
Theophylline	10	1000	70	900	50	≤60
Trenbolone	70	140	70	135	30	≤4

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Trenbolone acetate	55	130	60	130	30	≤0.3
Valsartan	70	130	70	130	30	≤4
Verapamil	70	145	70	140	30	≤0.15
Surrogate Standards						
d_5-Alprazolam	45	130	45	130	30	
d_6-Amitriptyline	10	130	20	130	40	
d_8-Benzoylecgonine	10	170	20	160	40	
d_3-Benztropine	20	140	25	130	40	
d_3-Cocaine	25	140	30	130	50	
d_7-DEET	15	160	20	150	40	
d_5-Diazepam	15	160	25	150	40	
d_4-Hydrocortisone	40	240	45	230	50	
d_2-Methylprednisolone	15	160	20	150	60	
d_7-Metoprolol	25	140	30	140	30	
d_5-Norfluoxetine	20	130	20	130	50	
d_6-Paroxetine	7	150	9	140	60	
d_4-Promethazine	3	140	5	130	80	
d_5-Propoxyphene	30	130	40	130	30	
d_7-Propranolol	25	140	30	130	30	
$^{13}C_1$, $^{15}N_2$-Theophylline	20	200	25	180	60	
Recovery Standards						
$^{13}C_3$-Atrazine						

[1] OPR and IPR limits derived from actual method performance data according to EPA 821B98003, appendix D.

[2] Because of very low stability the accuracy of Ampicillin is not known. The analysis result is classified as “Information Value” only.

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Instrumental Acceptance Specifications

QC Parameter	Specification
Instrument Sensitivity	Daily, S:N ≥ 3:1 for all analytes for lowest calibration point.
Initial Calibration (native compounds)	Initial, (1/X) weighted linear regression (followed by regular Cal/Ver procedures and repeated as necessary to maintain Cal/Ver results within established acceptance ranges. Calculated concentrations 70-130%, one point per compound may be 60-140% Internal guideline - correlation coefficient >0.985. Calibration curves with lower correlation coefficient values meeting all above criteria may be accepted based on batch specific QC results and professional judgement
OPENING Calibration Verification	Every 20 samples, determined concentrations within 30% of actual concentrations. Professional judgment allowed for wider acceptance limits.
CLOSING Calibration Verification	Within OPENING Calibration Verification specifications. Allowable exception: results for the greater of 1 compound or 10% of the compounds on a Compound List (1,2,3,4,5) may fall outside the Opening Calibration Verification specification provided the RPD between the CLOSING result and the OPENING result is <30%.
Instrumental Carryover And Instrument Background	Every Initial Calibration, Cal/Ver, or SPM: < 0.3 % carryover and area response of analytes in instrument blank < 800 judged following two previous methanol blank injections.

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QUANTIFICATION AND DATA REPORTING PROCEDURES

Positive identification of target natives, surrogate standard and recovery standards require:

- ≥ 3:1 S:N for parent ion to daughter ion transition, on condition that the result is above the lowest calibration standard level.
- Compound retention time falls within 0.4 minutes of the predicted retention times from the daily calibration standard. Natives with labelled surrogate standards must elute within 0.1 minutes of the associated labelled surrogates.

Concentrations of the targets and surrogates are calculated by isotope dilution or internal standard quantification with linear regression calibration, using a 1/X weighting type, excluding origin. Sample specific detection limits (SDLs) are calculated by QuanLynx software using 3 times the signal of the noise in the target channel converted to an equivalent sample concentration.

General equation : $Y = \text{slope} \times X + \text{intercept}$

Where: $Y = \text{Response ratio} = \left(\frac{\text{area of Target}}{\text{area of Quant. Std}} \times \text{weight of Quant. std (ng)} \right)$

X = weight of target (ng)

Quant. Std = labelled surrogate or recovery standard

The slope and intercept are used to convert raw peak areas in sample chromatograms to final concentrations as follows:

$$\text{Sample Conc.} = \left(\frac{\text{area of Target}}{\text{area of Quant. Std}} \times \text{weight of Quant. Std (ng)} - \text{intercept} \right) \text{x} \left(\frac{1}{\text{slope}} \right) \text{x} \left(\frac{1}{\text{sample size (L)}} \right)$$

The recovery of surrogate standards, calculated from the determined concentration of the surrogate in the extract relative to the amount spiked, are monitored as an indication of overall data quality.

The lower reporting limit for each target compound is defined as the concentration equivalent to the lowest calibration standard analyzed or the SDL, whichever is greater.

Narrative Appendix – Details of Hydrocodone/Codeine Correction

Summary

There is significant analytical cross-interference between hydrocodone and codeine in Axys Method MLA-075. This interference arises from the chemical similarity of these compounds. The compounds have the same molecular weight and chemical formula $C_{18}H_{21}NO_3$. Due to this structural similarity, they are not chromatographically separated on the HPLC column used in this analysis. The quantitation transitions for each of these compounds also show mass spectrometric interferences from the presence of the other compound. AXYS has determined that the extent of this interference is constant across the concentration range of the method, except close to the reporting limit where there is increased uncertainty. As the extent of interference is constant, a correction can be applied to the concentration as detailed below. If the amount of area correction is more than half the original area response, AXYS reports the result as not detected with a detection limit equal to the calculated concentration value plus 1% (the 1% is added to facilitate automated data processing).

Note the raw data in this data package contains uncorrected areas and concentrations. All corrections have been made using automated procedures within the LIMS (Laboratory Information Management System).

Area Correction

$H = \frac{Y - aX}{1 - ab}$ $C = \frac{X - bY}{1 - ab}$ where

X , Y - Observed areas of codeine and hydrocodone

C, H - Corrected areas for codeine and hydrocodone

a, b – Cross Interference constants, a = 0.562 (codeine in hydrocodone) b = 0.172 (hydrocodone in codeine).

Correction of Linearity

Since ratio of codeine:hydrocodone concentration is constant in the linearity, linearity slope is reduced for each compound by a constant R = 0.730 for hydrocodone and 0.790 for codeine.

Concentration

$C_{corr} = \frac{C_{uncorr} * A_{corr}}{R * A_{uncorr}}$ A_{corr} is H or C, A_{uncorr} is X or Y, and R is the linearity correction.

Correction Limits

For hydrocodone, if $\frac{Y - H_{199}}{Y} > 0.5$, concentration will be reported as ND < Y.

For codeine, if $\frac{X - C_{152}}{X} > 0.5$, concentration will be reported as ND < X.

Example Calculations

Reported Codeine Concentration (ng/L)	Reported Hydrocodone Concentration (ng/L)	Reported Codeine Area	Reported Hydrocodone Area
47.0	6.78	32900	21019

After applying correction procedure

Corrected Codeine Area	Corrected Hydrocodone Area	Corrected Codeine Concentration (ng/L)	Corrected Hydrocodone Concentration (ng/L)
32418	2800	58.6	ND < 6.78

PHARMACEUTICAL AND PERSONAL CARE PRODUCT ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-075

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG30928

Analysis WG30278 and WG30337

19 November 2009

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19 November 2009

ORSANCO
AQUEOUS SAMPLES

PHARMACEUTICAL AND PERSONAL-CARE PRODUCTS ANALYSIS
AXYS METHOD: MLA-075
4562: L13550 -1 to -15

PROJECT NAME: EMERGING CONTAMINANTS IN MAINSTEM OHIO

19 November 2009

NARRATIVE

This narrative describes the analysis of fifteen aqueous samples for the determination of Pharmaceutical and Personal-Care Products using High Performance Liquid Chromatography coupled with tandem Mass Spectrometry (LC- MS/MS).

SAMPLE RECEIPT AND STORAGE

The samples were received on the 17th of September 2009. Details of sample conditions upon receipt are provided in the Sample Receiving Record form included in the sample documentation section of this data package. The temperature of the samples upon receipt was between 0 and 4 °C. The samples were stored at 4 °C prior to sample preparation and analysis

SAMPLE PREPARATION AND ANALYSIS

Samples and QC samples (a procedural blank and a lab-generated reference sample known as the Ongoing Precision and Recovery (OPR)) were analyzed in two analysis batches named WG30278 and WG30337. Composition of the analysis batches is shown on the Cover Page and Correlation Table, and on the Batch Lists that accompany the extraction workup sheets. .

Extraction and analysis procedures were in accordance with AXYS Method MLA-075: ***Analytical Procedure for the Analysis of Pharmaceutical and Personal Care Products in Solid and Aqueous Samples by LC-MS/MS***. A method summary (MSU-075) for the AXYS Method MLA-075 is included in this data package.

Two aliquots of accurately weighed sub-sample for each sample (approximately 1 liter) were spiked with labeled quantification standards and extracted with acetonitrile using sonication at pH 2 (in analysis batch WG30337) and pH 10 (in analysis batch WG30278), respectively. The resulting extracts were reduced in volume, reconstituted in water and cleaned up on Waters Oasis HLB SPE cartridges. The final extract was reduced in volume and spiked with labeled recovery (internal) standards prior to instrumental analysis. Analysis was performed on Waters 2690 or 2795 HPLC equipped with Micromass Quattro Ultima MS/MS using five instrument and LC conditions as shown in the table below.

Target Group	LC Column	Ionization	Acquisition	LC Conditions
List 1	Waters Xtera C18 (10.0 cm, 2.1 mm i.d., 3.5 µm particle size)	Positive Ion Electrospray	MRM mode, unit resolution	1
List 2	Waters Xtera C18 (10.0 cm, 2.1 mm i.d., 3.5 µm particle size)	Positive Ion Electrospray	MRM mode, unit resolution	2
List 3	Waters Xtera C18 (10.0 cm, 2.1 mm i.d., 3.5 µm particle size)	Negative Ion Electrospray	MRM mode, unit resolution	3
List 4	Waters Atlantis HILIC (10.0 cm, 2.1 mm i.d., 3.0 µm particle size)	Positive Ion Electrospray	MRM mode, unit resolution	4
List 5	Waters Xtera C18 (10.0 cm, 2.1 mm i.d., 3.5 µm particle size)	Positive Ion Electrospray	MRM mode, unit resolution	5

CALCULATION

Target analyte concentrations were determined by isotope dilution or internal standard quantification procedures using MassLynx software. Quantification was conducted by comparing the area of the quantification ion to that of the quantification standard (surrogate) and correcting for response factors.

For all target compounds, linear equations were determined from a multi-point calibration series prepared alongside the samples with 1/X weighting fit. Formulae used in the conversion of the raw chromatograms to concentration are provided in the method summary document.

Sample specific detection limits (SDLs) were calculated for each target analyte and used as the detection qualifier. If the MassLynx software selected an unrepresentative area for the detection limit calculation, the data interpretation chemist or the QA chemist made corrections. These corrections are hand noted on the quantification report pages attached to the chromatograms.

The lower reporting limit for each target compound is defined as the concentration equivalent to the lowest calibration standard analyzed, prorated for the extract volume and sample size, or the SDL, whichever is greater.

REPORTING CONVENTIONS

The AXYS contract number assigned for internal tracking was 4562. The samples were assigned a unique laboratory identifier of the form L13550-X, where X is a numeral. All data reports reference the unique AXYS IDs plus the client sample identifiers.

The following laboratory qualifier flags were used in this data package:

B	=	analyte found in the sample and the associated blank
U	=	identifies a compound that was not detected
V	=	surrogate recovery is not within method/contract control limit

Results are reported in concentration units of nanograms per litre (ng/L). Concentration and reporting limits are provided to three significant figures. Analysis results for each sample are provided on Analysis Report form 1A/2.

QA/QC NOTES

The samples and associated QC samples analyzed in an analysis batch were carried intact through the entire analytical process. The sample data were reviewed and evaluated in relation to the batch QC samples.

- Sample analyte concentrations are not blank corrected. The data should be evaluated with consideration to the procedural blank results.
- By virtue of the isotope dilution/internal standard quantification procedures, data are recovery corrected for possible losses during extraction and cleanup.
- The Signal/Noise (S/N) ratios were measured as '0' for some compounds in the QC samples and sample data. This has been determined to be a limitation of the software and would not affect the data.
- All linearity, calibration verification, OPR and labeled compound recovery specifications were met with the following exceptions:

List 1 Compounds (WG30337)

1. At least 5 calibration standard points are used in quantification of the initial calibration (filename: QA9J_186) for all analytes except Digoxin, Tylosin and Virginiamycin. These analytes were not detected in the samples. Digoxin and Tylosin were deemed not quantifiable and are flagged 'NQ'.

The detection limit for Virginiamycin was raised to the CS3 level. This compound was not detected in the samples. D6-Thiabendazole was excluded in the highest point (CS6) as it did not meet method specifications.

2. The recovery of $^{13}C_2$-15N- Acetaminophen and $^{13}C_3$Caffeine in the samples below did not meet the method criteria; this compound is flagged with a 'V'. As the isotope dilution method of quantification produces data that are recovery corrected, the slight variances from the method acceptance criteria are deemed not to affect the quantification of these analytes. Percent surrogate recoveries are used as general method performance indicator only.

AXYS ID	CLIENT ID	SURROGATE	RECOVERY %
L13550-1	Site 1	13C2-15N Acetominophen	180
L13550-2	Site 2	13C2-15N Acetominophen	167
		13C2-Caffeine	142
L13550-8	Site 8	13C2-15N Acetominophen	179
L13550-10	Site 10	13C2-15N Acetominophen	173
		13C2-Caffeine	175
L13550-11	Site 12	13C2-15N Acetominophen	171
L13550-13	Site 14	13C2-15N Acetominophen	167
L13550-15	Site 16	13C2-15N Acetominophen	185
		13C2-Caffeine	150

List 2 Compounds (WG30337)

Due to ion suppression or enhancement which caused significant drop of responses and/or non-linearity, some high-level calibration standards in the initial calibration (filename QB9K_199 S: 9 to S: 15) were excluded. A minimum of 5 calibration standard points was used to construct the linear equations for quantification of target analytes or to calculate response factor (RF) for quantification of labeled surrogates. As multi-point calibrations were used, sample data are deemed not significantly affected.

List 3 Compounds (WG30337)

1. At least 6 calibration points were used in quantification of the initial calibration (Data filename: QF9K_202 S: 7 to S: 13) for all the analytes except for Hydrochlorothiazide, which was quantified using 5 calibration points (CS0 to CS4). Since multiple calibrations were used, sample data are deemed not be significantly affected.

2. An interference was observed affecting Ibuprofen in samples Site 7 (AXYS ID: L13550-7), Site 14 (AXYS ID: L13550-13) and Site 16 (AXYS ID: L13550-15). This disturbance was observed only in these client samples and therefore the interference is likely matrix related. As the concentration for samples Site 7 and Site 14 were below the detection limit only Site 16 was affected. The reported concentrations of Ibuprofen in this sample should be interpreted as 'estimated' maximum value.

List 4 Compounds (WG30278)

1. Due to ion suppression which caused significant drop of responses, the highest level calibration standard CS6 (data filename QG9K_197 S:9) in the initial calibration was excluded for Atorvastatin and Clonidine, A minimum of 5 calibration standard points was used to construct the linear equations for quantification of target analytes. As multiple calibrations were used, sample data were deemed not be significantly affected.

2. Due to analytical cross-interference between Hydrocodone and Codeine, a correction has been added to these compounds on the report forms. Details of the Hydrocodone/Codeine correction are provided in the Narrative of the data package.

3. The recovery of surrogates detailed below did not meet the method criteria. These compounds are flagged with 'V'. As the isotope dilution method of quantification produces data that are recovery corrected, the slight variances from the method acceptance criteria are deemed not to affect the quantification of these analytes. Percent surrogate recoveries are used as a general method performance indicator only.

AXYS ID	CLIENT ID	SURROGATE	RECOVERY %
L13550-2	Site 2	D5-Enalpril	140
		D4-Clonidine	152
		D6-Codeine	169
		D3-Hydrocodone	169
L13550-4	Site 4	D5-Enalpril	140
		D4-Clonidine	142
		D6-Codeine	161
		D3-Hydrocodone	150
		D7-Atenolol	159

4. A Water Pretreatment Record is included in this data package for a test for residual chlorine. Although not required by this method, some bottles analyzed in WG30278 had been tested as a requirement of AXYS method MLA-068. This test is not likely to affect samples.

List 5 Compounds (WG30337)

1. Elevated level of Methylprednisolone was detected in the Lab Blank. The Lab Blank level should be taken into consideration in evaluation of sample Methylprednisolone data for significance.

2. At least 5 calibration points were used in quantification of the initial calibration (QE9J_190 S: 4 to S: 10) for all the analytes. Since multiple calibrations were used, sample data were deemed not be significantly affected.

3. Lowest level calibration standard CS0 for Prednisolone and DEET was excluded from the initial calibration. As a result, the CS1 level calibration was used as detection qualifier for these analytes in samples. Given that Prednisolone was not detected, sample data for this compound was not impacted by the variance. DEET was detected however in all client samples the concentrations were either in greater than that of the CS1 standard or not detected, sample data for these compounds were not impacted by the variances. Betamethasone was excluded in the CS0 and CS1 for the initial calibration and the CS2 level was used as detection qualifier for this compound. This compound was not detected in the samples.

4. The recoveries of native Hydrocortisone and Prednisone did not meet method specifications for the initial calibration and the opening calibration standard (datafile ID: QE9J_190 S:11). Sample data are not considered affected as this compound was not detected in any samples.

5. In the opening calibration standard for the first bracket (data filename: QE9J_190 S: 11), percent recovery of Betamethasone and Norverapmil were observe above method specifications. Data are not considered affected as these compounds were not detected in the samples.

6. In the opening calibration standard for the second bracket (data filename: QE9J_190 S: 25), percent recovery of Amlodipine and Simvastatin were observed above method specifications. Data are not considered affected as these compounds were not detected in the samples.

7. The recovery of d2-Methylprednisolone and d5-Norfluoxetine in some samples did not meet the method criteria; these compounds are flagged with a 'V and detailed in a table below'. As the isotope dilution method of quantification produces data that are recovery corrected, the slight variances from the method acceptance criteria are deemed not to affect the quantification of these analytes. Percent surrogate recoveries are used as general method performance indicator only.

AXYS ID	CLIENT ID	SURROGATE	RECOVERY %
L13550-7	Site 7	d2-Methylprednisolone	178
"	"	d5-Norfluoxetine	147
L13550-8	Site 8	d5-Norfluoxetine	139
L13550-10	Site 10	"	132
L13550-11	Site 12	"	140
L13550-15	Site 16	"	136

ANALYTICAL DISCUSSION

List 1, 2, 3, 5 Compounds (WG30337)

No analytical difficulties were encountered.

List 4 Compounds (WG30278)

No analytical difficulties were encountered.

DATA PACKAGE

This data package has been assigned a unique identifier, DPWG30928, shown on the cover page. Included in this data package following the narrative is the following documentation:

- Method summary (MSU-075)
- Hydrocodone/Codeine correction summary
- Narrative Appendix
- Sample 'Cover Page' and 'Correlation Table'
- Sample Receiving Documentation
- Laboratory extraction worksheets
- Sample data reports (in order of AXYS Sample ID)
- Laboratory QC data reports
- Instrument run (injection) log
- Instrumental QC data reports (organized by analysis date)
- Sample raw data (in order of AXYS Sample ID)
- Laboratory QC raw data
- Instrumental QC raw data (organized by analysis date)

I certify that this data package is in compliance with the terms and conditions of the contract, both technically and for completeness, except for the conditions detailed above. In addition, I certify, that to the best of my knowledge and belief, the data as reported are true and accurate. The following signature, on behalf of AXYS Analytical Services Ltd, authorizes the release of the data contained in this data package.

Kristina Coleman

Signed: Kristina Coleman, QA/QC Chemist

19Nov09

Date Signed

AXYS Analytical Services Ltd.

SUMMARY OF AXYS METHOD MLA-075:

ANALYTICAL PROCEDURES FOR THE ANALYSIS OF PHARMACEUTICAL AND PERSONAL CARE COMPOUNDS IN SOLID AND AQUEOUS SAMPLES BY LC-MS/MS

ANALYTE LISTS

List 1 (Acid extraction, positive ESI)	
Acetaminophen	Norfloxacin
Ampicillin [1]	Norgestimate
Azithromycin	Ofloxacin
Caffeine	Ormetoprim
Carbadox	Oxacillin
Carbamazepine	Oxolinic acid
Cefotaxime	Penicillin G
Ciprofloxacin	Penicillin V
Clarithromycin	Roxithromycin
Clinafloxacin	Sarafloxacin
Cloxacillin	Sulfachloropyridazine
Dehydronifedipine	Sulfadiazine
Digoxigenin	Sulfadimethoxine
Digoxin	Sulfamerazine
Diltiazem	Sulfamethazine
1,7-Dimethylxanthine	Sulfamethizole
Diphenhydramine	Sulfamethoxazole
Enrofloxacin	Sulfanilamide
Erythromycin	Sulfathiazole
Flumequine	Thiabendazole
Fluoxetine	Trimethoprim
Lincomycin	Tylosin
Lomefloxacin	Virginiamycin
Miconazole	
List 2 (Tetracyclines, positive ESI)	
Anhydrochlortetracycline (ACTC)	4-Epichlortetracycline (ECTC)
Anhydrotetracycline (ATC)	4-Epioxytetracycline (EOTC)
Chlortetracycline (CTC)	4-Epitetracycline (ETC)
Demeclocycline	Isochlortetracycline (ICTC)
Doxycycline	Minocycline
4-Epianhydrochlortetracycline (EACTC)	Oxytetracycline (OTC)
4-Epianhydrotetracycline (EATC)	Tetracycline (TC)

List 3 (Acid extraction, negative ESI)	
Bisphenol A	2-hydroxy-ibuprofen
Furosemide	Ibuprofen
Gemfibrozil	Naproxen
Glipizide	Triclocarban
Glyburide	Triclosan
Hydrochlorothiazide	Warfarin
List 4 (Base extraction, positive ESI)	
Albuterol	Cotinine
Amphetamine	Enalapril
Atenolol	Hydrocodone
Atorvastatin	Metformin
Cimetidine	Oxycodone
Clonidine	Ranitidine
Codeine	Triamterene
List 5 (Acid Extraction, positive ESI)	
Alprazolam	Metoprolol
Amitriptyline	Norfluoxetine
Amlodipine	Norverapamil
Benzoylecgonine	Paroxetine
Benztropine	Prednisolone
Betamethasone	Prednisone
Cocaine	Promethazine
DEET (N,N-diethyl-m-toluamide)	Propoxyphene
Desmethyldiltiazem	Propranolol
Diazepam	Sertraline
Fluocinonide	Simvastatin
Fluticasone propionate	Theophylline
Hydrocortisone	Trenbolone
10-hydroxy-amitriptyline	Trenbolone acetate
Meprobamate	Valsartan
Methylprednisolone	Verapamil

[1] Due to instability accuracy of Ampicillin data is unknown.

EXTRACTION AND CLEANUP PROCEDURES

The analysis requires extraction at two different pH conditions: At pH 10 for analysis of fourteen analytes (List 4); and at pH 2.0 for the analysis of the other analytes (Lists 1, 2, 3, and 5). Prior to extraction and/or clean-up samples are adjusted to the required pH and spiked with surrogates.

Solid samples are repeatedly extracted by sonication with aqueous buffered acetonitrile and pure acetonitrile, concentrated by rotary evaporation, and diluted with ultra pure water to 200 mL. The acidic extract is treated with EDTA. The extracts are cleaned up by solid phase extraction (SPE), filtered, and analyzed by LC/ESI-MS/MS in positive and negative ionization modes requiring a total of five runs to analyze the complete list of analytes.

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All aqueous samples are filtered and the aqueous portion is cleaned up by solid phase extraction before analysis by LC/ESI-MS/MS.

Aqueous samples with no or limited visible particulate (e.g. surface water, ground water, wastewater treatment final effluent, typically with < 100 mg/L TSS) normally can be processed with up to 1L samples sizes. The sample is filtered and routinely only the aqueous phase is analyzed. However, upon specific agreement a separate extraction may be performed on the solids phase. The solids extract may in this case either be carried through the analysis individually as a separate sample that is reported separately, or the aqueous extract and the solids extract may be combined just prior to clean-up and reported as a combined aqueous/solids phase result.

For mixed phase aqueous/solids samples with significant solids and distinct aqueous and solids phases such as wastewater influent or process streams the sample may either be analyzed as an aqueous phase only or as two separate samples, one aqueous and one solid.

ANALYSIS BY LC-MS/MS

The analysis is performed on a high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer. Instrument calibration is performed using a series of calibration solutions (7 points) covering the working concentration range of the instrument specific for the individual compounds of interest. The LC/MS/MS is run in MRM (Multiple Reaction Monitoring) mode and quantification is performed by recording the peak areas of the applicable parent ion/daughter ion transitions. Some analytes are analyzed in the ESI positive mode and some are analyzed in the ESI negative mode.

List 1 – Acid Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Sulfanilamide	2.5	190.0	155.8	$^{13}C_6$-Sulfamethazine
Acetaminophen	4.6	152.2	110.0	$^{13}C_2$-^{15}N-Acetaminophen
Sulfadiazine	6.0	251.2	156.1	$^{13}C_6$-Sulfamethazine
1,7-Dimethylxanthine	6.9	181.2	124.0	$^{13}C_3$-Caffeine
Sulfathiazole	7.7	256.3	156.0	$^{13}C_6$-Sulfamethoxazole
Sulfamerazine	8.7	265.0	156.0	$^{13}C_6$-Sulfamethazine
Caffeine	9.3	195.0	138.0	$^{13}C_3$-Caffeine
Lincomycin	9.3	407.5	126.0	$^{13}C_3$-Trimethoprim
Sulfamethizole	10.0	271.0	156.0	$^{13}C_6$-Sulfamethoxazole
Thiabendazole	10.0	202.1	175.1	d_6-Thiabendazole
Trimethoprim	10.0	291.0	230.0	$^{13}C_3$-Trimethoprim
Sulfamethazine	10.1	279.0	156.0	$^{13}C_6$-Sulfamethazine
Cefotaxime	10.2	456.4	396.1	$^{13}C_3$-Trimethoprim
Carbadox	10.5	263.2	231.2	$^{13}C_3$-Trimethoprim
Ormetoprim	10.5	275.3	259.1	$^{13}C_3$-Trimethoprim
Norfloxacin	10.7	320.0	302.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Ofloxacin	10.8	362.2	318.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sulfachloropyridazine	10.8	285.0	156.0	$^{13}C_6$-Sulfamethazine
Ciprofloxacin	10.9	332.2	314.2	$^{13}C_3$, ^{15}N-Ciprofloxacin
Lomefloxacin	11.2	352.2	308.1	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sulfamethoxazole	11.2	254.0	156.0	$^{13}C_6$-Sulfamethoxazole

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Enrofloxacin	11.5	360.0	316.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sarafloxacin	11.9	386.0	299.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Clinafloxacin	12.1	366.3	348.1	$^{13}C_3$, ^{15}N-Ciprofloxacin
Digoxigenin	12.6	391.2	355.2	$^{13}C_3$-Trimethoprim
Oxolinic Acid	13.1	261.8	243.8	$^{13}C_3$-Trimethoprim
Sulfadimethoxine	13.2	311.0	156.0	$^{13}C_6$-Sulfamethoxazole
Diphenhydramine	14.5	256.8	168.1	$^{13}C_3$-Trimethoprim
Penicillin G	14.6	367.5	160.2	$^{13}C_3$-Trimethoprim
Azithromycin	14.8	749.9	591.6	$^{13}C_3$-Trimethoprim
Flumequine	15.2	262.0	173.7	$^{13}C_3$-Trimethoprim
Ampicillin	15.3	350.3	160.2	$^{13}C_3$-Trimethoprim
Carbamazepine	15.3	237.4	194.2	$^{13}C_3$-Trimethoprim
Diltiazem	15.3	415.5	178.0	$^{13}C_3$-Trimethoprim
Penicillin V	15.4	383.4	160.2	$^{13}C_3$-Trimethoprim
Erythromycin [1]	15.9	734.4	158	not quantified
Tylosin	16.3	916.0	772.0	$^{13}C_6$-Sulfamethazine
Oxacillin	16.4	434.3	160.1	$^{13}C_3$-Trimethoprim
Dehydronifedipine	16.5	345.5	284.1	$^{13}C_3$-Trimethoprim
Digoxin	16.6	803.1	283.0	$^{13}C_3$-Trimethoprim
Cloxacillin	16.9	469.1	160.1	$^{13}C_3$-Trimethoprim
Fluoxetine	16.9	310.3	148.0	d_5-Fluoxetine
Virginiamycin	17.3	508.0	355.0	$^{13}C_3$-Trimethoprim
Clarithromycin	17.5	748.9	158.2	$^{13}C_6$-Sulfamethazine
Erythromycin - H_2O [1]	17.7	716.4	158	$^{13}C_2$-Erythromycin - H_2O
Roxithromycin	17.8	837.0	679.0	$^{13}C_6$-Sulfamethazine
Miconazole	20.1	417.0	161.0	$^{13}C_3$-Trimethoprim
Norgestimate	21.7	370.5	124.0	$^{13}C_3$-Trimethoprim
Surrogate Standard				
$^{13}C_2$-^{15}N-Acetaminophen	4.5	155.2	111.0	$^{13}C_3$-Atrazine
$^{13}C_3$-Caffeine	9.3	198.0	140.0	$^{13}C_3$-Atrazine
d_6-Thiabendazole	9.8	208.1	180.1	$^{13}C_3$-Atrazine
$^{13}C_3$-Trimethoprim	10.0	294.0	233.0	$^{13}C_3$-Atrazine
$^{13}C_6$-Sulfamethazine	10.1	285.1	162.1	$^{13}C_3$-Atrazine
$^{13}C_3$, ^{15}N-Ciprofloxacin	10.9	336.1	318.2	$^{13}C_3$-Atrazine
$^{13}C_6$-Sulfamethoxazole	11.2	260.0	162.0	$^{13}C_3$-Atrazine
$^{13}C_2$-Erythromycin [1]	15.9	736.4	160.0	monitor for less than 5%
d_5-Fluoxetine	16.8	315.3	153.0	$^{13}C_3$-Atrazine
$^{13}C_2$-Erythromycin - H_2O [1]	17.7	718.4	160.0	$^{13}C_3$-Atrazine
Recovery Standard				
$^{13}C_3$-Atrazine	15.9	219.5	176.9 (134.0)	External Standard

[1] Because of intramolecular dehydration during the analytical procedure erythromycin is quantified as the dehydration product “erythromycin – H_2O” [5]. The peak area of the $^{13}C_2$-Erythromycin is monitored and must be less than 5% of the $^{13}C_2$-Erythromycin - H_2O peak area. If it is greater, the Erythromycin - H_2O result is flagged as ‘accuracy unknown’.

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List 2 – Acid Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Minocycline	5.1	458.0	441.0	d_6-Thiabendazole
Epitetracycline (ETC)	8.1	445.2	410.2	d_6-Thiabendazole
Epioxytetracycline (EOTC)	8.6	461.2	426.2	d_6-Thiabendazole
Oxytetracycline (OTC)	9.4	461.2	426.2	d_6-Thiabendazole
Tetracycline (TC)	9.9	445.2	410.2	d_6-Thiabendazole
Demeclocycline	11.7	465.0	430.0	d_6-Thiabendazole
Isochlortetracycline (ICTC) [1]	11.9	479.0	462.0	d_6-Thiabendazole
Epichlortetracycline (ECTC)	12.0	479.0	444.0	d_6-Thiabendazole
Chlortetracycline (CTC)	14.1	479.0	444.0	d_6-Thiabendazole
Doxycycline	16.7	445.2	428.2	d_6-Thiabendazole
Epianhydrotetracycline (EATC)	17.0	426.8	409.8	d_6-Thiabendazole
Anhydrotetracycline (ATC)	18.8	426.8	409.8	d_6-Thiabendazole
Epianhydrochlortetracycline (EACTC)	20.7	461.2	444.0	d_6-Thiabendazole
Anhydrochlortetracycline (ACTC)	22.1	461.2	444.0	d_6-Thiabendazole
Surrogate Standard				
d_6-Thiabendazole	7.1	208.1	180.1	$^{13}C_3$-Atrazine
Recovery Standard				
$^{13}C_3$-Atrazine	21.2	219.5	176.9	External Standard

[1] Isochlortetracycline (ICTC) is reported as the sum ICTC + ECTC due to a common transition ion.

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List 3 – Acid Extraction, Negative Electrospray Ionization (-)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Hydrochlorathiazide	2.3	296.0	268.0	^{13}C-d_3-Naproxen
Hydrochlorathiazide*	2.3	296.0	204.8	^{13}C-d_3-Naproxen
Furosemide	3.4	329.0	284.8	^{13}C-d_3-Naproxen
Furosemide*	3.4	329.0	204.7	^{13}C-d_3-Naproxen
2-hydroxy-ibuprofen	4.2	221.1	176.8	$^{13}C_3$-Ibuprofen
Bisphenol A	6.5	227.0	211.9	d6-Bisphenol A
Bisphenol A*	6.5	227.0	132.9	d6-Bisphenol A
Glipizide	6.9	444.2	319.0	d11-Glipizide
Glipizide*	6.9	444.2	169.8	d11-Glipizide
Naproxen	7.0	228.9	168.6	^{13}C-d_3-Naproxen
Warfarin	7.4	307.0	161.0	d_5-Warfarin
Glyburide	8.8	492.1	169.8	d3-Glyburide
Glyburide*	8.8	492.1	367.0	d3-Glyburide
Ibuprofen	8.8	205.1	161.1	$^{13}C_3$-Ibuprofen
Gemfibrozil	9.9	249.0	121.0	d_6-Gemfibrozil
Triclocarban	10.1	312.9	159.7	$^{13}C_6$-Triclocarban
Triclosan	10.2	286.8	35.0	$^{13}C_{12}$-Triclosan
Surrogate Standard				
d_6-Bisphenol A	6.5	233.0	214.8	$^{13}C_6$-2,4,5-T
d_6-Bisphenol A*	6.5	233.0	137.8	$^{13}C_6$-2,4,5-T
d_{11}-Glipizide	6.8	455.0	319.0	$^{13}C_6$-2,4,5-T
d_{11}-Glipizide*	6.8	455.0	169.8	$^{13}C_6$-2,4,5-T
^{13}C-d_3-Naproxen	7.0	232.9	168.6	$^{13}C_6$-2,4,5-T
d_5-Warfarin	7.4	312	161.0	$^{13}C_6$-2,4,5-T
d_3-Glyburide	8.7	495.0	169.9	$^{13}C_6$-2,4,5-T
d_3-Glyburide*	8.7	495.0	370.1	$^{13}C_6$-2,4,5-T
$^{13}C_3$-Ibuprofen	8.8	208.2	163.1	$^{13}C_6$-2,4,5-T
d_6-Gemfibrozil	9.9	255	121	$^{13}C_6$-2,4,5-T
$^{13}C_6$-Triclocarban	10.1	318.9	159.7	$^{13}C_6$-2,4,5-T
$^{13}C_{12}$-Triclosan	10.2	298.8	35	$^{13}C_6$-2,4,5-T
Recovery Standard				
$^{13}C_6$-2,4,5-Trichlorophen-oxyacetic acid ($^{13}C_6$-2,4,5-T)	4.9	258.8	200.7	External Standard

* Indicates secondary transition for possible diagnostic use.

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List 4 – Base Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Cotinine	4.0	177.0	98.0	d3-Cotinine
Cimetidine	4.7	253.1	159.0	d3-Cimetidine
Triamterene	5.4	254.1	236.9	d4-Clonidine
Triamterene*	5.4	254.1	103.7	d4-Clonidine
Enalapril	6.5	377.2	233.9	d5-Enalapril
Enalapril*	6.5	377.2	159.8	d5-Enalapril
Oxycodone	6.7	316.2	240.9	d6-Oxycodone
Oxycodone*	6.7	316.2	298.0	d6-Oxycodone
Clonidine	6.8	230.0	212.5	d4-Clonidine
Clonidine*	6.8	230.0	43.9	d4-Clonidine
Amphetamine	8.1	136.1	90.8	d5-Amphetamine
Amphetamine*	8.1	136.1	118.9	d5-Amphetamine
Albuterol	8.3	240.0	148.0	d_3-Albuterol
Codeine	8.4	300.0	152.0	d6-Codeine
Hydrocodone	8.6	300.2	198.8	d3-Hydrocodone
Hydrocodone*	8.6	300.2	170.6	d3-Hydrocodone
Atorvastatin	8.9	559.3	440.0	d5-Enalapril
Atorvastatin*	8.9	559.3	466.0	d5-Enalapril
Atenolol	9.0	267.2	144.7	d7-Atenolol
Atenolol*	9.0	267.2	189.7	d7-Atenolol
Metformin	9.5	131.1	60.1	d_6-Metformin
Ranitidine	18.8	315.0	175.9	d_3-Albuterol
Surrogate Standards				
d_3-Cotinine	4.0	180.0	79.9	d3-Amitriptyline
d_3-Cotinine*	4.0	180.0	101.0	d3-Amitriptyline
d_3-Cimetidine	4.7	256.0	161.8	d3-Amitriptyline
d_3-Cimetidine*	4.7	256.0	94.8	d3-Amitriptyline
d_5-Enalapril	6.5	382.0	238.8	d3-Amitriptyline
d_5-Enalapril*	6.5	382.0	164.8	d3-Amitriptyline
d_6-Oxycodone	6.7	322.1	262.0	d3-Amitriptyline
d_6-Oxycodone*	6.7	322.1	304.1	d3-Amitriptyline
d_4-Clonidine	6.8	234.0	216.7	d3-Amitriptyline
d_4-Clonidine*	6.8	234.0	47.9	d3-Amitriptyline
d_5-Amphetamine	8.1	141.1	92.9	d3-Amitriptyline
d_5-Amphetamine*	8.1	141.1	123.9	d3-Amitriptyline
d_3-Albuterol	8.3	243.0	151.0	d3-Amitriptyline
d_6-Codeine	8.4	306.0	151.8	d3-Amitriptyline
d_6-Codeine*	8.4	306.0	217.9	d3-Amitriptyline
d_3-Hydrocodone	8.6	303.1	198.9	d3-Amitriptyline
d_3-Hydrocodone*	8.6	303.1	170.8	d3-Amitriptyline
d_7-Atenolol	9.0	274.0	144.7	d3-Amitriptyline
d_7-Atenolol*	9.0	274.0	189.7	d3-Amitriptyline
d_6-Metformin	9.5	137.1	60.1	d3-Amitriptyline
Recovery Standards				

d_3-Amitriptyline	7.9	281.0	232.7	External Standard
d_3-Amitriptyline*	7.9	281.0	90.7	External Standard

* Indicates secondary transition for possible diagnostic use.

List 5 – Acid Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention	Parent Ion Mass	Daughter Ion Mass	Quantified against
Theophylline	2.7	181.1	123.8	13C-15N2-Theophylline
Theophylline*	2.7	181.1	95.8	13C-15N2-Theophylline
Benzoylecgonine	5.7	290.1	167.8	d8-Benzoylecgonine
Benzoylecgonine*	5.7	290.1	104.8	d8-Benzoylecgonine
Metoprolol	8.4	268.2	190.7	d7-Metoprolol
Metoprolol*	8.4	268.2	115.7	d7-Metoprolol
Cocaine	9.2	304.1	181.8	d3-Cocaine
Cocaine*	9.2	304.1	81.9	d3-Cocaine
Meprobamate	11.1	219.0	157.8	d7-Metoprolol
Meprobamate*	11.1	219.0	96.9	d7-Metoprolol
10-hydroxy-amitriptyline	12.0	294.2	215.0	d7-Propranolol
10-hydroxy-amitriptyline*	12.0	294.2	276.0	d7-Propranolol
Propranolol	14.6	260.2	115.8	d7-Propranolol
Propranolol*	14.6	260.2	182.7	d7-Propranolol
Prednisone	16.6	359.2	341.0	d7-Propranolol
Prednisone*	16.6	359.2	146.7	d7-Propranolol
Prednisolone	17.5	361.2	343.0	d7-Propranolol
Prednisolone*	17.5	361.2	324.7	d7-Propranolol
Hydrocortisone	17.6	363.2	120.7	d4-Hydrocortisone
Hydrocortisone*	17.6	363.2	326.7	d4-Hydrocortisone
Desmethyldiltiazem	18.8	401.2	177.8	d4-Promethazine
Desmethyldiltiazem*	18.8	401.2	149.5	d4-Promethazine
Promethazine	18.8	285.1	197.8	d4-Promethazine
Promethazine*	18.8	285.1	85.7	d4-Promethazine
DEET	20.6	192.0	118.6	d7-DEET
DEET	20.6	192.0	90.7	d7-DEET
Paroxetine	20.6	330.2	191.8	d6-Paroxetine
Paroxetine*	20.6	330.2	69.8	d6-Paroxetine
Norverapamil	21.0	441.3	164.7	d7-Propranolol
Norverapamil*	21.0	441.3	149.7	d7-Propranolol
Methylprednisolone	21.4	375.2	357.0	d2-Methylprednisolone
Methylprednisolone*	21.4	375.2	339.0	d2-Methylprednisolone
Verapamil	21.4	455.3	164.8	d6-Amitriptyline
Verapamil*	21.4	455.3	149.8	d6-Amitriptyline
Betamethasone	21.7	393.2	355.1	d6-Amitriptyline
Betamethasone*	21.7	393.2	373.0	d6-Amitriptyline
Propoxyphene	21.8	340.2	57.9	d5-Propoxyphene
Propoxyphene*	21.8	340.2	266.1	d5-Propoxyphene
Amitriptyline	22.4	278.2	232.8	d6-Amitriptyline
Amitriptyline*	22.4	278.2	90.7	d6-Amitriptyline
Trenbolone	22.5	271.2	198.7	d5-Alprazolam

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Trenbolone*	22.5	271.2	252.8	d5-Alprazolam
Benztropine	22.9	308.2	166.7	d3-Benztropine
Benztropine*	22.9	308.2	151.7	d3-Benztropine
Alprazolam	23.3	309.1	280.9	d5-Alprazolam
Alprazolam*	23.3	309.1	204.9	d5-Alprazolam
Amlodipine	23.8	409.1	237.8	d5-Norfluoxetine
Amlodipine*	23.8	409.1	293.8	d5-Norfluoxetine
Norfluoxetine	24.7	296.1	133.7	d5-Norfluoxetine
Sertraline	26.4	306.1	274.8	d7-Propranolol
Sertraline*	26.4	306.1	158.7	d7-Propranolol
Diazepam	29.1	285.1	192.8	d5-Diazepam
Diazepam*	29.1	285.1	153.8	d5-Diazepam
Valsartan	31.8	436.2	235.0	d5-Propoxyphene
Valsartan*	31.8	436.2	291.0	d5-Propoxyphene
Fluocinonide	34.8	495.2	337.0	d5-Alprazolam
Fluocinonide*	34.8	495.2	475.0	d5-Alprazolam
Trenbolone acetate	37.8	313.2	253.0	d5-Alprazolam
Trenbolone acetate*	37.8	313.2	271.0	d5-Alprazolam
Fluticasone propionate	37.9	501.2	293.0	d7-Metoprolol
Fluticasone propionate*	37.9	501.2	313.0	d7-Metoprolol
Simvastatin	40.1	419.3	285.0	d5-Propoxyphene
Simvastatin*	40.1	419.3	198.9	d5-Propoxyphene
Surrogate Standards				
^{13}C, $^{15}N_2$-Theophylline	2.7	184.0	124.7	$^{13}C_3$-Atrazine
^{13}C, $^{15}N_2$-Theophylline*	2.7	184.0	96.8	$^{13}C_3$-Atrazine
d_8-Benzoylecgonine	5.6	298.1	170.9	$^{13}C_3$-Atrazine
d_8-Benzoylecgonine*	5.6	298.1	109.8	$^{13}C_3$-Atrazine
d_7-Metoprolol	8.3	275.0	190.8	$^{13}C_3$-Atrazine
d_7-Metoprolol*	8.3	275.0	122.7	$^{13}C_3$-Atrazine
d_3-Cocaine	9.2	307.1	184.9	$^{13}C_3$-Atrazine
d_3-Cocaine*	9.2	30.7.1	84.8	$^{13}C_3$-Atrazine
d_7-Propranolol	14.4	267.0	116.0	$^{13}C_3$-Atrazine
d_7-Propranolol*	14.4	267.0	188.7	$^{13}C_3$-Atrazine
d_4-Hydrocortisone	17.6	367.0	120.8	$^{13}C_3$-Atrazine
d_4-Hydrocortisone*	17.6	367.0	331.0	$^{13}C_3$-Atrazine
d_4-Promethazine	18.6	289.0	201.8	$^{13}C_3$-Atrazine
d_4-Promethazine*	18.6	289.0	86.0	$^{13}C_3$-Atrazine
d_7-DEET	20.6	199.1	125.8	$^{13}C_3$-Atrazine
d_7-DEET*	20.6	199.1	97.8	$^{13}C_3$-Atrazine
d_6-Paroxetine	20.6	336.0	197.8	$^{13}C_3$-Atrazine
d_6-Paroxetine*	20.6	336.0	75.8	$^{13}C_3$-Atrazine
d_2-Methylprednisolone	21.4	377.0	359.0	$^{13}C_3$-Atrazine
d_2-Methylprednisolone*	21.4	377.0	341.0	$^{13}C_3$-Atrazine
d_5-Propoxyphene	21.8	245.2	266.1	$^{13}C_3$-Atrazine
d_5-Propoxyphene*	21.8	345.2	57.9	$^{13}C_3$-Atrazine
d_6-Amitriptyline	22.4	284.0	233.0	$^{13}C_3$-Atrazine
d_6-Amitriptyline*	22.4	284.0	90.8	$^{13}C_3$-Atrazine
d_3-Benztropine	22.9	311.0	166.7	$^{13}C_3$-Atrazine
d_3-Benztropine*	22.9	311.0	151.7	$^{13}C_3$-Atrazine
d_5-Alprazolam	23.1	314.1	285.9	$^{13}C_3$-Atrazine
d_5-Alprazolam*	23.1	314.1	209.9	$^{13}C_3$-Atrazine

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d_5-Norfluoxetine	24.7	301.0	138.7	$^{13}C_3$-Atrazine
d_5-Diazepam	29.1	290.1	197.9	$^{13}C_3$-Atrazine
d_5-Diazepam*	29.1	290.1	153.8	$^{13}C_3$-Atrazine
Recovery Standards				
$^{13}C_3$-Atrazine	18.8	219.5	176.9	External Standard
$^{13}C_3$-Atrazine *	18.8	219.5	134.0	External Standard

* Indicates secondary transition for possible diagnostic use.

QUALITY ACCEPTANCE CRITERIA

QC Acceptance Limits

	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
List 1 Compounds (APOS)						
Acetaminophen	70	140	70	140	30	≤60
Ampicillin [2]						
Azithromycin	10	130	10	130	130	≤1.5
Caffeine	25	160	35	150	60	≤15
Carbadox	25	180	35	180	40	≤1.5
Carbamazepine	25	200	35	200	40	≤1.5
Cefotaxime	10	300	10	300	60	≤6
Ciprofloxacin	25	180	35	180	40	≤6
Clarithromycin	50	160	50	160	30	≤1.5
Clinafloxacin	25	300	35	300	70	≤6
Cloxacillin	35	160	40	150	50	≤3
Dehydronifedipine	35	160	40	160	30	≤0.6
Digoxigenin	50	150	60	140	30	≤6
Digoxin	10	300	10	300	30	≤15
Diltiazem	20	160	25	160	50	≤0.3
1,7-Dimethylxanthine	30	300	40	300	60	≤150
Diphenhydramine	30	200	35	180	50	≤0.6
Enrofloxacin	30	220	40	220	40	≤3
Erythromycin - H_2O	70	130	70	130	30	≤0.3
Flumequine	40	160	50	160	30	≤1.5
Fluoxetine	60	150	70	140	30	≤1.5
Lincomycin	10	300	10	300	70	≤3
Lomefloxacin	50	250	60	250	30	≤3
Miconazole	35	130	40	130	30	≤1.5
Norfloxacin	10	250	25	220	40	≤15
Norgestimate	35	130	40	130	30	≤3
Ofloxacin	60	250	70	250	30	≤1.5
Ormetoprim	70	150	70	150	30	≤0.6
Oxacillin	20	130	20	130	40	≤3
Oxolinic Acid	60	150	70	150	30	≤0.6
Penicillin G	10	130	10	130	40	≤3
Penicillin V	40	140	50	140	30	≤3
Roxithromycin	50	140	50	140	30	≤0.3

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Sarafloxacin	50	200	60	180	30	≤15
Sulfachloropyridazine	60	160	70	160	30	≤1.5
Sulfadiazine	70	130	70	130	30	≤1.5
Sulfadimethoxine	35	160	40	160	30	≤0.3
Sulfamerazine	60	140	60	140	30	≤0.6
Sulfamethazine	70	130	70	130	30	≤0.6
Sulfamethizole	30	140	35	140	30	≤0.6
Sulfamethoxazole	70	130	70	130	30	≤0.6
Sulfanilamide	2	160	3	150	150	≤15
Sulfathiazole	30	180	30	160	50	≤1.5
Thiabendazole	60	150	60	150	30	≤1.5
Trimethoprim	50	150	60	150	30	≤1.5
Tylosin	10	180	20	180	40	≤6
Virginiamycin	15	300	15	250	90	≤3
Surrogate Standard						
$^{13}C_2$, ^{15}N-Acetaminophen	30	160	40	150	30	
$^{13}C_3$-Caffeine	40	140	50	140	30	
d_{10}-Carbamazepine-10,11-epoxide						
$^{13}C_3$, ^{15}N-Ciprofloxacin	7	150	9	140	70	
$^{13}C_2$-Erythromycin - H_2O	35	130	35	130	30	
d_5-Fluoxetine	40	130	50	130	30	
$^{13}C_6$-Sulfamethazine	30	160	35	150	40	
$^{13}C_6$-Sulfamethoxazole	30	140	40	130	30	
d_6-Thiabendazole	25	180	30	160	50	
$^{13}C_3$-Trimethoprim	30	140	40	130	30	
Recovery Standard						
$^{13}C_3$-Atrazine						
List 2 Compounds (TCYS)						
Anhydrochlortetracycline (ACTC)	15	200	20	180	70	≤15
Anhydrotetracycline (ATC)	20	160	20	150	50	≤15
Chlortetracycline (CTC)	30	250	35	250	60	≤6
Demeclocycline	35	180	35	160	50	≤15
Doxycycline	35	180	40	180	40	≤6
Epianhydrochlortetracycline (EACTC)	6	130	7	130	70	≤60
Epianhydrotetracycline (EATC)	15	200	20	200	60	≤15
Epichlortetracycline (ECTC)	25	180	30	160	50	≤15
Epioxytetracycline (EOTC)	25	180	35	160	40	≤6
Epitetracycline (ETC)	35	200	40	180	40	≤6
Isochlortetracycline (ICTC)	25	180	35	160	40	≤6
Minocycline	1	250	2	200	110	≤60
Oxytetracycline (OTC)	20	200	30	200	40	≤6
Tetracycline (TC)	20	200	30	180	40	≤6
Surrogate Standard						
d_6-Thiabendazole	25	140	25	130	50	

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Recovery Standard						
$^{13}C_3$-Atrazine						
	OPR Recovery and surrogate recovery in sample		**IPR**			**Blank Level (ng)**
			Average Recovery (%)		**RSD (%)**	
	Low	**High**	**Low**	**High**		
List 3 Compounds (ANEG)						
Bisphenol A	70	130	70	130	30	≤2500
Furosemide	65	130	70	130	30	≤40
Gemfibrozil	60	140	70	130	30	≤1.5
Glipizide	55	170	60	160	30	≤6
Glyburide	50	180	55	170	30	≤3
Hydroxychlorothiazide	70	200	70	180	30	≤20
2-hydroxy-ibuprofen	70	130	70	130	30	≤80
Ibuprofen	70	130	70	130	30	≤15
Naproxen	50	150	60	150	30	≤3
Triclocarban	60	140	70	130	30	≤3
Triclosan	70	130	70	130	30	≤60
Warfarin	70	140	70	140	30	≤1.5
Surrogate Standards						
d_6-Bisphenol A	50	170	60	160	30	
d_6-Gemfibrozil	50	150	55	140	30	
d_3-Glyburide	20	160	25	150	40	
d_{11}-Glipizide	30	180	35	170	50	
$^{13}C_3$-Ibuprofen	50	140	55	140	30	
^{13}C-d_3-Naproxen	30	150	35	140	30	
$^{13}C_6$-Triclocarban	20	160	25	150	50	
$^{13}C_{12}$-Triclosan	20	160	30	150	40	
d_5-Warfarin	35	250	50	250	30	
Recovery Standard						
$^{13}C_6$-2,4,5-Trichloro-phenoxyacetic acid						
List 4 Compounds (BPOS)						
Albuterol	50	160	50	160	30	≤0.3
Amphetamine	50	160	60	150	30	≤1.5
Atenolol	70	130	70	130	30	≤0.6
Atorvastatin	20	130	25	130	40	≤1.5
Cimetidine	15	130	20	130	50	≤0.6
Clonidine	70	130	70	130	30	≤1.5
Codeine	70	130	70	130	30	≤3
Cotinine	70	130	70	130	30	≤1.5
Enalapril	70	130	70	130	30	≤0.3
Hydrocodone	70	130	70	130	30	≤1.5
Metformin	70	160	70	160	30	≤30
Oxycodone	65	130	70	130	30	≤0.6
Ranitidine	25	140	30	140	50	≤0.6

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Triamterene	70	140	70	140	30	≤0.3
Surrogate Standards						
d_3-Albuterol	20	140	30	130	30	
d_5-Amphetamine	20	130	25	130	40	
d_7-Atenolol	70	130	70	130	30	
d_3-Cimetidine	15	130	15	130	50	
d_4-Clonidine	70	130	70	130	30	
d_6-Codeine	70	130	70	130	30	
d_3-Cotinine	70	140	70	135	30	
d_5-Enalapril	65	130	70	130	30	
d_3-Hydrocodone	70	130	70	130	30	
d_6-Metformin	3	130	4	130	130	
d_6-Oxycodone	50	150	60	140	30	
Recovery Standards						
d_3-Amitriptyline						
List 5 Compounds (APOS)						
Alprazolam	70	130	70	130	30	≤0.3
Amitriptyline	70	130	70	130	30	≤0.3
Amlodipine	45	130	50	130	30	≤1.5
Benzoylecgonine	70	130	70	130	30	≤0.3
Benztropine	70	130	70	130	30	≤0.3
Betamethasone	20	240	30	220	40	≤1.5
Cocaine	70	130	70	130	30	≤0.15
DEET	70	130	70	130	30	≤0.15
Desmethyldiltiazem	3	350	5	320	80	≤0.15
Diazepam	70	130	70	130	30	≤0.3
Fluocinonide	7	230	9	220	70	≤6
Fluticasone propionate	20	160	25	150	50	≤2
Hydrocortisone	15	220	20	200	80	≤60
10-hydroxy-amitriptyline	70	130	70	130	30	≤0.15
Meprobamate	65	150	70	140	30	≤4
Methylprednisolone	35	240	40	220	50	≤4
Metoprolol	70	130	70	130	30	≤1.5
Norfluoxetine	70	130	70	130	30	≤1.5
Norverapamil	55	130	60	130	30	≤0.15
Paroxetine	70	130	70	130	30	≤4
Prednisolone	35	240	40	220	50	≤6
Prednisone	50	180	60	170	30	≤20
Promethazine	70	130	70	130	30	≤0.4
Propoxyphene	70	130	70	130	30	≤0.3
Propranolol	70	150	70	150	30	≤2
Sertraline	50	130	55	130	30	≤0.4
Simvastatin	1	150	1	140	100	≤20
Theophylline	10	1000	70	900	50	≤60
Trenbolone	70	140	70	135	30	≤4

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Trenbolone acetate	55	130	60	130	30	≤0.3
Valsartan	70	130	70	130	30	≤4
Verapamil	70	145	70	140	30	≤0.15
Surrogate Standards						
d_5-Alprazolam	45	130	45	130	30	
d_6-Amitriptyline	10	130	20	130	40	
d_8-Benzoylecgonine	10	170	20	160	40	
d_3-Benztropine	20	140	25	130	40	
d_3-Cocaine	25	140	30	130	50	
d_7-DEET	15	160	20	150	40	
d_5-Diazepam	15	160	25	150	40	
d_4-Hydrocortisone	40	240	45	230	50	
d_2-Methylprednisolone	15	160	20	150	60	
d_7-Metoprolol	25	140	30	140	30	
d_5-Norfluoxetine	20	130	20	130	50	
d_6-Paroxetine	7	150	9	140	60	
d_4-Promethazine	3	140	5	130	80	
d_5-Propoxyphene	30	130	40	130	30	
d_7-Propranolol	25	140	30	130	30	
$^{13}C_1$, $^{15}N_2$-Theophylline	20	200	25	180	60	
Recovery Standards						
$^{13}C_3$-Atrazine						

[1] OPR and IPR limits derived from actual method performance data according to EPA 821B98003, appendix D.

[2] Because of very low stability the accuracy of Ampicillin is not known. The analysis result is classified as “Information Value” only.

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Instrumental Acceptance Specifications

QC Parameter	Specification
Instrument Sensitivity	Daily, S:N ≥ 3:1 for all analytes for lowest calibration point.
Initial Calibration (native compounds)	Initial, (1/X) weighted linear regression (followed by regular Cal/Ver procedures and repeated as necessary to maintain Cal/Ver results within established acceptance ranges. Calculated concentrations 70-130%, one point per compound may be 60-140% Internal guideline - correlation coefficient >0.985. Calibration curves with lower correlation coefficient values meeting all above criteria may be accepted based on batch specific QC results and professional judgement
OPENING Calibration Verification	Every 20 samples, determined concentrations within 30% of actual concentrations. Professional judgment allowed for wider acceptance limits.
CLOSING Calibration Verification	Within OPENING Calibration Verification specifications. Allowable exception: results for the greater of 1 compound or 10% of the compounds on a Compound List (1,2,3,4,5) may fall outside the Opening Calibration Verification specification provided the RPD between the CLOSING result and the OPENING result is <30%.
Instrumental Carryover And Instrument Background	Every Initial Calibration, Cal/Ver, or SPM: < 0.3 % carryover and area response of analytes in instrument blank < 800 judged following two previous methanol blank injections.

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QUANTIFICATION AND DATA REPORTING PROCEDURES

Positive identification of target natives, surrogate standard and recovery standards require:

- ≥ 3:1 S:N for parent ion to daughter ion transition, on condition that the result is above the lowest calibration standard level.
- Compound retention time falls within 0.4 minutes of the predicted retention times from the daily calibration standard. Natives with labelled surrogate standards must elute within 0.1 minutes of the associated labelled surrogates.

Concentrations of the targets and surrogates are calculated by isotope dilution or internal standard quantification with linear regression calibration, using a 1/X weighting type, excluding origin. Sample specific detection limits (SDLs) are calculated by QuanLynx software using 3 times the signal of the noise in the target channel converted to an equivalent sample concentration.

General equation : $Y = \text{slope} \times X + \text{intercept}$

Where: $Y = \text{Response ratio} = \left(\frac{\text{area of Target}}{\text{area of Quant. Std}} \times \text{weight of Quant. std (ng)} \right)$

X = weight of target (ng)

Quant. Std = labelled surrogate or recovery standard

The slope and intercept are used to convert raw peak areas in sample chromatograms to final concentrations as follows:

$$\text{Sample Conc.} = \left(\frac{\text{area of Target}}{\text{area of Quant. Std}} \times \text{weight of Quant. Std (ng)} - \text{intercept} \right) \text{x} \left(\frac{1}{\text{slope}} \right) \text{x} \left(\frac{1}{\text{sample size (L)}} \right)$$

The recovery of surrogate standards, calculated from the determined concentration of the surrogate in the extract relative to the amount spiked, are monitored as an indication of overall data quality.

The lower reporting limit for each target compound is defined as the concentration equivalent to the lowest calibration standard analyzed or the SDL, whichever is greater.

Narrative Appendix – Details of Hydrocodone/Codeine Correction

Summary

There is significant analytical cross-interference between hydrocodone and codeine in Axys Method MLA-075. This interference arises from the chemical similarity of these compounds. The compounds have the same molecular weight and chemical formula $C_{18}H_{21}NO_3$. Due to this structural similarity, they are not chromatographically separated on the HPLC column used in this analysis. The quantitation transitions for each of these compounds also show mass spectrometric interferences from the presence of the other compound. AXYS has determined that the extent of this interference is constant across the concentration range of the method, except close to the reporting limit where there is increased uncertainty. As the extent of interference is constant, a correction can be applied to the concentration as detailed below. If the amount of area correction is more than half the original area response, AXYS reports the result as not detected with a detection limit equal to the calculated concentration value plus 1% (the 1% is added to facilitate automated data processing).

Note the raw data in this data package contains uncorrected areas and concentrations. All corrections have been made using automated procedures within the LIMS (Laboratory Information Management System).

Area Correction

$H = \frac{Y - aX}{1 - ab}$ $C = \frac{X - bY}{1 - ab}$ where

X , Y - Observed areas of codeine and hydrocodone

C, H - Corrected areas for codeine and hydrocodone

a, b – Cross Interference constants, a = 0.562 (codeine in hydrocodone) b = 0.172 (hydrocodone in codeine).

Correction of Linearity

Since ratio of codeine:hydrocodone concentration is constant in the linearity, linearity slope is reduced for each compound by a constant R = 0.730 for hydrocodone and 0.790 for codeine.

Concentration

$C_{corr} = \frac{C_{uncorr} * A_{corr}}{R * A_{uncorr}}$ A_{corr} is H or C, A_{uncorr} is X or Y, and R is the linearity correction.

Correction Limits

For hydrocodone, if $\frac{Y - H_{199}}{Y} > 0.5$, concentration will be reported as ND < Y.

For codeine, if $\frac{X - C_{152}}{X} > 0.5$, concentration will be reported as ND < X.

Example Calculations

Reported Codeine Concentration (ng/L)	Reported Hydrocodone Concentration (ng/L)	Reported Codeine Area	Reported Hydrocodone Area
47.0	6.78	32900	21019

After applying correction procedure

Corrected Codeine Area	Corrected Hydrocodone Area	Corrected Codeine Concentration (ng/L)	Corrected Hydrocodone Concentration (ng/L)
32418	2800	58.6	ND < 6.78

PHARMACEUTICAL AND PERSONAL CARE PRODUCT ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-075

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG31221

Analysis WG30659, WG30661

10 December 2009

PHARMACEUTICAL AND PERSONAL CARE PRODUCT ANALYSIS

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Analysis WG30659, WG30661

Prepared for:
ORSANCO

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CANADA

Contact: Candice Navaroli
Project Manager

10 December 2009

OHIO RIVER VALLEY WATER SANITATION COMMISSION
AQUEOUS SAMPLES

PHARMACEUTICAL AND PERSONAL-CARE PRODUCTS ANALYSIS
AXYS METHOD: MLA-075
4562: L13813-1 and -2

Project Name: Emerging Contaminants in Mainstem Ohio

10 December 2009

NARRATIVE

This narrative describes the analysis of two aqueous samples for the determination of pharmaceutical and personal-care products using High Performance Liquid Chromatography coupled with tandem Mass Spectrometry (LC- MS/MS).

SAMPLE RECEIPT AND STORAGE

The samples were received on the 22nd and 23rd of October 2009. The temperature for sample Site 25 (AXYS ID L13813-2) was 5 °C on receipt, which was slightly above the method requirement <4°C. Temperature effect on the samples is unknown. Analyses of the sample proceeded as per client's instruction. Details of sample conditions upon receipt are provided on the Sample Receiving Record forms included in the sample documentation section of this data package. The samples were stored at 4 °C prior to sample preparation and analysis.

SAMPLE PREPARATION AND ANALYSIS

Extraction and analysis procedures were in accordance with AXYS Method MLA-075: ***Analytical Procedure for the Analysis of Pharmaceutical and Personal Care Products in Solid and Aqueous Samples by LC-MS/MS.*** A method summary (MSU-075) for the AXYS Method MLA-075 in included in this data package.

Samples and QC samples (a procedural blank and a lab-generated reference sample known as the Ongoing Precision and Recovery (OPR) per analysis batch) were analyzed in two analysis batches named WG30659 and WG30661 for acid- and base-extracted pharmaceutical products, respectively. Composition of each analysis batch is shown on the Cover Page and Correlation Table, and on the Batch List that accompanies the extraction workup sheets.

Seastar ultra pure water was used as the matrix for the Procedural Blanks and the OPRs.

Two aliquots of accurately weighed sub-sample for each sample (approximately 1 liter) were adjusted to pH2.0-3.5 and pH 10 in analysis batches WG30659 and WG30661, respectively. Each extract was spiked with labeled quantification standards and extracted/cleaned up on Waters Oasis HLB SPE cartridges. The final extract was reduced in volume and spiked with labeled recovery (internal) standards prior to instrumental analysis.

Analysis was performed on Waters 2690 or 2795 HPLC equipped with Micromass Quattro Ultima MS/MS using five instrument and LC conditions for various lists of analytes as shown in table below.

Instrument and LC Conditions

Analyte Group	LC Column	Ionization	Acquisition	LC Conditions
List 1	Waters Xtera C18 (10.0 cm, 2.1 mm i.d., 3.5 µm particle size)	Positive Ion Electrospray	MRM mode, unit resolution	1
List 2	Waters Xtera C18 (10.0 cm, 2.1 mm i.d., 3.5 µm particle size)	Positive Ion Electrospray	MRM mode, unit resolution	2
List 3	Waters Xtera C18MS (10.0 cm, 2.1 mm i.d., 3.5 µm particle size)	Negative Ion Electrospray	MRM mode, unit resolution	3
List 4	Waters Atlantis HILIC (10.0 cm, 2.1 mm i.d., 3.0 µm particle size)	Positive Ion Electrospray	MRM mode, unit resolution	4
List 5	Waters Xtera C18 (10.0 cm, 2.1 mm i.d., 3.5 µm particle size)	Positive Ion Electrospray	MRM mode, unit resolution	5

CALCULATION

Target analyte concentrations were determined by isotope dilution or internal standard quantification procedures using MassLynx software. Quantification was conducted by comparing the area of the quantification ion to that of the quantification standard (surrogate) and correcting for response factors.

For all target compounds, linear equations were determined from a multi-point calibration series with 1/X weighting fit and expressed as below:

$$Y = \text{slope} \times X + \text{intercept}$$

$$\text{Where: } Y = \text{Response ratio} = \left(\frac{\text{area of Target}}{\text{area of Quant. Std}} \times \text{weight of Quant. std (ng)} \right)$$

X = weight of target (ng)

The slope and intercept were used to convert raw peak areas in sample chromatograms to final concentrations (ng/L) as follows:

$$\text{Sample Conc.} = \left(\frac{\text{area of Target}}{\text{area of Quant. Std}} \times \text{weight of Quant. Std (ng)} - \text{intercept} \right) x \left(\frac{1}{\text{slope}} \right) x \left(\frac{1}{\text{samplesize(L)}} \right)$$

The recovery of the surrogate standard was calculated and monitored as an indication of overall method performance.

Sample specific detection limits (SDLs) were calculated for each target analyte and used as the detection qualifier. If the MassLynx software selected an unrepresentative area for the detection limit calculation, the data interpretation chemist or the QA chemist made corrections. These corrections are hand noted on the quantification report pages attached to the chromatograms.

The lower reporting limit for each target compound is defined as the concentration equivalent to the lowest calibration standard (LMCL) analyzed, prorated for the extract volume and sample size, or the SDL, whichever is greater.

REPORTING CONVENTIONS

The AXYS contract number assigned for internal tracking was 4562. The samples were assigned a unique laboratory identifier of the form L13813-X, where X is a numeral. All data reports reference the unique AXYS IDs plus the client sample identifiers.

Any extra work required and performed after the initial instrumental analysis of the sample's extract is given an extra "test suffix" code. The single letter code per extra work performed is added to the AXYS sample ID as a suffix, and is combined with any other applicable test suffix codes. The extra work codes used to report data in this package include:

i = instrumental re-analysis of sample extract.

The following laboratory qualifier flags were used in this data package:

B = analyte found in sample and associated blank
N = authentic recovery is not within method/contract control limits
U = identifies a compound that was not detected.

Results are reported in concentration units of nanograms per liter (ng/L). Concentration and reporting limits are provided to three significant figures.

QA/QC NOTES

The samples and associated QC samples analyzed in an analysis batch were carried intact through the entire analytical process. The sample data were reviewed and evaluated in relation to the batch QC samples.

- Sample analyte concentrations are not blank corrected. The data should be evaluated with consideration of the procedural blank results.
- By virtue of the isotope dilution/internal standard quantification procedures, data are recovery corrected for possible losses during extraction and cleanup.
- All linearity, calibration verification, OPR and labeled compound recovery specifications were met with the following exceptions:

  List 1 Compounds (WG30659): Concentration of Enrofloxacin detected in the Lab Blank (AXYS ID WG30659-101) was slightly above the method control limits. Given that Enrofloxacin was not detected in all client samples, sample Enrofloxacin data were not affected.

  List 5 Compounds (WG30659): The recovery of Trenbolone in the OPR (AXYS ID WG30659-102) was above the method upper control limit. This compound is flagged with an 'N' on the report form. Given that Trenbolone was not detected in all client samples, sample Trenbolone data were not impacted.

Due to ion suppression that caused significant drop of responses, some high-level calibration standards in the initial calibrations were excluded. However, a minimum of 5 calibration standard points was used to construct the linear equations for quantification of target analytes or to calculate response factor (RF) for quantification of labeled surrogates except for Digoxin. Four calibration points were used to construct linear calibration equations for Digoxin. Since multiple calibrations were used, sample data were deemed not be significantly affected.

CS0 initial calibrations for Virginiamycin (data filename QA9J_209 S:3), EATC, Minocycline (QB9K_214 S:4), Atorvastatin and Clonidine (QG9K_217 S:7), and CS0 and CS1 initial calibrations for DEET (QE9J_203 S:3 and S:4 respectively) were excluded as their responses were lower than method requirements. As a result, CS1 level calibration was the LMCL for all these analytes except DEET and data reported to the higher of CS1 calibration level or SDL; CS2 level calibration was the LMCL for DEET and data reported to the higher of CS2 calibration level or SDL.

The Signal/Noise (S/N) ratios were measured as '0' for some compounds in QC and sample data. This is determined to be a limitation of the software that noise is not picked. The noise for those compounds was inspected and S/N values met the method specifications. Data is not affected.

Concentrations and detection limits of Codeine and Hydrocodone have been recalculated to remove cross-interferences between the two analytes. Details of the recalculation are provided in a Narrative Appendix following this narrative.

ANALYTICAL DISCUSSION

List 1 Compounds (WG30659)

No analytical difficulty was encountered.

List 2 Compounds (WG30659)

No analytical difficulty was encountered.

List 3 Compounds (WG30659)

No analytical difficulty was encountered.

List 4 Compounds (WG30661)

Extracts for all client samples and QC samples were re-analyzed on instrument for confirmative purpose. Extracts for the QC samples were routinely re-analyzed for a second time on instrument. Data obtained in the re-analysis are reported (indicated by the suffix 'i' or 'i2' on the AXYS ID).

List 5 Compounds (WG30659)

No analytical difficulty was encountered.

DATA PACKAGE

This data package has been assigned a unique identifier, DPWG31221, shown on the cover page. Included in this data package following the narrative is the following documentation:

- Method summary
- Sample 'Cover Page' and 'Correlation Table'
- Sample Receiving Documentation
- Laboratory extraction worksheets
- Sample data reports (in order of AXYS Sample ID)
- Laboratory QC data reports
- Instrument run (injection) log
- Instrumental QC data reports (organized by analysis date)
- Sample raw data (in order of AXYS Sample ID)
- Laboratory QC raw data
- Instrumental QC raw data (organized by analysis date)

I certify that this data package is in compliance with the terms and conditions of the contract, both technically and for completeness, except for the conditions detailed above. In addition, I certify, that to the best of my knowledge and belief, the data as reported are true and accurate. The following signature, on behalf of AXYS Analytical Services Ltd, authorizes the release of the data contained in this data package.

Signed: (Matthew) Ziqing Ou, PhD, QA/QC Chemist

December 15, 2009
Date Signed

Narrative Appendix – Details of Hydrocodone/Codeine Correction

Summary

There is significant analytical cross-interference between hydrocodone and codeine in Axys Method MLA-075. This interference arises from the chemical similarity of these compounds. The compounds have the same molecular weight and chemical formula $C_{18}H_{21}NO_3$. Due to this structural similarity, they are not chromatographically separated on the HPLC column used in this analysis. The quantitation transitions for each of these compounds also show mass spectrometric interferences from the presence of the other compound. AXYS has determined that the extent of this interference is constant across the concentration range of the method, except close to the reporting limit where there is increased uncertainty. As the extent of interference is constant, a correction can be applied to the concentration as detailed below. If the amount of area correction is more than half the original area response, AXYS reports the result as not detected at the level of the detected result multiplied by a factor of 1%.

Note the raw data in this data package contains uncorrected areas and concentrations. All corrections have been made using automated procedures within the LIMS (Laboratory Information Management System).

Area Correction

$$H = \frac{Y - aX}{1 - ab} \text{ and } C = \frac{X - bY}{1 - ab}$$

where
X , Y - Observed areas of codeine and hydrocodone
C, H - Corrected areas for codeine and hydrocodone
a, b – Cross Interference constants, a = 0.562 (codeine in hydrocodone) b = 0.172 (hydrocodone in codeine).

Correction of Linearity

Since ratio of codeine:hydrocodone concentration is constant in the linearity, linearity slope is reduced for each compound by a constant R = 0.730 for hydrocodone and 0.790 for codeine.

Correction of Concentrations

$$C_{corr} = \frac{C_{uncorr} * A_{corr}}{R * A_{uncorr}}$$

where A_{corr} is H or C, A_{uncorr} is X or Y, and R is the linearity correction.

Correction of Detection Limits

For hydrocodone, if $\frac{Y - H_{199}}{Y} > 0.5$, concentration will be reported as ND < Y.

For codeine, if $\frac{X - C_{152}}{X} > 0.5$, concentration will be reported as ND < X.

Example Calculations

Reported Codeine Concentration (ng/L)	Reported Hydrocodone Concentration (ng/L)	Reported Codeine Area	Reported Hydrocodone Area
47.0	6.78	32900	21019

After applying correction procedure

Corrected Codeine Area	Corrected Hydrocodone Area	Corrected Codeine Concentration (ng/L)	Corrected Hydrocodone Concentration (ng/L)
32418	2800	58.6	ND < 6.78

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SUMMARY OF AXYS METHOD MLA-075:

ANALYTICAL PROCEDURES FOR THE ANALYSIS OF PHARMACEUTICAL AND PERSONAL CARE COMPOUNDS IN SOLID AND AQUEOUS SAMPLES BY LC-MS/MS

ANALYTE LISTS

List 1 (Acid extraction, positive ESI)	
Acetaminophen	Norfloxacin
Ampicillin [1]	Norgestimate
Azithromycin	Ofloxacin
Caffeine	Ormetoprim
Carbadox	Oxacillin
Carbamazepine	Oxolinic acid
Cefotaxime	Penicillin G
Ciprofloxacin	Penicillin V
Clarithromycin	Roxithromycin
Clinafloxacin	Sarafloxacin
Cloxacillin	Sulfachloropyridazine
Dehydronifedipine	Sulfadiazine
Digoxigenin	Sulfadimethoxine
Digoxin	Sulfamerazine
Diltiazem	Sulfamethazine
1,7-Dimethylxanthine	Sulfamethizole
Diphenhydramine	Sulfamethoxazole
Enrofloxacin	Sulfanilamide
Erythromycin	Sulfathiazole
Flumequine	Thiabendazole
Fluoxetine	Trimethoprim
Lincomycin	Tylosin
Lomefloxacin	Virginiamycin
Miconazole	
List 2 (Tetracyclines, positive ESI)	
Anhydrochlortetracycline (ACTC)	4-Epichlortetracycline (ECTC)
Anhydrotetracycline (ATC)	4-Epioxytetracycline (EOTC)
Chlortetracycline (CTC)	4-Epitetracycline (ETC)
Demeclocycline	Isochlortetracycline (ICTC)
Doxycycline	Minocycline
4-Epianhydrochlortetracycline (EACTC)	Oxytetracycline (OTC)
4-Epianhydrotetracycline (EATC)	Tetracycline (TC)

List 3 (Acid extraction, negative ESI)	
Bisphenol A	2-hydroxy-ibuprofen
Furosemide	Ibuprofen
Gemfibrozil	Naproxen
Glipizide	Triclocarban
Glyburide	Triclosan
Hydrochlorothiazide	Warfarin
List 4 (Base extraction, positive ESI)	
Albuterol	Cotinine
Amphetamine	Enalapril
Atenolol	Hydrocodone
Atorvastatin	Metformin
Cimetidine	Oxycodone
Clonidine	Ranitidine
Codeine	Triamterene
List 5 (Acid Extraction, positive ESI)	
Alprazolam	Metoprolol
Amitriptyline	Norfluoxetine
Amlodipine	Norverapamil
Benzoylecgonine	Paroxetine
Benztropine	Prednisolone
Betamethasone	Prednisone
Cocaine	Promethazine
DEET (N,N-diethyl-m-toluamide)	Propoxyphene
Desmethyldiltiazem	Propranolol
Diazepam	Sertraline
Fluocinonide	Simvastatin
Fluticasone propionate	Theophylline
Hydrocortisone	Trenbolone
10-hydroxy-amitriptyline	Trenbolone acetate
Meprobamate	Valsartan
Methylprednisolone	Verapamil

[1] Due to instability accuracy of Ampicillin data is unknown.

EXTRACTION AND CLEANUP PROCEDURES

The analysis requires extraction at two different pH conditions: At pH 10 for analysis of fourteen analytes (List 4); and at pH 2.0 for the analysis of the other analytes (Lists 1, 2, 3, and 5). Prior to extraction and/or clean-up samples are adjusted to the required pH and spiked with surrogates.

Solid samples are repeatedly extracted by sonication with aqueous buffered acetonitrile and pure acetonitrile, concentrated by rotary evaporation, and diluted with ultra pure water to 200 mL. The acidic extract is treated with EDTA. The extracts are cleaned up by solid phase extraction (SPE), filtered, and analyzed by LC/ESI-MS/MS in positive and negative ionization modes requiring a total of five runs to analyze the complete list of analytes.

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All aqueous samples are filtered and the aqueous portion is cleaned up by solid phase extraction before analysis by LC/ESI-MS/MS.

Aqueous samples with no or limited visible particulate (e.g. surface water, ground water, wastewater treatment final effluent, typically with < 100 mg/L TSS) normally can be processed with up to 1L samples sizes. The sample is filtered and routinely only the aqueous phase is analyzed. However, upon specific agreement a separate extraction may be performed on the solids phase. The solids extract may in this case either be carried through the analysis individually as a separate sample that is reported separately, or the aqueous extract and the solids extract may be combined just prior to clean-up and reported as a combined aqueous/solids phase result.

For mixed phase aqueous/solids samples with significant solids and distinct aqueous and solids phases such as wastewater influent or process streams the sample may either be analyzed as an aqueous phase only or as two separate samples, one aqueous and one solid.

ANALYSIS BY LC-MS/MS

The analysis is performed on a high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer. Instrument calibration is performed using a series of calibration solutions (7 points) covering the working concentration range of the instrument specific for the individual compounds of interest. The LC/MS/MS is run in MRM (Multiple Reaction Monitoring) mode and quantification is performed by recording the peak areas of the applicable parent ion/daughter ion transitions. Some analytes are analyzed in the ESI positive mode and some are analyzed in the ESI negative mode.

List 1 – Acid Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Sulfanilamide	2.5	190.0	155.8	$^{13}C_6$-Sulfamethazine
Acetaminophen	4.6	152.2	110.0	$^{13}C_2$-^{15}N-Acetaminophen
Sulfadiazine	6.0	251.2	156.1	$^{13}C_6$-Sulfamethazine
1,7-Dimethylxanthine	6.9	181.2	124.0	$^{13}C_3$-Caffeine
Sulfathiazole	7.7	256.3	156.0	$^{13}C_6$-Sulfamethoxazole
Sulfamerazine	8.7	265.0	156.0	$^{13}C_6$-Sulfamethazine
Caffeine	9.3	195.0	138.0	$^{13}C_3$-Caffeine
Lincomycin	9.3	407.5	126.0	$^{13}C_3$-Trimethoprim
Sulfamethizole	10.0	271.0	156.0	$^{13}C_6$-Sulfamethoxazole
Thiabendazole	10.0	202.1	175.1	d_6-Thiabendazole
Trimethoprim	10.0	291.0	230.0	$^{13}C_3$-Trimethoprim
Sulfamethazine	10.1	279.0	156.0	$^{13}C_6$-Sulfamethazine
Cefotaxime	10.2	456.4	396.1	$^{13}C_3$-Trimethoprim
Carbadox	10.5	263.2	231.2	$^{13}C_3$-Trimethoprim
Ormetoprim	10.5	275.3	259.1	$^{13}C_3$-Trimethoprim
Norfloxacin	10.7	320.0	302.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Ofloxacin	10.8	362.2	318.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sulfachloropyridazine	10.8	285.0	156.0	$^{13}C_6$-Sulfamethazine
Ciprofloxacin	10.9	332.2	314.2	$^{13}C_3$, ^{15}N-Ciprofloxacin
Lomefloxacin	11.2	352.2	308.1	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sulfamethoxazole	11.2	254.0	156.0	$^{13}C_6$-Sulfamethoxazole

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Enrofloxacin	11.5	360.0	316.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Sarafloxacin	11.9	386.0	299.0	$^{13}C_3$, ^{15}N-Ciprofloxacin
Clinafloxacin	12.1	366.3	348.1	$^{13}C_3$, ^{15}N-Ciprofloxacin
Digoxigenin	12.6	391.2	355.2	$^{13}C_3$-Trimethoprim
Oxolinic Acid	13.1	261.8	243.8	$^{13}C_3$-Trimethoprim
Sulfadimethoxine	13.2	311.0	156.0	$^{13}C_6$-Sulfamethoxazole
Diphenhydramine	14.5	256.8	168.1	$^{13}C_3$-Trimethoprim
Penicillin G	14.6	367.5	160.2	$^{13}C_3$-Trimethoprim
Azithromycin	14.8	749.9	591.6	$^{13}C_3$-Trimethoprim
Flumequine	15.2	262.0	173.7	$^{13}C_3$-Trimethoprim
Ampicillin	15.3	350.3	160.2	$^{13}C_3$-Trimethoprim
Carbamazepine	15.3	237.4	194.2	$^{13}C_3$-Trimethoprim
Diltiazem	15.3	415.5	178.0	$^{13}C_3$-Trimethoprim
Penicillin V	15.4	383.4	160.2	$^{13}C_3$-Trimethoprim
Erythromycin [1]	15.9	734.4	158	not quantified
Tylosin	16.3	916.0	772.0	$^{13}C_6$-Sulfamethazine
Oxacillin	16.4	434.3	160.1	$^{13}C_3$-Trimethoprim
Dehydronifedipine	16.5	345.5	284.1	$^{13}C_3$-Trimethoprim
Digoxin	16.6	803.1	283.0	$^{13}C_3$-Trimethoprim
Cloxacillin	16.9	469.1	160.1	$^{13}C_3$-Trimethoprim
Fluoxetine	16.9	310.3	148.0	d_5-Fluoxetine
Virginiamycin	17.3	508.0	355.0	$^{13}C_3$-Trimethoprim
Clarithromycin	17.5	748.9	158.2	$^{13}C_6$-Sulfamethazine
Erythromycin - H_2O [1]	17.7	716.4	158	$^{13}C_2$-Erythromycin - H_2O
Roxithromycin	17.8	837.0	679.0	$^{13}C_6$-Sulfamethazine
Miconazole	20.1	417.0	161.0	$^{13}C_3$-Trimethoprim
Norgestimate	21.7	370.5	124.0	$^{13}C_3$-Trimethoprim
Surrogate Standard				
$^{13}C_2$-^{15}N-Acetaminophen	4.5	155.2	111.0	$^{13}C_3$-Atrazine
$^{13}C_3$-Caffeine	9.3	198.0	140.0	$^{13}C_3$-Atrazine
d_6-Thiabendazole	9.8	208.1	180.1	$^{13}C_3$-Atrazine
$^{13}C_3$-Trimethoprim	10.0	294.0	233.0	$^{13}C_3$-Atrazine
$^{13}C_6$-Sulfamethazine	10.1	285.1	162.1	$^{13}C_3$-Atrazine
$^{13}C_3$, ^{15}N-Ciprofloxacin	10.9	336.1	318.2	$^{13}C_3$-Atrazine
$^{13}C_6$-Sulfamethoxazole	11.2	260.0	162.0	$^{13}C_3$-Atrazine
$^{13}C_2$-Erythromycin [1]	15.9	736.4	160.0	monitor for less than 5%
d_5-Fluoxetine	16.8	315.3	153.0	$^{13}C_3$-Atrazine
$^{13}C_2$-Erythromycin - H_2O [1]	17.7	718.4	160.0	$^{13}C_3$-Atrazine
Recovery Standard				
$^{13}C_3$-Atrazine	15.9	219.5	176.9 (134.0)	External Standard

[1] Because of intramolecular dehydration during the analytical procedure erythromycin is quantified as the dehydration product "erythromycin – H_2O" [5]. The peak area of the $^{13}C_2$-Erythromycin is monitored and must be less than 5% of the $^{13}C_2$-Erythromycin - H_2O peak area. If it is greater, the Erythromycin - H_2O result is flagged as 'accuracy unknown'.

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List 2 – Acid Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Minocycline	5.1	458.0	441.0	d_6-Thiabendazole
Epitetracycline (ETC)	8.1	445.2	410.2	d_6-Thiabendazole
Epioxytetracycline (EOTC)	8.6	461.2	426.2	d_6-Thiabendazole
Oxytetracycline (OTC)	9.4	461.2	426.2	d_6-Thiabendazole
Tetracycline (TC)	9.9	445.2	410.2	d_6-Thiabendazole
Demeclocycline	11.7	465.0	430.0	d_6-Thiabendazole
Isochlortetracycline (ICTC) [1]	11.9	479.0	462.0	d_6-Thiabendazole
Epichlortetracycline (ECTC)	12.0	479.0	444.0	d_6-Thiabendazole
Chlortetracycline (CTC)	14.1	479.0	444.0	d_6-Thiabendazole
Doxycycline	16.7	445.2	428.2	d_6-Thiabendazole
Epianhydrotetracycline (EATC)	17.0	426.8	409.8	d_6-Thiabendazole
Anhydrotetracycline (ATC)	18.8	426.8	409.8	d_6-Thiabendazole
Epianhydrochlortetracycline (EACTC)	20.7	461.2	444.0	d_6-Thiabendazole
Anhydrochlortetracycline (ACTC)	22.1	461.2	444.0	d_6-Thiabendazole
Surrogate Standard				
d_6-Thiabendazole	7.1	208.1	180.1	$^{13}C_3$-Atrazine
Recovery Standard				
$^{13}C_3$-Atrazine	21.2	219.5	176.9	External Standard

[1] Isochlortetracycline (ICTC) is reported as the sum ICTC + ECTC due to a common transition ion.

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List 3 – Acid Extraction, Negative Electrospray Ionization (-)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Hydrochlorathiazide	2.3	296.0	268.0	^{13}C-d_3-Naproxen
Hydrochlorathiazide*	2.3	296.0	204.8	^{13}C-d_3-Naproxen
Furosemide	3.4	329.0	284.8	^{13}C-d_3-Naproxen
Furosemide*	3.4	329.0	204.7	^{13}C-d_3-Naproxen
2-hydroxy-ibuprofen	4.2	221.1	176.8	$^{13}C_3$-Ibuprofen
Bisphenol A	6.5	227.0	211.9	d6-Bisphenol A
Bisphenol A*	6.5	227.0	132.9	d6-Bisphenol A
Glipizide	6.9	444.2	319.0	d11-Glipizide
Glipizide*	6.9	444.2	169.8	d11-Glipizide
Naproxen	7.0	228.9	168.6	^{13}C-d_3-Naproxen
Warfarin	7.4	307.0	161.0	d_5-Warfarin
Glyburide	8.8	492.1	169.8	d3-Glyburide
Glyburide*	8.8	492.1	367.0	d3-Glyburide
Ibuprofen	8.8	205.1	161.1	$^{13}C_3$-Ibuprofen
Gemfibrozil	9.9	249.0	121.0	d_6-Gemfibrozil
Triclocarban	10.1	312.9	159.7	$^{13}C_6$-Triclocarban
Triclosan	10.2	286.8	35.0	$^{13}C_{12}$-Triclosan
Surrogate Standard				
d_6-Bisphenol A	6.5	233.0	214.8	$^{13}C_6$-2,4,5-T
d_6-Bisphenol A*	6.5	233.0	137.8	$^{13}C_6$-2,4,5-T
d_{11}-Glipizide	6.8	455.0	319.0	$^{13}C_6$-2,4,5-T
d_{11}-Glipizide*	6.8	455.0	169.8	$^{13}C_6$-2,4,5-T
^{13}C-d_3-Naproxen	7.0	232.9	168.6	$^{13}C_6$-2,4,5-T
d_5-Warfarin	7.4	312	161.0	$^{13}C_6$-2,4,5-T
d_3-Glyburide	8.7	495.0	169.9	$^{13}C_6$-2,4,5-T
d_3-Glyburide*	8.7	495.0	370.1	$^{13}C_6$-2,4,5-T
$^{13}C_3$-Ibuprofen	8.8	208.2	163.1	$^{13}C_6$-2,4,5-T
d_6-Gemfibrozil	9.9	255	121	$^{13}C_6$-2,4,5-T
$^{13}C_6$-Triclocarban	10.1	318.9	159.7	$^{13}C_6$-2,4,5-T
$^{13}C_{12}$-Triclosan	10.2	298.8	35	$^{13}C_6$-2,4,5-T
Recovery Standard				
$^{13}C_6$-2,4,5-Trichlorophen-oxyacetic acid ($^{13}C_6$-2,4,5-T)	4.9	258.8	200.7	External Standard

* Indicates secondary transition for possible diagnostic use.

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List 4 – Base Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention Time (min)	Parent Ion Mass	Daughter Ion Mass	Quantified against
Cotinine	4.0	177.0	98.0	d3-Cotinine
Cimetidine	4.7	253.1	159.0	d3-Cimetidine
Triamterene	5.4	254.1	236.9	d4-Clonidine
Triamterene*	5.4	254.1	103.7	d4-Clonidine
Enalapril	6.5	377.2	233.9	d5-Enalapril
Enalapril*	6.5	377.2	159.8	d5-Enalapril
Oxycodone	6.7	316.2	240.9	d6-Oxycodone
Oxycodone*	6.7	316.2	298.0	d6-Oxycodone
Clonidine	6.8	230.0	212.5	d4-Clonidine
Clonidine*	6.8	230.0	43.9	d4-Clonidine
Amphetamine	8.1	136.1	90.8	d5-Amphetamine
Amphetamine*	8.1	136.1	118.9	d5-Amphetamine
Albuterol	8.3	240.0	148.0	d_3-Albuterol
Codeine	8.4	300.0	152.0	d6-Codeine
Hydrocodone	8.6	300.2	198.8	d3-Hydrocodone
Hydrocodone*	8.6	300.2	170.6	d3-Hydrocodone
Atorvastatin	8.9	559.3	440.0	d5-Enalapril
Atorvastatin*	8.9	559.3	466.0	d5-Enalapril
Atenolol	9.0	267.2	144.7	d7-Atenolol
Atenolol*	9.0	267.2	189.7	d7-Atenolol
Metformin	9.5	131.1	60.1	d_6-Metformin
Ranitidine	18.8	315.0	175.9	d_3-Albuterol
Surrogate Standards				
d_3-Cotinine	4.0	180.0	79.9	d3-Amitriptyline
d_3-Cotinine*	4.0	180.0	101.0	d3-Amitriptyline
d_3-Cimetidine	4.7	256.0	161.8	d3-Amitriptyline
d_3-Cimetidine*	4.7	256.0	94.8	d3-Amitriptyline
d_5-Enalapril	6.5	382.0	238.8	d3-Amitriptyline
d_5-Enalapril*	6.5	382.0	164.8	d3-Amitriptyline
d_6-Oxycodone	6.7	322.1	262.0	d3-Amitriptyline
d_6-Oxycodone*	6.7	322.1	304.1	d3-Amitriptyline
d_4-Clonidine	6.8	234.0	216.7	d3-Amitriptyline
d_4-Clonidine*	6.8	234.0	47.9	d3-Amitriptyline
d_5-Amphetamine	8.1	141.1	92.9	d3-Amitriptyline
d_5-Amphetamine*	8.1	141.1	123.9	d3-Amitriptyline
d_3-Albuterol	8.3	243.0	151.0	d3-Amitriptyline
d_6-Codeine	8.4	306.0	151.8	d3-Amitriptyline
d_6-Codeine*	8.4	306.0	217.9	d3-Amitriptyline
d_3-Hydrocodone	8.6	303.1	198.9	d3-Amitriptyline
d_3-Hydrocodone*	8.6	303.1	170.8	d3-Amitriptyline
d_7-Atenolol	9.0	274.0	144.7	d3-Amitriptyline
d_7-Atenolol*	9.0	274.0	189.7	d3-Amitriptyline
d_6-Metformin	9.5	137.1	60.1	d3-Amitriptyline
Recovery Standards				

d_3-Amitriptyline	7.9	281.0	232.7	External Standard
d_3-Amitriptyline*	7.9	281.0	90.7	External Standard

* Indicates secondary transition for possible diagnostic use.

List 5 – Acid Extraction, Positive Electrospray Ionization (+)ESI Analytes, Ions and Quantification References

Target Analyte	Typical Retention	Parent Ion Mass	Daughter Ion Mass	Quantified against
Theophylline	2.7	181.1	123.8	13C-15N2-Theophylline
Theophylline*	2.7	181.1	95.8	13C-15N2-Theophylline
Benzoylecgonine	5.7	290.1	167.8	d8-Benzoylecgonine
Benzoylecgonine*	5.7	290.1	104.8	d8-Benzoylecgonine
Metoprolol	8.4	268.2	190.7	d7-Metoprolol
Metoprolol*	8.4	268.2	115.7	d7-Metoprolol
Cocaine	9.2	304.1	181.8	d3-Cocaine
Cocaine*	9.2	304.1	81.9	d3-Cocaine
Meprobamate	11.1	219.0	157.8	d7-Metoprolol
Meprobamate*	11.1	219.0	96.9	d7-Metoprolol
10-hydroxy-amitriptyline	12.0	294.2	215.0	d7-Propranolol
10-hydroxy-amitriptyline*	12.0	294.2	276.0	d7-Propranolol
Propranolol	14.6	260.2	115.8	d7-Propranolol
Propranolol*	14.6	260.2	182.7	d7-Propranolol
Prednisone	16.6	359.2	341.0	d7-Propranolol
Prednisone*	16.6	359.2	146.7	d7-Propranolol
Prednisolone	17.5	361.2	343.0	d7-Propranolol
Prednisolone*	17.5	361.2	324.7	d7-Propranolol
Hydrocortisone	17.6	363.2	120.7	d4-Hydrocortisone
Hydrocortisone*	17.6	363.2	326.7	d4-Hydrocortisone
Desmethyldiltiazem	18.8	401.2	177.8	d4-Promethazine
Desmethyldiltiazem*	18.8	401.2	149.5	d4-Promethazine
Promethazine	18.8	285.1	197.8	d4-Promethazine
Promethazine*	18.8	285.1	85.7	d4-Promethazine
DEET	20.6	192.0	118.6	d7-DEET
DEET	20.6	192.0	90.7	d7-DEET
Paroxetine	20.6	330.2	191.8	d6-Paroxetine
Paroxetine*	20.6	330.2	69.8	d6-Paroxetine
Norverapamil	21.0	441.3	164.7	d7-Propranolol
Norverapamil*	21.0	441.3	149.7	d7-Propranolol
Methylprednisolone	21.4	375.2	357.0	d2-Methylprednisolone
Methylprednisolone*	21.4	375.2	339.0	d2-Methylprednisolone
Verapamil	21.4	455.3	164.8	d6-Amitriptyline
Verapamil*	21.4	455.3	149.8	d6-Amitriptyline
Betamethasone	21.7	393.2	355.1	d6-Amitriptyline
Betamethasone*	21.7	393.2	373.0	d6-Amitriptyline
Propoxyphene	21.8	340.2	57.9	d5-Propoxyphene
Propoxyphene*	21.8	340.2	266.1	d5-Propoxyphene
Amitriptyline	22.4	278.2	232.8	d6-Amitriptyline
Amitriptyline*	22.4	278.2	90.7	d6-Amitriptyline
Trenbolone	22.5	271.2	198.7	d5-Alprazolam

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Trenbolone*	22.5	271.2	252.8	d5-Alprazolam
Benztropine	22.9	308.2	166.7	d3-Benztropine
Benztropine*	22.9	308.2	151.7	d3-Benztropine
Alprazolam	23.3	309.1	280.9	d5-Alprazolam
Alprazolam*	23.3	309.1	204.9	d5-Alprazolam
Amlodipine	23.8	409.1	237.8	d5-Norfluoxetine
Amlodipine*	23.8	409.1	293.8	d5-Norfluoxetine
Norfluoxetine	24.7	296.1	133.7	d5-Norfluoxetine
Sertraline	26.4	306.1	274.8	d7-Propranolol
Sertraline*	26.4	306.1	158.7	d7-Propranolol
Diazepam	29.1	285.1	192.8	d5-Diazepam
Diazepam*	29.1	285.1	153.8	d5-Diazepam
Valsartan	31.8	436.2	235.0	d5-Propoxyphene
Valsartan*	31.8	436.2	291.0	d5-Propoxyphene
Fluocinonide	34.8	495.2	337.0	d5-Alprazolam
Fluocinonide*	34.8	495.2	475.0	d5-Alprazolam
Trenbolone acetate	37.8	313.2	253.0	d5-Alprazolam
Trenbolone acetate*	37.8	313.2	271.0	d5-Alprazolam
Fluticasone propionate	37.9	501.2	293.0	d7-Metoprolol
Fluticasone propionate*	37.9	501.2	313.0	d7-Metoprolol
Simvastatin	40.1	419.3	285.0	d5-Propoxyphene
Simvastatin*	40.1	419.3	198.9	d5-Propoxyphene
Surrogate Standards				
^{13}C, $^{15}N_2$-Theophylline	2.7	184.0	124.7	$^{13}C_3$-Atrazine
^{13}C, $^{15}N_2$-Theophylline*	2.7	184.0	96.8	$^{13}C_3$-Atrazine
d_8-Benzoylecgonine	5.6	298.1	170.9	$^{13}C_3$-Atrazine
d_8-Benzoylecgonine*	5.6	298.1	109.8	$^{13}C_3$-Atrazine
d_7-Metoprolol	8.3	275.0	190.8	$^{13}C_3$-Atrazine
d_7-Metoprolol*	8.3	275.0	122.7	$^{13}C_3$-Atrazine
d_3-Cocaine	9.2	307.1	184.9	$^{13}C_3$-Atrazine
d_3-Cocaine*	9.2	30.7.1	84.8	$^{13}C_3$-Atrazine
d_7-Propranolol	14.4	267.0	116.0	$^{13}C_3$-Atrazine
d_7-Propranolol*	14.4	267.0	188.7	$^{13}C_3$-Atrazine
d_4-Hydrocortisone	17.6	367.0	120.8	$^{13}C_3$-Atrazine
d_4-Hydrocortisone*	17.6	367.0	331.0	$^{13}C_3$-Atrazine
d_4-Promethazine	18.6	289.0	201.8	$^{13}C_3$-Atrazine
d_4-Promethazine*	18.6	289.0	86.0	$^{13}C_3$-Atrazine
d_7-DEET	20.6	199.1	125.8	$^{13}C_3$-Atrazine
d_7-DEET*	20.6	199.1	97.8	$^{13}C_3$-Atrazine
d_6-Paroxetine	20.6	336.0	197.8	$^{13}C_3$-Atrazine
d_6-Paroxetine*	20.6	336.0	75.8	$^{13}C_3$-Atrazine
d_2-Methylprednisolone	21.4	377.0	359.0	$^{13}C_3$-Atrazine
d_2-Methylprednisolone*	21.4	377.0	341.0	$^{13}C_3$-Atrazine
d_5-Propoxyphene	21.8	245.2	266.1	$^{13}C_3$-Atrazine
d_5-Propoxyphene*	21.8	345.2	57.9	$^{13}C_3$-Atrazine
d_6-Amitriptyline	22.4	284.0	233.0	$^{13}C_3$-Atrazine
d_6-Amitriptyline*	22.4	284.0	90.8	$^{13}C_3$-Atrazine
d_3-Benztropine	22.9	311.0	166.7	$^{13}C_3$-Atrazine
d_3-Benztropine*	22.9	311.0	151.7	$^{13}C_3$-Atrazine
d_5-Alprazolam	23.1	314.1	285.9	$^{13}C_3$-Atrazine
d_5-Alprazolam*	23.1	314.1	209.9	$^{13}C_3$-Atrazine

d_5-Norfluoxetine	24.7	301.0	138.7	$^{13}C_3$-Atrazine
d_5-Diazepam	29.1	290.1	197.9	$^{13}C_3$-Atrazine
d_5-Diazepam*	29.1	290.1	153.8	$^{13}C_3$-Atrazine
Recovery Standards				
$^{13}C_3$-Atrazine	18.8	219.5	176.9	External Standard
$^{13}C_3$-Atrazine *	18.8	219.5	134.0	External Standard

* Indicates secondary transition for possible diagnostic use.

QUALITY ACCEPTANCE CRITERIA

QC Acceptance Limits

	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
List 1 Compounds (APOS)						
Acetaminophen	70	140	70	140	30	≤60
Ampicillin [2]						
Azithromycin	10	130	10	130	130	≤1.5
Caffeine	25	160	35	150	60	≤15
Carbadox	25	180	35	180	40	≤1.5
Carbamazepine	25	200	35	200	40	≤1.5
Cefotaxime	10	300	10	300	60	≤6
Ciprofloxacin	25	180	35	180	40	≤6
Clarithromycin	50	160	50	160	30	≤1.5
Clinafloxacin	25	300	35	300	70	≤6
Cloxacillin	35	160	40	150	50	≤3
Dehydronifedipine	35	160	40	160	30	≤0.6
Digoxigenin	50	150	60	140	30	≤6
Digoxin	10	300	10	300	30	≤15
Diltiazem	20	160	25	160	50	≤0.3
1,7-Dimethylxanthine	30	300	40	300	60	≤150
Diphenhydramine	30	200	35	180	50	≤0.6
Enrofloxacin	30	220	40	220	40	≤3
Erythromycin - H_2O	70	130	70	130	30	≤0.3
Flumequine	40	160	50	160	30	≤1.5
Fluoxetine	60	150	70	140	30	≤1.5
Lincomycin	10	300	10	300	70	≤3
Lomefloxacin	50	250	60	250	30	≤3
Miconazole	35	130	40	130	30	≤1.5
Norfloxacin	10	250	25	220	40	≤15
Norgestimate	35	130	40	130	30	≤3
Ofloxacin	60	250	70	250	30	≤1.5
Ormetoprim	70	150	70	150	30	≤0.6
Oxacillin	20	130	20	130	40	≤3
Oxolinic Acid	60	150	70	150	30	≤0.6
Penicillin G	10	130	10	130	40	≤3
Penicillin V	40	140	50	140	30	≤3
Roxithromycin	50	140	50	140	30	≤0.3

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Sarafloxacin	50	200	60	180	30	≤15
Sulfachloropyridazine	60	160	70	160	30	≤1.5
Sulfadiazine	70	130	70	130	30	≤1.5
Sulfadimethoxine	35	160	40	160	30	≤0.3
Sulfamerazine	60	140	60	140	30	≤0.6
Sulfamethazine	70	130	70	130	30	≤0.6
Sulfamethizole	30	140	35	140	30	≤0.6
Sulfamethoxazole	70	130	70	130	30	≤0.6
Sulfanilamide	2	160	3	150	150	≤15
Sulfathiazole	30	180	30	160	50	≤1.5
Thiabendazole	60	150	60	150	30	≤1.5
Trimethoprim	50	150	60	150	30	≤1.5
Tylosin	10	180	20	180	40	≤6
Virginiamycin	15	300	15	250	90	≤3
Surrogate Standard						
$^{13}C_2$, ^{15}N-Acetaminophen	30	160	40	150	30	
$^{13}C_3$-Caffeine	40	140	50	140	30	
d_{10}-Carbamazepine-10,11-epoxide						
$^{13}C_3$, ^{15}N-Ciprofloxacin	7	150	9	140	70	
$^{13}C_2$-Erythromycin - H_2O	35	130	35	130	30	
d_5-Fluoxetine	40	130	50	130	30	
$^{13}C_6$-Sulfamethazine	30	160	35	150	40	
$^{13}C_6$-Sulfamethoxazole	30	140	40	130	30	
d_6-Thiabendazole	25	180	30	160	50	
$^{13}C_3$-Trimethoprim	30	140	40	130	30	
Recovery Standard						
$^{13}C_3$-Atrazine						
List 2 Compounds (TCYS)						
Anhydrochlortetracycline (ACTC)	15	200	20	180	70	≤15
Anhydrotetracycline (ATC)	20	160	20	150	50	≤15
Chlortetracycline (CTC)	30	250	35	250	60	≤6
Demeclocycline	35	180	35	160	50	≤15
Doxycycline	35	180	40	180	40	≤6
Epianhydrochlortetracycline (EACTC)	6	130	7	130	70	≤60
Epianhydrotetracycline (EATC)	15	200	20	200	60	≤15
Epichlortetracycline (ECTC)	25	180	30	160	50	≤15
Epioxytetracycline (EOTC)	25	180	35	160	40	≤6
Epitetracycline (ETC)	35	200	40	180	40	≤6
Isochlortetracycline (ICTC)	25	180	35	160	40	≤6
Minocycline	1	250	2	200	110	≤60
Oxytetracycline (OTC)	20	200	30	200	40	≤6
Tetracycline (TC)	20	200	30	180	40	≤6
Surrogate Standard						
d_6-Thiabendazole	25	140	25	130	50	

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Recovery Standard						
$^{13}C_3$-Atrazine						
	OPR Recovery and surrogate recovery in sample		**IPR**			**Blank Level (ng)**
			Average Recovery (%)		**RSD (%)**	
	Low	**High**	**Low**	**High**		
List 3 Compounds (ANEG)						
Bisphenol A	70	130	70	130	30	≤2500
Furosemide	65	130	70	130	30	≤40
Gemfibrozil	60	140	70	130	30	≤1.5
Glipizide	55	170	60	160	30	≤6
Glyburide	50	180	55	170	30	≤3
Hydroxychlorothiazide	70	200	70	180	30	≤20
2-hydroxy-ibuprofen	70	130	70	130	30	≤80
Ibuprofen	70	130	70	130	30	≤15
Naproxen	50	150	60	150	30	≤3
Triclocarban	60	140	70	130	30	≤3
Triclosan	70	130	70	130	30	≤60
Warfarin	70	140	70	140	30	≤1.5
Surrogate Standards						
d_6-Bisphenol A	50	170	60	160	30	
d_6-Gemfibrozil	50	150	55	140	30	
d_3-Glyburide	20	160	25	150	40	
d_{11}-Glipizide	30	180	35	170	50	
$^{13}C_3$-Ibuprofen	50	140	55	140	30	
^{13}C-d_3-Naproxen	30	150	35	140	30	
$^{13}C_6$-Triclocarban	20	160	25	150	50	
$^{13}C_{12}$-Triclosan	20	160	30	150	40	
d_5-Warfarin	35	250	50	250	30	
Recovery Standard						
$^{13}C_6$-2,4,5-Trichloro-phenoxyacetic acid						
List 4 Compounds (BPOS)						
Albuterol	50	160	50	160	30	≤0.3
Amphetamine	50	160	60	150	30	≤1.5
Atenolol	70	130	70	130	30	≤0.6
Atorvastatin	20	130	25	130	40	≤1.5
Cimetidine	15	130	20	130	50	≤0.6
Clonidine	70	130	70	130	30	≤1.5
Codeine	70	130	70	130	30	≤3
Cotinine	70	130	70	130	30	≤1.5
Enalapril	70	130	70	130	30	≤0.3
Hydrocodone	70	130	70	130	30	≤1.5
Metformin	70	160	70	160	30	≤30
Oxycodone	65	130	70	130	30	≤0.6
Ranitidine	25	140	30	140	50	≤0.6

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Triamterene	70	140	70	140	30	≤0.3
Surrogate Standards						
d_3-Albuterol	20	140	30	130	30	
d_5-Amphetamine	20	130	25	130	40	
d_7-Atenolol	70	130	70	130	30	
d_3-Cimetidine	15	130	15	130	50	
d_4-Clonidine	70	130	70	130	30	
d_6-Codeine	70	130	70	130	30	
d_3-Cotinine	70	140	70	135	30	
d_5-Enalapril	65	130	70	130	30	
d_3-Hydrocodone	70	130	70	130	30	
d_6-Metformin	3	130	4	130	130	
d_6-Oxycodone	50	150	60	140	30	
Recovery Standards						
d_3-Amitriptyline						
List 5 Compounds (APOS)						
Alprazolam	70	130	70	130	30	≤0.3
Amitriptyline	70	130	70	130	30	≤0.3
Amlodipine	45	130	50	130	30	≤1.5
Benzoylecgonine	70	130	70	130	30	≤0.3
Benztropine	70	130	70	130	30	≤0.3
Betamethasone	20	240	30	220	40	≤1.5
Cocaine	70	130	70	130	30	≤0.15
DEET	70	130	70	130	30	≤0.15
Desmethyldiltiazem	3	350	5	320	80	≤0.15
Diazepam	70	130	70	130	30	≤0.3
Fluocinonide	7	230	9	220	70	≤6
Fluticasone propionate	20	160	25	150	50	≤2
Hydrocortisone	15	220	20	200	80	≤60
10-hydroxy-amitriptyline	70	130	70	130	30	≤0.15
Meprobamate	65	150	70	140	30	≤4
Methylprednisolone	35	240	40	220	50	≤4
Metoprolol	70	130	70	130	30	≤1.5
Norfluoxetine	70	130	70	130	30	≤1.5
Norverapamil	55	130	60	130	30	≤0.15
Paroxetine	70	130	70	130	30	≤4
Prednisolone	35	240	40	220	50	≤6
Prednisone	50	180	60	170	30	≤20
Promethazine	70	130	70	130	30	≤0.4
Propoxyphene	70	130	70	130	30	≤0.3
Propranolol	70	150	70	150	30	≤2
Sertraline	50	130	55	130	30	≤0.4
Simvastatin	1	150	1	140	100	≤20
Theophylline	10	1000	70	900	50	≤60
Trenbolone	70	140	70	135	30	≤4

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	OPR Recovery and surrogate recovery in sample		IPR			Blank Level (ng)
			Average Recovery (%)		RSD (%)	
	Low	High	Low	High		
Trenbolone acetate	55	130	60	130	30	≤0.3
Valsartan	70	130	70	130	30	≤4
Verapamil	70	145	70	140	30	≤0.15
Surrogate Standards						
d_5-Alprazolam	45	130	45	130	30	
d_6-Amitriptyline	10	130	20	130	40	
d_8-Benzoylecgonine	10	170	20	160	40	
d_3-Benztropine	20	140	25	130	40	
d_3-Cocaine	25	140	30	130	50	
d_7-DEET	15	160	20	150	40	
d_5-Diazepam	15	160	25	150	40	
d_4-Hydrocortisone	40	240	45	230	50	
d_2-Methylprednisolone	15	160	20	150	60	
d_7-Metoprolol	25	140	30	140	30	
d_5-Norfluoxetine	20	130	20	130	50	
d_6-Paroxetine	7	150	9	140	60	
d_4-Promethazine	3	140	5	130	80	
d_5-Propoxyphene	30	130	40	130	30	
d_7-Propranolol	25	140	30	130	30	
$^{13}C_1$, $^{15}N_2$-Theophylline	20	200	25	180	60	
Recovery Standards						
$^{13}C_3$-Atrazine						

[1] OPR and IPR limits derived from actual method performance data according to EPA 821B98003, appendix D.

[2] Because of very low stability the accuracy of Ampicillin is not known. The analysis result is classified as "Information Value" only.

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Instrumental Acceptance Specifications

QC Parameter	Specification
Instrument Sensitivity	Daily, S:N ≥ 3:1 for all analytes for lowest calibration point.
Initial Calibration (native compounds)	Initial, (1/X) weighted linear regression (followed by regular Cal/Ver procedures and repeated as necessary to maintain Cal/Ver results within established acceptance ranges. Calculated concentrations 70-130%, one point per compound may be 60-140% Internal guideline - correlation coefficient >0.985. Calibration curves with lower correlation coefficient values meeting all above criteria may be accepted based on batch specific QC results and professional judgement
OPENING Calibration Verification	Every 20 samples, determined concentrations within 30% of actual concentrations. Professional judgment allowed for wider acceptance limits.
CLOSING Calibration Verification	Within OPENING Calibration Verification specifications. Allowable exception: results for the greater of 1 compound or 10% of the compounds on a Compound List (1,2,3,4,5) may fall outside the Opening Calibration Verification specification provided the RPD between the CLOSING result and the OPENING result is <30%.
Instrumental Carryover And Instrument Background	Every Initial Calibration, Cal/Ver, or SPM: < 0.3 % carryover and area response of analytes in instrument blank < 800 judged following two previous methanol blank injections.

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QUANTIFICATION AND DATA REPORTING PROCEDURES

Positive identification of target natives, surrogate standard and recovery standards require:

- ≥ 3:1 S:N for parent ion to daughter ion transition, on condition that the result is above the lowest calibration standard level.
- Compound retention time falls within 0.4 minutes of the predicted retention times from the daily calibration standard. Natives with labelled surrogate standards must elute within 0.1 minutes of the associated labelled surrogates.

Concentrations of the targets and surrogates are calculated by isotope dilution or internal standard quantification with linear regression calibration, using a 1/X weighting type, excluding origin. Sample specific detection limits (SDLs) are calculated by QuanLynx software using 3 times the signal of the noise in the target channel converted to an equivalent sample concentration.

General equation : $Y = \text{slope} \times X + \text{intercept}$

Where: $Y = \text{Response ratio} = \left(\frac{\text{area of Target}}{\text{area of Quant. Std}} \times \text{weight of Quant. std (ng)} \right)$

X = weight of target (ng)

Quant. Std = labelled surrogate or recovery standard

The slope and intercept are used to convert raw peak areas in sample chromatograms to final concentrations as follows:

$$\text{Sample Conc.} = \left(\frac{\text{area of Target}}{\text{area of Quant. Std}} \times \text{weight of Quant. Std (ng)} - \text{intercept} \right) \text{x} \left(\frac{1}{\text{slope}} \right) \text{x} \left(\frac{1}{\text{sample size (L)}} \right)$$

The recovery of surrogate standards, calculated from the determined concentration of the surrogate in the extract relative to the amount spiked, are monitored as an indication of overall data quality.

The lower reporting limit for each target compound is defined as the concentration equivalent to the lowest calibration standard analyzed or the SDL, whichever is greater.

HORMONE AND STEROL ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-068

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG30912

Analysis WG30292, WG30417, WG30695

25 November 2009

HORMONE AND STEROL ANALYSIS

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Contact: Candice Navaroli
Project Manager

25 November 2009

OHIO RIVER VALLEY WATER SANITATION COMMISSION
AQUEOUS SAMPLES

HORMONES AND STEROLS ANALYSIS
METHOD: MLA-068
4562: L13603-1 to -8

Project Name: Emerging Contaminants in Mainstem Ohio

24 November 2009

NARRATIVE

This narrative describes the analysis of eight aqueous samples for hormones and sterols by high-resolution gas chromatography / high-resolution mass spectrometry (HR-GC/MS).

SAMPLE RECEIPT AND STORAGE

The samples were received on September 23rd 2009. Temperature for two samples, Site 23 and Site 24 (AXYS ID L13603-7 and -8 respectively) was 6°C on receipt, which was above the method requirement <4°C. Temperature effect on the samples is unknown. Analyses of the samples proceeded as per client's instruction. Details of sample conditions on receipt are provided on the Sample Receiving Record forms included in this data package. The sampless were were stored at 4°C in dark prior to extraction and analysis.

SAMPLE PREPARATION AND ANALYSIS

The samples were pretreated as described on Sample Pretreatment Record forms included in this data package.

Extraction and analysis procedures were in general accordance with AXYS Method MLA-068, *Analytical Method for the Determination of Sterols and Hormones with BSTFA Derivatization by GC/MS and GC/HRMS*. A summary (MSU-029) of the method is included following this narrative.

Samples and QC samples (a procedural blank and a lab-generated reference sample known as the Ongoing Precision and Recovery (OPR) per analysis batch) were analyzed in three analysis batches named WG30292, WG30417 and WG30695. Composition of each analysis batch is shown on the Cover Page and Correlation Table, and on the Batch List that accompanies the extraction workup sheets.

Ultra pure Seastar water was used as the matrix for the procedural blank and the OPR.

An accurately-weighed sub-sample of approximately 1L was spiked with labeled quantification standards, and extracted/cleaned up using SPE cartridges. The extract was then derivatized. The final extract was reduced in volume and spiked with labeled internal standards (referred to as the "recovery standard" in the method summary) prior to instrumental analysis.

CALCULATIONS

Target analyte concentrations were determined by either isotope dilution or internal standard quantification procedures, using Micromass OPUSQuan software. Formulae used in the conversion of the raw chromatograms to concentration are provided in the method summary document.

Sample specific detection limits (SDLs) were calculated for each target analyte and used as the detection qualifier. SDLs were determined from the analysis data by converting three times the height of the average noise signal to a response, using the area/height ratio of the labeled standard, and then to a concentration following the same procedures used to convert target peak responses to concentrations.

REPORTING CONVENTIONS

The AXYS contract number assigned for internal tracking was 4562. Samples were assigned a unique laboratory identifier L13603-X, where X is a numeral. All data reports reference this unique AXYS ID plus the client sample identifier. To assist in locating data a table correlating the client sample number with AXYS sample ID is included following this narrative.

Any extra work required and performed after the initial instrumental analysis of the sample's extract was given an extra "test suffix" code. The single letter code per extra work performed was added to the AXYS sample ID as a suffix, and was combined with any other applicable test suffix codes. The extra work codes used to report data in this package include:

N = extract was diluted in a new vial followed by instrumental re-analysis
R = repeat analysis using fresh sub-sample.

The laboratory qualifiers used are as follows:

B = analyte found in the sample and the associated blank
D = data from analysis of diluted extract
E = analyte response above the instrument calibration linearity range
J = indicates an estimated value where the concentration of the analyte is less than the LMCL but greater than the SDL
K = identifies a target that could not be confirmed by virtue of not satisfying all method required criteria, the reported value may be interpreted as an estimated maximum analyte concentration.
N = recovery of the analyte was outside the method control limits
U = identifies a compound that was not detected
V = surrogate recovery is not within method/contract control limits
X = result reported elsewhere.

Data are reported to three significant figures, in units of nanograms per Liter (ng/L).

QA/QC NOTES

Client samples and associated QC samples analyzed in one analysis batch were carried intact through the entire analytical process. The sample data were reviewed and evaluated in relation to the batch QC samples.

- Sample analyte concentrations are not blank-corrected. Sample data should be evaluated with consideration of the procedural blank results.
- By virtue of the isotope dilution quantification procedures, data are recovery corrected for possible losses during extraction and cleanup procedure except for androsterone, desogestrel, androstenedione and testosterone. These compounds are quantified against recovery (internal) standard and their concentrations are not recovery corrected.
- All linearity, calibration verification, OPR and labeled compound recovery specifications were met with the following exceptions:

WG30292

Percent recoveries of D6-Norethindrone, D9-Progesterone and/or D6-Norgestrel in samples Site 18, Site 19, Site 20 and Site 22 (AXYS ID L13603-2, -3, -4 and -6, respectively) were above the method upper control limits. These labeled surrogates are flagged with a 'V' on report forms. Since the isotope dilution method of quantification produces data that are recovery corrected, the variances from the method acceptance criteria are deemed not to affect the quantification of analytes. Percent surrogate recoveries

are used as general method performance indicator only. In addition, all analytes associated with those surrogates were not detected in these samples.

WG30417

The percent recovery of Stigmasterol in the OPR (AXYS ID WG30417-102) was 153%, which was slightly above the method nominal upper control limit 152%. It is flagged with an 'N' on the report form. Stigmasterol data for samples in this analysis batch might be similarly overestimated.

ANALYTICAL DISCUSSION

To bring area responses of Cholesterol, Stigmasterol and/or beta-Sitosterol to within the instrument calibration linearity range, extracts for samples Site 20 and Site 24 (AXYS ID L13603-4 and -8 respectively) were diluted in a new vial and re-analyzed on instrument. Data obtained from the analysis of the diluted extracts for these compounds are reported (as indicated by the suffix 'N' added on the AXYS ID). Dilution factor is listed on the report form.

Sample Site 21 (AXYS ID L13603-5) was initially analyzed in WG30292. As not all method specifications were met in initial analysis, repeat analysis of the sample was conducted in WG30695 using fresh sub-sample. Repeat analysis was successful. Data from the repeat analysis are reported (as indicated by the suffix 'R' added on the AXYS ID).

DATA PACKAGE

This data package is assigned a unique data package identification workgroup, DPWG30912, shown on the front page. The following documents are included:

- Method summary
- Sample Cover Page and Correlation Table
- Sample Receiving Documentation
- Sample pretreatment records
- Laboratory extraction logs for each sample
- Sample data reports (in order of AXYS Sample ID)
- Laboratory QC data reports
- Instrumental QC data reports (organized by analysis date)
- Sample raw data (in order of AXYS Sample ID)
- Laboratory QC raw data
- Instrumental QC raw data (organized by analysis date)

I certify that this data package is in compliance with the terms and conditions of the contract, both technically and for completeness, except for the conditions detailed above. In addition, I certify, that to the best of my knowledge and belief, the data as reported are true and accurate. The following signature, on behalf of AXYS Analytical Services Ltd, authorizes the release of the data contained in this data package.

Signed: (Matthew) Ziqing Ou, PhD, QA/QC Chemist

November 25, 2009
Date Signed

METHOD SUMMARY

ANALYSIS OF STEROLS AND HORMONES IN WATER AND SOLIDS SAMPLES BY METHOD MLA-068

List of Analytes (Sterols and Hormones)

Androstenedione
Androsterone
Campesterol
Cholestanol
Cholesterol
Coprostanol
Desmosterol
Desogestrel
17α-Dihydroequilin
Epicoprostanol
Equilenin
Equilin
Ergosterol
17α-Estradiol
17β-Estradiol
β-Estradiol-3-Benzoate
Estriol
Estrone
17α-Ethinyl Estradiol
Mestranol
Norethindrone
Norgestrel
Progesterone
β-Sitosterol
β-Stigmastanol
Stigmasterol
Testosterone

Extraction and Cleanup Procedures

This method is applicable for the determination of a suite of sterols and hormones in solid, biosolid and aqueous samples. A “clean water” analysis option is provided for aqueous samples where very low detection limits are desired. Samples are pre-treated and separated into aqueous and solid phases as necessary to ensure that the sub-samples are representative and that the analytes are efficiently extracted. Before extraction the samples are spiked with a suite of isotopically labelled surrogate standards. Biosolids are spiked with a higher amount of cholesterol-d7 surrogate standard. Water is extracted by shaking with dichloromethane. “Clean water” samples are spiked with lower amounts of surrogate standards and processed to a lower final extract volume. Solids and biosolids are dried with anhydrous sodium sulphate and Soxhlet extracted with 40:60 hexane:acetone. The extract is solvent exchanged to 5% toluene in hexane, and sulphur is removed by treatment with activated copper. Column cleanup is performed on a layered alumina/Florisil column and the analytes eluted into a methanol fraction. Extracts from high level samples may need to be split. Derivatization is performed of sample extracts and calibration solutions at 55ºC using BSTFA:TMCS (99:1) in a pyridine solution. The derivatization converts all analytes except progesterone and androstenedione into the corresponding TMS ethers. An isotopically labelled recovery (internal) standard is added and the extract is analysed by gas chromatography/mass spectrometry (GC/MS or GC/HRMS).

HRGC/HRMS Analysis

Instrumental analysis of the derivatized final extract is performed by split/splitless injection on a high-resolution gas chromatograph (HRGC) equipped with a Restek RT_x-5 capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) coupled to either a high-resolution (HRMS) or a low-resolution (LRMS) mass spectrometer. The HRMS is operated at a static (5000) mass resolution in the electron ionization (EI) mode using Voltage SIR Detection. LRMS is operated at unit mass resolution in the EI mode using multiple ion detection (MID). Two characteristic ions for each target analyte and surrogate standard are acquired and summed.

Initial calibration is performed using a multi-point calibration series of solutions that encompass the working concentration range. Calibration is verified at least once every twelve hours by analysis of a mid-level calibration solution.

Quantification Procedures

Target concentrations are determined with respect to labelled surrogate standards as shown in Table 1 (LRMS) and Table 2 (HRMS). Mean relative response factors (RRF) determined from the initial calibration runs are used to convert raw peak areas in sample chromatograms to final concentrations as follows:

$$\text{Concentration of target} = \left(\frac{\text{area of Target}}{\text{area of Surrogate}}\right) \times \left(\frac{\text{weight of Surrogate}}{\text{weight of Sample}}\right) \times \left(\frac{1}{\text{RRF}}\right)$$

$$\text{where} \quad \text{RRF} = \left(\frac{\text{area of Target}}{\text{area of Surrogate}}\right) \times \left(\frac{\text{concentration of Surrogate}}{\text{concentration of Target}}\right)$$

Final concentrations are recovery corrected by the method of quantification. Recoveries of surrogates are determined similarly against the recovery (internal) standard and are used as general indicators of overall analytical quality.

Estimated sample specific detection limits (SDLs) are shown in Table 3.

QA/QC

Samples are analyzed in batches consisting of a maximum of twenty samples, one procedural blank and one spiked matrix (OPR) sample. Sample duplicates or matrix spike/matrix spike duplicate (MS/MSD) pairs may be analyzed on an individual contract basis. The batch is carried through the complete analytical process as a unit. For sample data to be reportable, the batch QC data must meet the established acceptance criteria presented on the analysis reports.

All aspects of the method are described in detail in AXYS' document MLA-068 "Analytical Method for the Determination of Sterols and Hormones with BSTFA Derivatization by GC/MS and GC/HRMS".

Table 1. Analyte Ions Monitored, Surrogates Used, and RRF Determination for Sterols and Hormones by GC/LRMS

Analyte Name	Quantification Standard	Typical Retention Time	RT Win (sec)	RT Standard	mass1	mass2	m1/m2 ratio
Androsterone	17β-Estradiol -d4	21:17	6	17β-Estradiol -d4	347	348	3.56
Desogestrel	17β-Estradiol -d4	21:32	6	17β-Estradiol -d4	353	354	2.97
17α-Estradiol	17β-Estradiol -d4	22:25	6	17β-Estradiol -d4	416	417	2.80
Estrone	17β-Estradiol -d4	22:25	6	17β-Estradiol -d4	342	343	3.50
Equilin	17β-Estradiol -d4	22:31	6	17β-Estradiol -d4	340	341	3.36
Androstenedione	17β-Estradiol -d4	22:37	6	17β-Estradiol -d4	286	287	4.75
17β-Estradiol	17β-Estradiol -d4	22:52	6	17β-Estradiol -d4	416	417	2.71
Testosterone	17β-Estradiol -d4	22:58	8	17β-Estradiol -d4	360	361	3.37
Equilenin	17β-Estradiol -d4	23:22	8	17β-Estradiol -d4	338	339	3.48
Mestranol	Mestranol -d4	23:26	6	Mestranol -d4	367	368	3.35
Norethindrone	Norethindrone -d6	23:33	6	Norethindrone -d6	355	356	3.49
17α-Dihydroequilin	Norethindrone -d6	23:59	6	Norethindrone -d6	307	308	3.74
17α-Ethinyl-Estradiol	17α-Ethinyl-Estradiol -d4	24:09	6	17α-Ethinyl-Estradiol -d4	425	426	2.70
Progesterone	Progesterone -d9	24:40	6	Progesterone -d9	314	315	4.45
Norgestrel	Norgestrel -d6	24:53	6	Norgestrel -d6	355	356	2.91
Estriol	Norgestrel -d6	25:27	6	Norgestrel -d6	504	505	2.28
Coprostanol	Cholesterol -d7	27:48	6	Cholesterol -d7	370	371	3.43
Epicoprostanol	Cholesterol -d7	27:59	6	Cholesterol -d7	370	371	3.49
Cholesterol	Cholesterol -d7	29:36	10	Cholesterol -d7	368	369	3.30
Cholestanol	Cholesterol -d7	29:46	6	Cholesterol -d7	445	446	2.65
Desmosterol	Cholesterol -d7	30:14	8	Cholesterol -d7	343	344	3.03
Ergosterol	Cholesterol -d7	30:54	6	Cholesterol -d7	363	364	3.51
Campesterol	Cholesterol -d7	31:08	6	Cholesterol -d7	382	383	3.19
Stigmasterol	Cholesterol -d7	31:31	6	Cholesterol -d7	484	485	2.43
β-Sitosterol	Cholesterol -d7	32:10	6	Cholesterol -d7	486	487	2.38
β-Stigmastanol	Cholesterol -d7	32:17	6	Cholesterol -d7	488	489	2.29
β-Estradiol-3-Benzoate	Norgestrel -d6	34:56	12	Norgestrel -d6	105	106	13.07
Bisphenol-A-Propane -d6	Pyrene -d10	18:48	20	Pyrene -d10	360	361	2.96
Diethyl Stilbesterol -d8	Pyrene -d10	20:05	20	Pyrene -d10	420	421	2.76
17β-Estradiol -d4	Pyrene -d10	22:50	20	Pyrene -d10	420	421	2.78
Mestranol -d4	Pyrene -d10	23:25	20	Pyrene -d10	371	372	3.38
Norethindrone -d6	Pyrene -d10	23:29	20	Pyrene -d10	361	362	3.47
17α-Ethinyl-Estradiol -d4	Pyrene -d10	24:07	20	Pyrene -d10	429	430	2.70
Progesterone -d9	Pyrene -d10	24:32	20	Pyrene -d10	323	324	4.39
Norgestrel -d6	Pyrene -d10	24:49	20	Pyrene -d10	361	362	3.03
Cholesterol -d7	Pyrene -d10	29:30	20	Pyrene -d10	375	376	3.34
Pyrene -d10		18:01	100		212	213	5.86

Table 2. Analyte Ions Monitored, Surrogates Used, and RRF Determination for Sterols and Hormones by GC/HRMS

Analyte Name	Quantification Standard	Typical Retention Time	RT Win (sec)	RT Standard	Acquired in Function	mass1	mass2	m1/m2 ratio
Androsterone	17β-Estradiol -d4	21:08	6	17β-Estradiol -d4	2	347.2406	348.2440	3.36
Desogestrel	17β-Estradiol -d4	21:26	6	17β-Estradiol -d4	2	353.2300	354.2334	2.60
17α-Estradiol	17β-Estradiol -d4	22:29	6	17β-Estradiol -d4	2	416.2566	417.2600	2.67
Estrone	17β-Estradiol -d4	22:34	6	17β-Estradiol -d4	2	342.2015	343.2048	3.42
Equilin	17β-Estradiol -d4	22:40	6	17β-Estradiol -d4	2	340.1858	341.1892	3.32
Androstenedione	17β-Estradiol -d4	22:36	6	17β-Estradiol -d4	2	358.2328	359.2361	2.54
17β-Estradiol	17β-Estradiol -d4	23:02	6	17β-Estradiol -d4	2	416.2566	417.2600	1.64
Testosterone	17β-Estradiol -d4	23:16	8	17β-Estradiol -d4	2	360.2484	361.2518	3.40
Equilenin	17β-Estradiol -d4	23:45	8	17β-Estradiol -d4	3	338.1702	339.1739	3.30
Mestranol	Mestranol -d4	23:47	6	Mestranol -d4	3	367.2093	368.2127	3.35
Norethindrone	Norethindrone -d6	23:58	6	Norethindrone -d6	3	355.2093	356.2127	3.10
17α-Dihydroequilin	17α-Ethinyl-Estradiol -d4	24:28	6	17α-Ethinyl-Estradiol -d4	4	307.1913	308.1946	3.94
17α-Ethinyl-Estradiol	17α-Ethinyl-Estradiol -d4	24:38	6	17α-Ethinyl-Estradiol -d4	4	425.2332	426.2365	2.65
Progesterone	Progesterone -d9	24:40	6	Progesterone -d9	4	314.2246	315.2279	4.45
Norgestrel	Norgestrel -d6	25:40	6	Norgestrel -d6	4	355.2093	356.2127	2.70
Estriol	Norgestrel -d6	26:15	6	Norgestrel -d6	5	504.2910	505.2944	2.25
Coprostanol	Cholesterol -d7	28:57	6	Cholesterol -d7	5	370.3631	371.3664	3.40
Epicoprostanol	Cholesterol -d7	29:05	6	Cholesterol -d7	5	370.3631	371.3664	3.40
Cholesterol	Cholesterol -d7	30:21	10	Cholesterol -d7	5	368.3474	369.3508	2.80
Cholestanol	Cholesterol -d7	30:29	6	Cholesterol -d7	5	445.3865	446.3899	2.70
Desmosterol	Cholesterol -d7	30:51	8	Cholesterol -d7	5	441.3552	442.3586	2.90
Ergosterol	Cholesterol -d7	31:26	6	Cholesterol -d7	5	363.3447	364.3475	3.50
Campesterol	Cholesterol -d7	31:41	6	Cholesterol -d7	5	382.3631	383.3664	3.05
Stigmasterol	Cholesterol -d7	32:05	6	Cholesterol -d7	5	484.4100	485.4134	2.70
β-Sitosterol	Cholesterol -d7	32:54	6	Cholesterol -d7	5	486.4257	487.4290	2.70
β-Stigmastanol	Cholesterol -d7	33:03	6	Cholesterol -d7	5	488.4413	489.4447	2.55
β-Estradiol-3-Benzoate	Norgestrel -d6	36:16	12	Norgestrel -d6	6	105.0340	106.0374	11.50
Bisphenol-A-Propane -d6	Pyrene -d10	18:24	20	Pyrene -d10	2	360.1894	361.1927	2.90
Diethyl Stilbesterol -d8	Pyrene -d10	19:45	20	Pyrene -d10	2	420.2755	421.2789	2.90
17β-Estradiol -d4	Pyrene -d10	23:00	20	Pyrene -d10	2	420.2817	421.2851	2.90
Mestranol -d4	Pyrene -d10	23:44	20	Pyrene -d10	3	371.2344	372.2378	3.31
Norethindrone -d6	Pyrene -d10	23:54	20	Pyrene -d10	3	361.2470	362.2503	3.45
17α-Ethinyl-Estradiol -d4	Pyrene -d10	24:36	20	Pyrene -d10	4	429.2583	430.2616	3.45
Progesterone -d9	Pyrene -d10	24:56	20	Pyrene -d10	4	323.2811	324.2844	4.44
Norgestrel -d6	Pyrene -d10	25:35	20	Pyrene -d10	4	361.2470	362.2503	2.90
Cholesterol -d7	Pyrene -d10	30:15	20	Pyrene -d10	5	375.3913	376.3947	3.20
Pyrene -d10		17:42	100		1	213.1400	214.0000	12.00

Table 3. Detection limits for sterols and hormones determined by GC-high resolution MS

Sample type	Analyte	Estimated detection limit
Aqueous (< 1 % solids)	Androsterone, Desogestrel, 17α-Estradiol, Estrone, Equilin, 17β-Estradiol, Testosterone, Equilenin, Mestranol, Norethindrone, 17α-Dihydroequilin, 17α-Ethinyl Estradiol, Estriol, Coprostanol, Epicoprostanol, Campesterol, and β-Estradiol-3-Benzoate	1 ng/L
	Norgestrel	2 ng/L
	β-Stigmastanol	3 ng/L
	Androstenedione, Progesterone, Ergosterol	5 ng/L
	Cholestanol, Desmosterol, Stigmasterol	10 ng/L
	β-Sitosterol	200 ng/L
	Cholesterol	500 ng/L
Aqueous, "Clean water" option	Androsterone, Desogestrel, 17α-Estradiol, Estrone, Equilin, 17β-Estradiol, Testosterone, Equilenin, Mestranol, Norethindrone, 17α-Dihydroequilin, 17α-Ethinyl Estradiol, Estriol, Coprostanol, Epicoprostanol, Campesterol, and β-Estradiol-3-Benzoate	0.04 ng/L
	Norgestrel	0.08 ng/L
	β-Stigmastanol	0.12 ng/L
	Androstenedione, Progesterone, Ergosterol	0.2 ng/L
	Cholestanol, Desmosterol, Stigmasterol	0.4 ng/L
	β-Sitosterol	8 ng/L
	Cholesterol	20 ng/L

(Table 3, continued)

Sample type	Analyte	Estimated detection limit
Solids, except biosolids	Androsterone, Desogestrel, 17α-Estradiol, Estrone, Equilin, 17β-Estradiol, Testosterone, Equilenin, Mestranol, Norethindrone, 17α-Dihydroequilin, 17α-Ethinyl Estradiol, Estriol, Coprostanol, Epicoprostanol, Campesterol, and β-Estradiol-3-Benzoate	0.1 ng/g dry
	Norgestrel	0.2 ng/g dry
	β-Stigmastanol	0.3 ng/g dry
	Androstenedione, Progesterone, Ergosterol	0.5 ng/g dry
	Cholestanol, Desmosterol, Stigmasterol	1 ng/g dry
	β-Sitosterol	20 ng/g dry
	Cholesterol	50 ng/g dry
Biosolids	Androsterone, Desogestrel, 17α-Estradiol, Estrone, Equilin, 17β-Estradiol, Testosterone, Equilenin, Mestranol, Norethindrone, 17α-Dihydroequilin, 17α-Ethinyl Estradiol, Estriol, Coprostanol, Epicoprostanol, Campesterol, and β-Estradiol-3-Benzoate	4 ng/g dry
	Norgestrel	8 ng/g dry
	β-Stigmastanol	12 ng/g dry
	Androstenedione, Progesterone, Ergosterol	20 ng/g dry
	Cholestanol, Desmosterol, Stigmasterol	40 ng/g dry
	β-Sitosterol	800 ng/g dry
	Cholesterol	2000 ng/g dry

NOTE: Low resolution detection limits are approximately 5 times higher than the high resolution detection limits above.

HORMONE AND STEROL ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-068

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG30973

Analysis WG30228, WG30695

24 November 2009

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24 November 2009

OHIO RIVER VALLEY WATER SANITATION COMMISSION
AQUEOUS SAMPLES

STEROLS ANALYSIS
METHOD: MLA-068
4562: L13550-1 to 15

24 November 2009

NARRATIVE

This narrative describes the analysis of fifteen aqueous samples for sterols and hormones by high-resolution gas chromatography / high-resolution mass spectrometry (HR-GC/MS).

SAMPLE RECEIPT AND STORAGE

The samples were received on September 17th 2009. Details of sample conditions on receipt are provided on the Sample Receiving Records. The samples were stored at 4°C prior to extraction and analysis.

SAMPLE PREPARATION AND ANALYSIS

The samples were analyzed between two batches, WG30228 and WG30695, the compositions of which are shown on the cover page and correlation table, and on the batch accompanying the extraction workup sheets. Each batch contained a procedural blank and a lab-generated reference sample known as the Ongoing Precision and Recovery (OPR), prepared from ultra pure Seastar water.

Extraction and analysis procedures were in general accordance with Axys Method MLA-068, Analytical Method for the Determination of Sterols and Hormones with BSTFA Derivatization by GC/MS and GC/HRMS. A summary of the method (MSU-029) is supplied.

An accurately weighed subsample of approximately 1L was spiked with the routine suite of labeled quantification standards, and then extracted/cleaned up by solid phase extraction. The resulting eluate was derivatized, reduced in volume, and then spiked with labeled internal standards (referred to as the "recovery standard" in the method summary) before being submitted for instrumental analysis.

CALCULATIONS

Target analyte concentrations were determined by internal standard quantification procedures, using Micromass OPUSQuan software. Formulae used in the conversion of the raw chromatograms to concentration are provided in the method summary document.

Androsterone, desogestrel, androstenedione, and testosterone are quantified against the so-called recovery standard, the internal standard added prior to instrumental analysis. These four analytes are therefore not recovery-corrected. The remaining analytes are quantified against internal standards added at the beginning of the analysis, and are therefore recovery-corrected.

Sample specific detection limits (SDLs) were calculated for each target analyte and used as the detection qualifier. SDLs were determined from the analysis data by converting three times the height of the average noise signal to a response, using the area/height ratio of the labeled standard, and then to a concentration following the same procedures used to convert target peak responses to concentrations.

REPORTING CONVENTIONS

For internal tracking, Axys assigned ORSANCO a contract number 4562. Axys logged the samples under unique laboratory identifiers L13550-1 to -15. Each report references both the Axys ID and the ORSANCO sample identifier.

If an extract receives more than one instrumental analysis, a suffix is added to the Axys ID for each additional GC-MS run ("additional work"). The additional work suffixes used in this data package are:

- i = the extract was re-analyzed on the GC-MS
- N = the extract was serially diluted, and the dilution re-analyzed on the GC-MS
- R = the extraction and analysis was repeated starting from a fresh sub-sample

The laboratory qualifiers used are as follows:

- B = the analyte was detected in the corresponding blank and in the sample
- D = dilution data
- E = the extract concentration exceeds the calibrated range of the GC-MS; refer to dilution data
- J = the concentration is less than the LMCL
- K = a GC peak was detected that did not meet the criteria for identification as the target analyte; the reported value is the estimated maximum possible concentration.
- U = identifies a compound that was not detected
- V = the recovery of the surrogate falls outside the method control limits
- X = the result is found on another report

Results are reported to three significant figures, in units of nanograms per Liter (ng/L).

ANALYTICAL DISCUSSION

The samples were originally extracted and analyzed for hormones and sterols in batch WG30228. The hormone results from this batch were reported. Because of various QC issues in the sterol data, the samples were re-extracted from fresh subsamples in batch WG30695. All sterol results are taken from repeat batch WG30695.

WG30228
All samples required one or more additional runs on the GC-MS ("re-injections") before all instrumental QC criteria were met. Re-injections are indicated by the suffix "i" or "i2" added to the Axys IDs.

WG30695
In the initial instrumental analysis of the Site 12 and Site 16 extracts, the concentrations of stigmasterol exceeded the calibrated range of the GC-MS, and are flagged "E" accordingly. The extracts were diluted according to the procedure outlined on the Additional Work Summary included among the extraction workup sheets. (Dilutions are indicated by the suffix "N" added to the Axys IDs). Stigmasterol was reported from the dilution data, the remaining analytes from the initial analyses.

QA/QC NOTES AND DISCUSSION

QC samples (a procedural blank and an OPR) were prepared alongside the client samples and carried through the same analytical procedures. The sample data were evaluated in comparison to the corresponding batch QC samples.

- Sample analyte concentrations are not blank-corrected. Data should be compared to the corresponding blank.
- Androsterone, desogestrel, androstenedione, and testosterone are not recovery-corrected. All other analytes are recovery-corrected for possible losses through the extraction and clean up steps of the analytical procedure.
- All linearity, calibration verification, OPR and labeled compound recovery specifications were met with the following exceptions:

  In the samples listed below, the recoveries of d6-norethindrone exceeded the upper method control limit. d6-Norgestrel in Site 1 was also over-recovered. Although dilution would remedy the apparent over-recoveries, no additional work was performed because the native analytes quantified against the high surrogates -- norethindrone, norgestrel, estriol, and β-estradiol-3-benzoate – are not detected. The high surrogate recoveries do not affect the data.

CLIENT ID	AXYS ID
Site 1	L13550-1
Site 3	L13550-3
Site 4	L13550-4
Site 6	L13550-6
Site 7	L13550-7
Site 8	L13550-8
Site 10	L13550-10
Site 12	L13550-11
Site 14	L13550-13
Site 15	L13550-14

DATA PACKAGE

This data package, assigned a unique data package identifier DPWG30973 shown on the front page, includes:

- Method summary
- Sample Cover Page and Correlation Table
- Sample Receiving Documentation
- Sample homogenization and pretreatment records
- Laboratory extraction logs for each sample
- Sample data reports (in order of Axys Sample ID)
- Laboratory QC data reports
- Instrumental QC data reports (organized by analysis date)
- Sample raw data (in order of Axys Sample ID)
- Laboratory QC raw data
- Instrumental QC raw data (organized by analysis date)

I certify that this data package complies with the terms and conditions of the contract, both technically and for completeness, except for the conditions detailed above. In addition, I certify, that to the best of my knowledge and belief, the data as reported are true and accurate. The following signature, on behalf of AXYS Analytical Services Ltd, authorizes the release of the data contained in this data package.

Brian Watson
Signed: Brian Watson, B.Sc., QC Chemist

24 November 2009
Date Signed

METHOD SUMMARY

ANALYSIS OF STEROLS AND HORMONES IN WATER AND SOLIDS SAMPLES BY METHOD MLA-068

List of Analytes (Sterols and Hormones)

Androstenedione
Androsterone
Campesterol
Cholestanol
Cholesterol
Coprostanol
Desmosterol
Desogestrel
17α-Dihydroequilin
Epicoprostanol
Equilenin
Equilin
Ergosterol
17α-Estradiol
17β-Estradiol
β-Estradiol-3-Benzoate
Estriol
Estrone
17α-Ethinyl Estradiol
Mestranol
Norethindrone
Norgestrel
Progesterone
β-Sitosterol
β-Stigmastanol
Stigmasterol
Testosterone

Extraction and Cleanup Procedures

This method is applicable for the determination of a suite of sterols and hormones in solid, biosolid and aqueous samples. A “clean water” analysis option is provided for aqueous samples where very low detection limits are desired. Samples are pre-treated and separated into aqueous and solid phases as necessary to ensure that the sub-samples are representative and that the analytes are efficiently extracted. Before extraction the samples are spiked with a suite of isotopically labelled surrogate standards. Biosolids are spiked with a higher amount of cholesterol-d7 surrogate standard. Water is extracted by shaking with dichloromethane. “Clean water” samples are spiked with lower amounts of surrogate standards and processed to a lower final extract volume. Solids and biosolids are dried with anhydrous sodium sulphate and Soxhlet extracted with 40:60 hexane:acetone. The extract is solvent exchanged to 5% toluene in hexane, and sulphur is removed by treatment with activated copper. Column cleanup is performed on a layered alumina/Florisil column and the analytes eluted into a methanol fraction. Extracts from high level samples may need to be split. Derivatization is performed of sample extracts and calibration solutions at 55ºC using BSTFA:TMCS (99:1) in a pyridine solution. The derivatization converts all analytes except progesterone and androstenedione into the corresponding TMS ethers. An isotopically labelled recovery (internal) standard is added and the extract is analysed by gas chromatography/mass spectrometry (GC/MS or GC/HRMS).

HRGC/HRMS Analysis

Instrumental analysis of the derivatized final extract is performed by split/splitless injection on a high-resolution gas chromatograph (HRGC) equipped with a Restek RT_x-5 capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) coupled to either a high-resolution (HRMS) or a low-resolution (LRMS) mass spectrometer. The HRMS is operated at a static (5000) mass resolution in the electron ionization (EI) mode using Voltage SIR Detection. LRMS is operated at unit mass resolution in the EI mode using multiple ion detection (MID). Two characteristic ions for each target analyte and surrogate standard are acquired and summed.

Initial calibration is performed using a multi-point calibration series of solutions that encompass the working concentration range. Calibration is verified at least once every twelve hours by analysis of a mid-level calibration solution.

Quantification Procedures

Target concentrations are determined with respect to labelled surrogate standards as shown in Table 1 (LRMS) and Table 2 (HRMS). Mean relative response factors (RRF) determined from the initial calibration runs are used to convert raw peak areas in sample chromatograms to final concentrations as follows:

$$\text{Concentration of target} = \left(\frac{\text{area of Target}}{\text{area of Surrogate}}\right) \times \left(\frac{\text{weight of Surrogate}}{\text{weight of Sample}}\right) \times \left(\frac{1}{\text{RRF}}\right)$$

$$\text{where} \quad \text{RRF} = \left(\frac{\text{area of Target}}{\text{area of Surrogate}}\right) \times \left(\frac{\text{concentration of Surrogate}}{\text{concentration of Target}}\right)$$

Final concentrations are recovery corrected by the method of quantification. Recoveries of surrogates are determined similarly against the recovery (internal) standard and are used as general indicators of overall analytical quality.

Estimated sample specific detection limits (SDLs) are shown in Table 3.

QA/QC

Samples are analyzed in batches consisting of a maximum of twenty samples, one procedural blank and one spiked matrix (OPR) sample. Sample duplicates or matrix spike/matrix spike duplicate (MS/MSD) pairs may be analyzed on an individual contract basis. The batch is carried through the complete analytical process as a unit. For sample data to be reportable, the batch QC data must meet the established acceptance criteria presented on the analysis reports.

All aspects of the method are described in detail in AXYS' document MLA-068 "Analytical Method for the Determination of Sterols and Hormones with BSTFA Derivatization by GC/MS and GC/HRMS".

Table 1. Analyte Ions Monitored, Surrogates Used, and RRF Determination for Sterols and Hormones by GC/LRMS

Analyte Name	Quantification Standard	Typical Retention Time	RT Win (sec)	RT Standard	mass1	mass2	m1/m2 ratio
Androsterone	17β-Estradiol -d4	21:17	6	17β-Estradiol -d4	347	348	3.56
Desogestrel	17β-Estradiol -d4	21:32	6	17β-Estradiol -d4	353	354	2.97
17α-Estradiol	17β-Estradiol -d4	22:25	6	17β-Estradiol -d4	416	417	2.80
Estrone	17β-Estradiol -d4	22:25	6	17β-Estradiol -d4	342	343	3.50
Equilin	17β-Estradiol -d4	22:31	6	17β-Estradiol -d4	340	341	3.36
Androstenedione	17β-Estradiol -d4	22:37	6	17β-Estradiol -d4	286	287	4.75
17β-Estradiol	17β-Estradiol -d4	22:52	6	17β-Estradiol -d4	416	417	2.71
Testosterone	17β-Estradiol -d4	22:58	8	17β-Estradiol -d4	360	361	3.37
Equilenin	17β-Estradiol -d4	23:22	8	17β-Estradiol -d4	338	339	3.48
Mestranol	Mestranol -d4	23:26	6	Mestranol -d4	367	368	3.35
Norethindrone	Norethindrone -d6	23:33	6	Norethindrone -d6	355	356	3.49
17α-Dihydroequilin	Norethindrone -d6	23:59	6	Norethindrone -d6	307	308	3.74
17α-Ethinyl-Estradiol	17α-Ethinyl-Estradiol -d4	24:09	6	17α-Ethinyl-Estradiol -d4	425	426	2.70
Progesterone	Progesterone -d9	24:40	6	Progesterone -d9	314	315	4.45
Norgestrel	Norgestrel -d6	24:53	6	Norgestrel -d6	355	356	2.91
Estriol	Norgestrel -d6	25:27	6	Norgestrel -d6	504	505	2.28
Coprostanol	Cholesterol -d7	27:48	6	Cholesterol -d7	370	371	3.43
Epicoprostanol	Cholesterol -d7	27:59	6	Cholesterol -d7	370	371	3.49
Cholesterol	Cholesterol -d7	29:36	10	Cholesterol -d7	368	369	3.30
Cholestanol	Cholesterol -d7	29:46	6	Cholesterol -d7	445	446	2.65
Desmosterol	Cholesterol -d7	30:14	8	Cholesterol -d7	343	344	3.03
Ergosterol	Cholesterol -d7	30:54	6	Cholesterol -d7	363	364	3.51
Campesterol	Cholesterol -d7	31:08	6	Cholesterol -d7	382	383	3.19
Stigmasterol	Cholesterol -d7	31:31	6	Cholesterol -d7	484	485	2.43
β-Sitosterol	Cholesterol -d7	32:10	6	Cholesterol -d7	486	487	2.38
β-Stigmastanol	Cholesterol -d7	32:17	6	Cholesterol -d7	488	489	2.29
β-Estradiol-3-Benzoate	Norgestrel -d6	34:56	12	Norgestrel -d6	105	106	13.07
Bisphenol-A-Propane -d6	Pyrene -d10	18:48	20	Pyrene -d10	360	361	2.96
Diethyl Stilbesterol -d8	Pyrene -d10	20:05	20	Pyrene -d10	420	421	2.76
17β-Estradiol -d4	Pyrene -d10	22:50	20	Pyrene -d10	420	421	2.78
Mestranol -d4	Pyrene -d10	23:25	20	Pyrene -d10	371	372	3.38
Norethindrone -d6	Pyrene -d10	23:29	20	Pyrene -d10	361	362	3.47
17α-Ethinyl-Estradiol -d4	Pyrene -d10	24:07	20	Pyrene -d10	429	430	2.70
Progesterone -d9	Pyrene -d10	24:32	20	Pyrene -d10	323	324	4.39
Norgestrel -d6	Pyrene -d10	24:49	20	Pyrene -d10	361	362	3.03
Cholesterol -d7	Pyrene -d10	29:30	20	Pyrene -d10	375	376	3.34
Pyrene -d10		18:01	100		212	213	5.86

Table 2. Analyte Ions Monitored, Surrogates Used, and RRF Determination for Sterols and Hormones by GC/HRMS

Analyte Name	Quantification Standard	Typical Retention Time	RT Win (sec)	RT Standard	Acquired in Function	mass1	mass2	m1/m2 ratio
Androsterone	17β-Estradiol -d4	21:08	6	17β-Estradiol -d4	2	347.2406	348.2440	3.36
Desogestrel	17β-Estradiol -d4	21:26	6	17β-Estradiol -d4	2	353.2300	354.2334	2.60
17α-Estradiol	17β-Estradiol -d4	22:29	6	17β-Estradiol -d4	2	416.2566	417.2600	2.67
Estrone	17β-Estradiol -d4	22:34	6	17β-Estradiol -d4	2	342.2015	343.2048	3.42
Equilin	17β-Estradiol -d4	22:40	6	17β-Estradiol -d4	2	340.1858	341.1892	3.32
Androstenedione	17β-Estradiol -d4	22:36	6	17β-Estradiol -d4	2	358.2328	359.2361	2.54
17β-Estradiol	17β-Estradiol -d4	23:02	6	17β-Estradiol -d4	2	416.2566	417.2600	1.64
Testosterone	17β-Estradiol -d4	23:16	8	17β-Estradiol -d4	2	360.2484	361.2518	3.40
Equilenin	17β-Estradiol -d4	23:45	8	17β-Estradiol -d4	3	338.1702	339.1739	3.30
Mestranol	Mestranol -d4	23:47	6	Mestranol -d4	3	367.2093	368.2127	3.35
Norethindrone	Norethindrone -d6	23:58	6	Norethindrone -d6	3	355.2093	356.2127	3.10
17α-Dihydroequilin	17α-Ethinyl-Estradiol -d4	24:28	6	17α-Ethinyl-Estradiol -d4	4	307.1913	308.1946	3.94
17α-Ethinyl-Estradiol	17α-Ethinyl-Estradiol -d4	24:38	6	17α-Ethinyl-Estradiol -d4	4	425.2332	426.2365	2.65
Progesterone	Progesterone -d9	24:40	6	Progesterone -d9	4	314.2246	315.2279	4.45
Norgestrel	Norgestrel -d6	25:40	6	Norgestrel -d6	4	355.2093	356.2127	2.70
Estriol	Norgestrel -d6	26:15	6	Norgestrel -d6	5	504.2910	505.2944	2.25
Coprostanol	Cholesterol -d7	28:57	6	Cholesterol -d7	5	370.3631	371.3664	3.40
Epicoprostanol	Cholesterol -d7	29:05	6	Cholesterol -d7	5	370.3631	371.3664	3.40
Cholesterol	Cholesterol -d7	30:21	10	Cholesterol -d7	5	368.3474	369.3508	2.80
Cholestanol	Cholesterol -d7	30:29	6	Cholesterol -d7	5	445.3865	446.3899	2.70
Desmosterol	Cholesterol -d7	30:51	8	Cholesterol -d7	5	441.3552	442.3586	2.90
Ergosterol	Cholesterol -d7	31:26	6	Cholesterol -d7	5	363.3447	364.3475	3.50
Campesterol	Cholesterol -d7	31:41	6	Cholesterol -d7	5	382.3631	383.3664	3.05
Stigmasterol	Cholesterol -d7	32:05	6	Cholesterol -d7	5	484.4100	485.4134	2.70
β-Sitosterol	Cholesterol -d7	32:54	6	Cholesterol -d7	5	486.4257	487.4290	2.70
β-Stigmastanol	Cholesterol -d7	33:03	6	Cholesterol -d7	5	488.4413	489.4447	2.55
β-Estradiol-3-Benzoate	Norgestrel -d6	36:16	12	Norgestrel -d6	6	105.0340	106.0374	11.50
Bisphenol-A-Propane -d6	Pyrene -d10	18:24	20	Pyrene -d10	2	360.1894	361.1927	2.90
Diethyl Stilbesterol -d8	Pyrene -d10	19:45	20	Pyrene -d10	2	420.2755	421.2789	2.90
17β-Estradiol -d4	Pyrene -d10	23:00	20	Pyrene -d10	2	420.2817	421.2851	2.90
Mestranol -d4	Pyrene -d10	23:44	20	Pyrene -d10	3	371.2344	372.2378	3.31
Norethindrone -d6	Pyrene -d10	23:54	20	Pyrene -d10	3	361.2470	362.2503	3.45
17α-Ethinyl-Estradiol -d4	Pyrene -d10	24:36	20	Pyrene -d10	4	429.2583	430.2616	3.45
Progesterone -d9	Pyrene -d10	24:56	20	Pyrene -d10	4	323.2811	324.2844	4.44
Norgestrel -d6	Pyrene -d10	25:35	20	Pyrene -d10	4	361.2470	362.2503	2.90
Cholesterol -d7	Pyrene -d10	30:15	20	Pyrene -d10	5	375.3913	376.3947	3.20
Pyrene -d10		17:42	100		1	213.1400	214.0000	12.00

Table 3. Detection limits for sterols and hormones determined by GC-high resolution MS

Sample type	Analyte	Estimated detection limit
Aqueous (< 1 % solids)	Androsterone, Desogestrel, 17α-Estradiol, Estrone, Equilin, 17β-Estradiol, Testosterone, Equilenin, Mestranol, Norethindrone, 17α-Dihydroequilin, 17α-Ethinyl Estradiol, Estriol, Coprostanol, Epicoprostanol, Campesterol, and β-Estradiol-3-Benzoate	1 ng/L
	Norgestrel	2 ng/L
	β-Stigmastanol	3 ng/L
	Androstenedione, Progesterone, Ergosterol	5 ng/L
	Cholestanol, Desmosterol, Stigmasterol	10 ng/L
	β-Sitosterol	200 ng/L
	Cholesterol	500 ng/L
Aqueous, "Clean water" option	Androsterone, Desogestrel, 17α-Estradiol, Estrone, Equilin, 17β-Estradiol, Testosterone, Equilenin, Mestranol, Norethindrone, 17α-Dihydroequilin, 17α-Ethinyl Estradiol, Estriol, Coprostanol, Epicoprostanol, Campesterol, and β-Estradiol-3-Benzoate	0.04 ng/L
	Norgestrel	0.08 ng/L
	β-Stigmastanol	0.12 ng/L
	Androstenedione, Progesterone, Ergosterol	0.2 ng/L
	Cholestanol, Desmosterol, Stigmasterol	0.4 ng/L
	β-Sitosterol	8 ng/L
	Cholesterol	20 ng/L

(Table 3, continued)

Sample type	Analyte	Estimated detection limit
Solids, except biosolids	Androsterone, Desogestrel, 17α-Estradiol, Estrone, Equilin, 17β-Estradiol, Testosterone, Equilenin, Mestranol, Norethindrone, 17α-Dihydroequilin, 17α-Ethinyl Estradiol, Estriol, Coprostanol, Epicoprostanol, Campesterol, and β-Estradiol-3-Benzoate	0.1 ng/g dry
	Norgestrel	0.2 ng/g dry
	β-Stigmastanol	0.3 ng/g dry
	Androstenedione, Progesterone, Ergosterol	0.5 ng/g dry
	Cholestanol, Desmosterol, Stigmasterol	1 ng/g dry
	β-Sitosterol	20 ng/g dry
	Cholesterol	50 ng/g dry
Biosolids	Androsterone, Desogestrel, 17α-Estradiol, Estrone, Equilin, 17β-Estradiol, Testosterone, Equilenin, Mestranol, Norethindrone, 17α-Dihydroequilin, 17α-Ethinyl Estradiol, Estriol, Coprostanol, Epicoprostanol, Campesterol, and β-Estradiol-3-Benzoate	4 ng/g dry
	Norgestrel	8 ng/g dry
	β-Stigmastanol	12 ng/g dry
	Androstenedione, Progesterone, Ergosterol	20 ng/g dry
	Cholestanol, Desmosterol, Stigmasterol	40 ng/g dry
	β-Sitosterol	800 ng/g dry
	Cholesterol	2000 ng/g dry

NOTE: Low resolution detection limits are approximately 5 times higher than the high resolution detection limits above.

HORMONE AND STEROL ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-068

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG31021

Analysis WG30695

24 November 2009

HORMONE AND STEROL ANALYSIS

AQUEOUS SAMPLES

AXYS METHOD: MLA-068

PROJECT: EMERGING CONTAMINANTS IN MAINSTEM OHIO

Contract: 4562
Data Package Identification: DPWG31021
Analysis WG30695

Prepared for:
ORSANCO

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CANADA

Contact: Candice Navaroli
Project Manager

24 November 2009

OHIO RIVER VALLEY WATER SANITATION COMMISSION
AQUEOUS SAMPLES

STEROLS ANALYSIS
METHOD: MLA-068
4562: L13813-1 & -2

25 November 2009

NARRATIVE

This narrative describes the analysis of two aqueous samples for sterols and hormones by high-resolution gas chromatography / high-resolution mass spectrometry (HR-GC/MS).

SAMPLE RECEIPT AND STORAGE

The samples were received on October 22nd and 23rd 2009. Details of sample conditions on receipt are provided on the Sample Receiving Records. One sample arrived at 5°C, marginally above the guideline of 4°C. The analysis was allowed to proceed (refer to the email correspondence with the client, included at the end of the Sample Receiving section). The samples were stored at 4°C prior to extraction and analysis.

SAMPLE PREPARATION AND ANALYSIS

The samples were analyzed in batch WG30695, whose composition is shown on the cover page and correlation table, and on the batch list accompanying the extraction workup sheets. The batch contained a procedural blank and a lab-generated reference sample known as the Ongoing Precision and Recovery (OPR), both prepared from ultra pure Seastar water.

Extraction and analysis procedures were in general accordance with Axys Method MLA-068, Analytical Method for the Determination of Sterols and Hormones with BSTFA Derivatization by GC/MS and GC/HRMS. A summary of the method (MSU-029) is supplied.

An accurately weighed subsample of approximately 1L was spiked with the routine suite of labeled quantification standards, and then extracted/cleaned up by solid phase extraction. The resulting eluate was derivatized, reduced in volume, and then spiked with labeled internal standards (referred to as the "recovery standard" in the method summary) before being submitted for instrumental analysis.

CALCULATIONS

Target analyte concentrations were determined by internal standard quantification procedures, using Micromass OPUSQuan software. Formulae used in the conversion of the raw chromatograms to concentration are provided in the method summary document.

Androsterone, desogestrel, androstenedione, and testosterone are quantified against the so-called recovery standard, the internal standard added prior to instrumental analysis. These four analytes are therefore not recovery-corrected. The remaining analytes are quantified against internal standards added at the beginning of the analysis, and are therefore recovery-corrected.

Sample specific detection limits (SDLs) were calculated for each target analyte and used as the detection qualifier. SDLs were determined from the analysis data by converting three times the height of the average noise signal to a response, using the area/height ratio of the labeled standard, and then to a concentration following the same procedures used to convert target peak responses to concentrations.

REPORTING CONVENTIONS

For internal tracking, Axys assigned ORSANCO a contract number 4562. Axys logged the samples under unique laboratory identifiers L13813-1 and -2. Each report references both the Axys ID and the ORSANCO sample identifier.

If an extract receives more than one instrumental analysis, a suffix is added to the Axys ID for each additional GC-MS run ("additional work"). The additional work suffixes used in this data package are:

N = the extract was serially diluted, and the dilution re-analyzed on the GC-MS

The laboratory qualifiers used are as follows:

B = the analyte was detected in the corresponding blank and in the sample
D = dilution data
E = the extract concentration exceeds the calibrated range of the GC-MS; refer to dilution data
J = the concentration is less than the LMCL
K = a GC peak was detected that did not meet the criteria for identification as the target analyte; the reported value is the estimated maximum possible concentration.
U = identifies a compound that was not detected
V = the recovery of the surrogate falls outside the method control limits
X = the result is found on another report

Results are reported to three significant figures, in units of nanograms per Liter (ng/L).

ANALYTICAL DISCUSSION

In the initial instrumental analysis of the Site 11 extract, there were interferences affecting some of the surrogates. The extracts were diluted according to the procedure outlined on the Additional Work Summary. (Dilutions are indicated by the suffix "N" added to the Axys IDs). The dilution was successful in reducing the interferences. In order to obtain the optimal set of data, 17α-dihydroequilin and mestranol were reported from the initial analysis, the remaining analytes from the dilution analysis.

QA/QC NOTES AND DISCUSSION

QC samples (a procedural blank and an OPR) were prepared alongside the client samples and carried through the same analytical procedures. The sample data were evaluated in comparison to the corresponding batch QC samples.

- Sample analyte concentrations are not blank-corrected. Data should be compared to the corresponding blank.
- Androsterone, desogestrel, androstenedione, and testosterone are not recovery-corrected. All other analytes are recovery-corrected for possible losses through the extraction and clean up steps of the analytical procedure.
- All linearity, calibration verification, OPR and labeled compound recovery specifications were met.

DATA PACKAGE

This data package, assigned a unique data package identifier DPWG31021 shown on the front page, includes:

- Method summary
- Sample Cover Page and Correlation Table
- Sample Receiving Documentation
- Sample homogenization and pretreatment records
- Laboratory extraction logs for each sample
- Sample data reports (in order of Axys Sample ID)
- Laboratory QC data reports
- Instrumental QC data reports (organized by analysis date)
- Sample raw data (in order of Axys Sample ID)
- Laboratory QC raw data
- Instrumental QC raw data (organized by analysis date)

I certify that this data package complies with the terms and conditions of the contract, both technically and for completeness, except for the conditions detailed above. In addition, I certify, that to the best of my knowledge and belief, the data as reported are true and accurate. The following signature, on behalf of AXYS Analytical Services Ltd, authorizes the release of the data contained in this data package.

Brian Watson
Signed: Brian Watson, B.Sc., QC Chemist

25 November 2009
Date Signed

METHOD SUMMARY

ANALYSIS OF STEROLS AND HORMONES IN WATER AND SOLIDS SAMPLES BY METHOD MLA-068

List of Analytes (Sterols and Hormones)

Androstenedione
Androsterone
Campesterol
Cholestanol
Cholesterol
Coprostanol
Desmosterol
Desogestrel
17α-Dihydroequilin
Epicoprostanol
Equilenin
Equilin
Ergosterol
17α-Estradiol
17β-Estradiol
β-Estradiol-3-Benzoate
Estriol
Estrone
17α-Ethinyl Estradiol
Mestranol
Norethindrone
Norgestrel
Progesterone
β-Sitosterol
β-Stigmastanol
Stigmasterol
Testosterone

Extraction and Cleanup Procedures

This method is applicable for the determination of a suite of sterols and hormones in solid, biosolid and aqueous samples. A “clean water” analysis option is provided for aqueous samples where very low detection limits are desired. Samples are pre-treated and separated into aqueous and solid phases as necessary to ensure that the sub-samples are representative and that the analytes are efficiently extracted. Before extraction the samples are spiked with a suite of isotopically labelled surrogate standards. Biosolids are spiked with a higher amount of cholesterol-d7 surrogate standard. Water is extracted by shaking with dichloromethane. “Clean water” samples are spiked with lower amounts of surrogate standards and processed to a lower final extract volume. Solids and biosolids are dried with anhydrous sodium sulphate and Soxhlet extracted with 40:60 hexane:acetone. The extract is solvent exchanged to 5% toluene in hexane, and sulphur is removed by treatment with activated copper. Column cleanup is performed on a layered alumina/Florisil column and the analytes eluted into a methanol fraction. Extracts from high level samples may need to be split. Derivatization is performed of sample extracts and calibration solutions at 55ºC using BSTFA:TMCS (99:1) in a pyridine solution. The derivatization converts all analytes except progesterone and androstenedione into the corresponding TMS ethers. An isotopically labelled recovery (internal) standard is added and the extract is analysed by gas chromatography/mass spectrometry (GC/MS or GC/HRMS).

HRGC/HRMS Analysis

Instrumental analysis of the derivatized final extract is performed by split/splitless injection on a high-resolution gas chromatograph (HRGC) equipped with a Restek RT_x-5 capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) coupled to either a high-resolution (HRMS) or a low-resolution (LRMS) mass spectrometer. The HRMS is operated at a static (5000) mass resolution in the electron ionization (EI) mode using Voltage SIR Detection. LRMS is operated at unit mass resolution in the EI mode using multiple ion detection (MID). Two characteristic ions for each target analyte and surrogate standard are acquired and summed.

Initial calibration is performed using a multi-point calibration series of solutions that encompass the working concentration range. Calibration is verified at least once every twelve hours by analysis of a mid-level calibration solution.

Quantification Procedures

Target concentrations are determined with respect to labelled surrogate standards as shown in Table 1 (LRMS) and Table 2 (HRMS). Mean relative response factors (RRF) determined from the initial calibration runs are used to convert raw peak areas in sample chromatograms to final concentrations as follows:

$$\text{Concentration of target} = \left(\frac{\text{area of Target}}{\text{area of Surrogate}}\right) \times \left(\frac{\text{weight of Surrogate}}{\text{weight of Sample}}\right) \times \left(\frac{1}{\text{RRF}}\right)$$

$$\text{where} \quad \text{RRF} = \left(\frac{\text{area of Target}}{\text{area of Surrogate}}\right) \times \left(\frac{\text{concentration of Surrogate}}{\text{concentration of Target}}\right)$$

Final concentrations are recovery corrected by the method of quantification. Recoveries of surrogates are determined similarly against the recovery (internal) standard and are used as general indicators of overall analytical quality.

Estimated sample specific detection limits (SDLs) are shown in Table 3.

QA/QC

Samples are analyzed in batches consisting of a maximum of twenty samples, one procedural blank and one spiked matrix (OPR) sample. Sample duplicates or matrix spike/matrix spike duplicate (MS/MSD) pairs may be analyzed on an individual contract basis. The batch is carried through the complete analytical process as a unit. For sample data to be reportable, the batch QC data must meet the established acceptance criteria presented on the analysis reports.

All aspects of the method are described in detail in AXYS' document MLA-068 "Analytical Method for the Determination of Sterols and Hormones with BSTFA Derivatization by GC/MS and GC/HRMS".

Table 1. Analyte Ions Monitored, Surrogates Used, and RRF Determination for Sterols and Hormones by GC/LRMS

Analyte Name	Quantification Standard	Typical Retention Time	RT Win (sec)	RT Standard	mass1	mass2	m1/m2 ratio
Androsterone	17β-Estradiol -d4	21:17	6	17β-Estradiol -d4	347	348	3.56
Desogestrel	17β-Estradiol -d4	21:32	6	17β-Estradiol -d4	353	354	2.97
17α-Estradiol	17β-Estradiol -d4	22:25	6	17β-Estradiol -d4	416	417	2.80
Estrone	17β-Estradiol -d4	22:25	6	17β-Estradiol -d4	342	343	3.50
Equilin	17β-Estradiol -d4	22:31	6	17β-Estradiol -d4	340	341	3.36
Androstenedione	17β-Estradiol -d4	22:37	6	17β-Estradiol -d4	286	287	4.75
17β-Estradiol	17β-Estradiol -d4	22:52	6	17β-Estradiol -d4	416	417	2.71
Testosterone	17β-Estradiol -d4	22:58	8	17β-Estradiol -d4	360	361	3.37
Equilenin	17β-Estradiol -d4	23:22	8	17β-Estradiol -d4	338	339	3.48
Mestranol	Mestranol -d4	23:26	6	Mestranol -d4	367	368	3.35
Norethindrone	Norethindrone -d6	23:33	6	Norethindrone -d6	355	356	3.49
17α-Dihydroequilin	Norethindrone -d6	23:59	6	Norethindrone -d6	307	308	3.74
17α-Ethinyl-Estradiol	17α-Ethinyl-Estradiol -d4	24:09	6	17α-Ethinyl-Estradiol -d4	425	426	2.70
Progesterone	Progesterone -d9	24:40	6	Progesterone -d9	314	315	4.45
Norgestrel	Norgestrel -d6	24:53	6	Norgestrel -d6	355	356	2.91
Estriol	Norgestrel -d6	25:27	6	Norgestrel -d6	504	505	2.28
Coprostanol	Cholesterol -d7	27:48	6	Cholesterol -d7	370	371	3.43
Epicoprostanol	Cholesterol -d7	27:59	6	Cholesterol -d7	370	371	3.49
Cholesterol	Cholesterol -d7	29:36	10	Cholesterol -d7	368	369	3.30
Cholestanol	Cholesterol -d7	29:46	6	Cholesterol -d7	445	446	2.65
Desmosterol	Cholesterol -d7	30:14	8	Cholesterol -d7	343	344	3.03
Ergosterol	Cholesterol -d7	30:54	6	Cholesterol -d7	363	364	3.51
Campesterol	Cholesterol -d7	31:08	6	Cholesterol -d7	382	383	3.19
Stigmasterol	Cholesterol -d7	31:31	6	Cholesterol -d7	484	485	2.43
β-Sitosterol	Cholesterol -d7	32:10	6	Cholesterol -d7	486	487	2.38
β-Stigmastanol	Cholesterol -d7	32:17	6	Cholesterol -d7	488	489	2.29
β-Estradiol-3-Benzoate	Norgestrel -d6	34:56	12	Norgestrel -d6	105	106	13.07
Bisphenol-A-Propane -d6	Pyrene -d10	18:48	20	Pyrene -d10	360	361	2.96
Diethyl Stilbesterol -d8	Pyrene -d10	20:05	20	Pyrene -d10	420	421	2.76
17β-Estradiol -d4	Pyrene -d10	22:50	20	Pyrene -d10	420	421	2.78
Mestranol -d4	Pyrene -d10	23:25	20	Pyrene -d10	371	372	3.38
Norethindrone -d6	Pyrene -d10	23:29	20	Pyrene -d10	361	362	3.47
17α-Ethinyl-Estradiol -d4	Pyrene -d10	24:07	20	Pyrene -d10	429	430	2.70
Progesterone -d9	Pyrene -d10	24:32	20	Pyrene -d10	323	324	4.39
Norgestrel -d6	Pyrene -d10	24:49	20	Pyrene -d10	361	362	3.03
Cholesterol -d7	Pyrene -d10	29:30	20	Pyrene -d10	375	376	3.34
Pyrene -d10		18:01	100		212	213	5.86

Table 2. Analyte Ions Monitored, Surrogates Used, and RRF Determination for Sterols and Hormones by GC/HRMS

Analyte Name	Quantification Standard	Typical Retention Time	RT Win (sec)	RT Standard	Acquired in Function	mass1	mass2	m1/m2 ratio
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Desogestrel	17β-Estradiol -d4	21:26	6	17β-Estradiol -d4	2	353.2300	354.2334	2.60
17α-Estradiol	17β-Estradiol -d4	22:29	6	17β-Estradiol -d4	2	416.2566	417.2600	2.67
Estrone	17β-Estradiol -d4	22:34	6	17β-Estradiol -d4	2	342.2015	343.2048	3.42
Equilin	17β-Estradiol -d4	22:40	6	17β-Estradiol -d4	2	340.1858	341.1892	3.32
Androstenedione	17β-Estradiol -d4	22:36	6	17β-Estradiol -d4	2	358.2328	359.2361	2.54
17β-Estradiol	17β-Estradiol -d4	23:02	6	17β-Estradiol -d4	2	416.2566	417.2600	1.64
Testosterone	17β-Estradiol -d4	23:16	8	17β-Estradiol -d4	2	360.2484	361.2518	3.40
Equilenin	17β-Estradiol -d4	23:45	8	17β-Estradiol -d4	3	338.1702	339.1739	3.30
Mestranol	Mestranol -d4	23:47	6	Mestranol -d4	3	367.2093	368.2127	3.35
Norethindrone	Norethindrone -d6	23:58	6	Norethindrone -d6	3	355.2093	356.2127	3.10
17α-Dihydroequilin	17α-Ethinyl-Estradiol -d4	24:28	6	17α-Ethinyl-Estradiol -d4	4	307.1913	308.1946	3.94
17α-Ethinyl-Estradiol	17α-Ethinyl-Estradiol -d4	24:38	6	17α-Ethinyl-Estradiol -d4	4	425.2332	426.2365	2.65
Progesterone	Progesterone -d9	24:40	6	Progesterone -d9	4	314.2246	315.2279	4.45
Norgestrel	Norgestrel -d6	25:40	6	Norgestrel -d6	4	355.2093	356.2127	2.70
Estriol	Norgestrel -d6	26:15	6	Norgestrel -d6	5	504.2910	505.2944	2.25
Coprostanol	Cholesterol -d7	28:57	6	Cholesterol -d7	5	370.3631	371.3664	3.40
Epicoprostanol	Cholesterol -d7	29:05	6	Cholesterol -d7	5	370.3631	371.3664	3.40
Cholesterol	Cholesterol -d7	30:21	10	Cholesterol -d7	5	368.3474	369.3508	2.80
Cholestanol	Cholesterol -d7	30:29	6	Cholesterol -d7	5	445.3865	446.3899	2.70
Desmosterol	Cholesterol -d7	30:51	8	Cholesterol -d7	5	441.3552	442.3586	2.90
Ergosterol	Cholesterol -d7	31:26	6	Cholesterol -d7	5	363.3447	364.3475	3.50
Campesterol	Cholesterol -d7	31:41	6	Cholesterol -d7	5	382.3631	383.3664	3.05
Stigmasterol	Cholesterol -d7	32:05	6	Cholesterol -d7	5	484.4100	485.4134	2.70
β-Sitosterol	Cholesterol -d7	32:54	6	Cholesterol -d7	5	486.4257	487.4290	2.70
β-Stigmastanol	Cholesterol -d7	33:03	6	Cholesterol -d7	5	488.4413	489.4447	2.55
β-Estradiol-3-Benzoate	Norgestrel -d6	36:16	12	Norgestrel -d6	6	105.0340	106.0374	11.50
Bisphenol-A-Propane -d6	Pyrene -d10	18:24	20	Pyrene -d10	2	360.1894	361.1927	2.90
Diethyl Stilbesterol -d8	Pyrene -d10	19:45	20	Pyrene -d10	2	420.2755	421.2789	2.90
17β-Estradiol -d4	Pyrene -d10	23:00	20	Pyrene -d10	2	420.2817	421.2851	2.90
Mestranol -d4	Pyrene -d10	23:44	20	Pyrene -d10	3	371.2344	372.2378	3.31
Norethindrone -d6	Pyrene -d10	23:54	20	Pyrene -d10	3	361.2470	362.2503	3.45
17α-Ethinyl-Estradiol -d4	Pyrene -d10	24:36	20	Pyrene -d10	4	429.2583	430.2616	3.45
Progesterone -d9	Pyrene -d10	24:56	20	Pyrene -d10	4	323.2811	324.2844	4.44
Norgestrel -d6	Pyrene -d10	25:35	20	Pyrene -d10	4	361.2470	362.2503	2.90
Cholesterol -d7	Pyrene -d10	30:15	20	Pyrene -d10	5	375.3913	376.3947	3.20
Pyrene -d10		17:42	100		1	213.1400	214.0000	12.00

Table 3. Detection limits for sterols and hormones determined by GC-high resolution MS

Sample type	Analyte	Estimated detection limit
Aqueous (< 1 % solids)	Androsterone, Desogestrel, 17α-Estradiol, Estrone, Equilin, 17β-Estradiol, Testosterone, Equilenin, Mestranol, Norethindrone, 17α-Dihydroequilin, 17α-Ethinyl Estradiol, Estriol, Coprostanol, Epicoprostanol, Campesterol, and β-Estradiol-3-Benzoate	1 ng/L
	Norgestrel	2 ng/L
	β-Stigmastanol	3 ng/L
	Androstenedione, Progesterone, Ergosterol	5 ng/L
	Cholestanol, Desmosterol, Stigmasterol	10 ng/L
	β-Sitosterol	200 ng/L
	Cholesterol	500 ng/L
Aqueous, "Clean water" option	Androsterone, Desogestrel, 17α-Estradiol, Estrone, Equilin, 17β-Estradiol, Testosterone, Equilenin, Mestranol, Norethindrone, 17α-Dihydroequilin, 17α-Ethinyl Estradiol, Estriol, Coprostanol, Epicoprostanol, Campesterol, and β-Estradiol-3-Benzoate	0.04 ng/L
	Norgestrel	0.08 ng/L
	β-Stigmastanol	0.12 ng/L
	Androstenedione, Progesterone, Ergosterol	0.2 ng/L
	Cholestanol, Desmosterol, Stigmasterol	0.4 ng/L
	β-Sitosterol	8 ng/L
	Cholesterol	20 ng/L

(Table 3, continued)

Sample type	Analyte	Estimated detection limit
Solids, except biosolids	Androsterone, Desogestrel, 17α-Estradiol, Estrone, Equilin, 17β-Estradiol, Testosterone, Equilenin, Mestranol, Norethindrone, 17α-Dihydroequilin, 17α-Ethinyl Estradiol, Estriol, Coprostanol, Epicoprostanol, Campesterol, and β-Estradiol-3-Benzoate	0.1 ng/g dry
	Norgestrel	0.2 ng/g dry
	β-Stigmastanol	0.3 ng/g dry
	Androstenedione, Progesterone, Ergosterol	0.5 ng/g dry
	Cholestanol, Desmosterol, Stigmasterol	1 ng/g dry
	β-Sitosterol	20 ng/g dry
	Cholesterol	50 ng/g dry
Biosolids	Androsterone, Desogestrel, 17α-Estradiol, Estrone, Equilin, 17β-Estradiol, Testosterone, Equilenin, Mestranol, Norethindrone, 17α-Dihydroequilin, 17α-Ethinyl Estradiol, Estriol, Coprostanol, Epicoprostanol, Campesterol, and β-Estradiol-3-Benzoate	4 ng/g dry
	Norgestrel	8 ng/g dry
	β-Stigmastanol	12 ng/g dry
	Androstenedione, Progesterone, Ergosterol	20 ng/g dry
	Cholestanol, Desmosterol, Stigmasterol	40 ng/g dry
	β-Sitosterol	800 ng/g dry
	Cholesterol	2000 ng/g dry

NOTE: Low resolution detection limits are approximately 5 times higher than the high resolution detection limits above.