Chemicals of Emerging Concern in Marine and Freshwater Fish in King County Quality Assurance Project Plan

May 2021

King County

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Prepared for:

Washington Department of Ecology

Submitted by:

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Water and Land Resources Division

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Each study funded by the U.S. Environmental Protection Agency (EPA) must have an approved Quality Assurance Project Plan (QAPP). The plan describes the objectives of the study and the procedures to be followed to achieve those objectives. After completing the study, the author will post the final report of the study to the internet. This QAPP describes a project selected by the EPA's National Estuary Program (NEP) in support of Near-Term Action (NTA) 2018-0518, under Ecology Agreement No. WQNEP-2020-KCWLRD-00050.

This Quality Assurance Project Plan is available upon request from the author.

Data for this project are available in EPA's Water Quality Exchange (WQX) database (https://www.epa.gov/waterdata/water-quality-data-wqx). This QAPP is valid through five years from the date of approval.

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Quality Assurance Project Plan

Chemicals of Emerging Concern in Marine and Freshwater Fish

Ecology Agreement No.: WQNEP-2020-KCWLRD-00050
NTA 2018-0518

by Jennifer Lanksbury, Jennifer White

Published May 2021

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1.0 ABSTRACT

A summary of the effort to collect data on chemicals of emerging concern (CECs) in fish is captured by the data quality objectives (DQOs), prepared consistent with EPA's seven step DQO process (EPA QA/G-4, Publication EPA/240/B-06/001; USEPA 2006).

Step 1. State the Problem: King County's fish tissue monitoring does not include data on pharmaceuticals and personal care products (PPCPs), chemicals in common household cleaners or antibacterials in soaps and other consumer products. The absence of such information is a data gap. Information to describe the presence/absence of such chemicals in the fish tissues routinely monitored through existing programs is needed to determine whether these CECs should be further studied in tissue (Section 2).

Step 2. Identify the Goals of the Study: The goal of the study is to describe basic information on presence/absence and concentrations of 142 PPCPs, triclosan, and octylphenol (OP), nonylphenol (NP) and nonylphenol ethoxylates (NPEs) in whole fish. The study objective is the capture of up to 18 whole fish samples for analysis. These will include nine largemouth bass (*Micropterus salmoides*) or smallmouth bass (*Micropterus dolomieu*) for the freshwater species, and nine marine fish (inclusive of QA samples) including one or more of the following species: brown (*Sebastes auriculatus*; preferred species), quillback (*S. maliger*), or copper rockfish (*S. caurinus*), or English sole (*Parophrys vetulus*) (Section 3).

Step 3. Identify Information Inputs: Information inputs include a description of the study area (Section 2) and existing reports of studies conducted regionally on related topics (Section 2.2); this information will be the basis for interpretation of results. Information inputs also include the laboratory's analytical method descriptions (Appendix A).

Step 4. Define the Boundaries of the Study: The spatial boundaries of the study are defined by the existing King County (2016) fish tissue monitoring work plan in Lake Union and Elliott Bay with a preference for locations within 2,500 m of King County Combined Sewer Overflows (CSOs) (Figure 2). The temporal boundary of the study is the second quarter of 2021, when regular monitoring sample collections will be conducted. Therefore, samples will represent a 2021 snapshot of the subject analytes in fish tissue at locations in King County where the target analytes are most likely to be found in tissues (Section 4).

Step 5. Develop the Analytic Approach: Analysis will include qualitative comparisons to results of other studies or to comparable data developed in the future; descriptive results on presence/absence of each analyte in each location and species; and evaluation of within-species individual level variation in each detected analyte.

Step 6. Specify Performance or Acceptance Criteria: Analytical data acceptance criteria are determined according to the laboratory quality assurance protocols and measurement quality objectives as described in Section 6 and Appendix A.

Step 7. Plan for Obtaining Data: This QAPP presents the plan for obtaining the data. The King County Fish Tissue Monitoring Work Plan (King County 2015a); sampling and analysis plans (King County 2015b; King County 2017) and the 2021 Sampling and Analysis Plan (SAP) addenda (in progress) define the activities and procedures associated with King County's long-term monitoring program.

2.0 BACKGROUND

The project described in this Quality Assurance Project Plan (QAPP) is a near-term action (NTA) to be conducted as part of the Puget Sound Partnership's Action Agenda for protection and recovery of Puget Sound. The project will be conducted according to specifications of Agreement No. WQNEP-2020-KCWLDR-00050 between the State of Washington Department of Ecology (Ecology) and King County – Water and Land Resources Division ("Agreement"). The project is partially funded by a grant from Ecology as part of its National Estuary Program (NEP) Stormwater Strategic Initiative (SI). Both King County's technical objectives and Ecology's related project requirements are reflected in this QAPP.

2.1 Introduction and Problem Statement

Over the past two decades, natural resource management agencies regionally and nationally have initiated programs to describe the distribution of "chemicals of emerging concern (CECs)" in environmental media. Surveys of sediment, water, wastewater and fish for presence/absence and concentrations of dozens of CECs in various environmental media have been reported, including for locations in Puget Sound (Long et al. 2013; Meador et al. 2016; O'Neill et al. 2016; James et al. 2020). The specific sources and fate of CECs originating in King County are not fully understood.

King County manages wastewater for the region and discharges effluent to receiving waters from three large and two small wastewater treatment plants (WWTPs) and several combined sewer overflow facilities[1] (CSOs). King County also manages stormwater entering receiving waters within unincorporated King County. The County monitors water, sediment, and biota in receiving waters for pollutants that may be associated with stormwater and wastewater. For certain subgroups of CECs, such as pharmaceuticals and personal care products (PPCPs), antibiotic soaps containing triclosan, and the active ingredients in cleaning products such as octylphenol, nonylphenol, and nonylphenol ethoxylates (target CECs), King County facilities may be an important pathway to fresh and marine waters. Currently, the King County long-term tissue monitoring program collects several freshwater and marine fish and invertebrate species for chemistry analysis but does not have the resources to include these types of chemicals.

The problem to be addressed by this study is a shortage of information on CECs in tissues of fish within waters of King County. Results will be used to help determine whether additional sampling for the target CECs is prudent or necessary, and to prioritize which chemicals should be the focus of future sampling or monitoring in fish tissue. Results will also be used for qualitative comparisons of concentrations or presence/absence of chemicals with recent studies of whole fish by others, between fish species from similar habitat types and with results of comparable sampling efforts in the future.

[1] King County owns 39 CSO outfalls

This project will build from existing tissue monitoring programs and generate data to fill a knowledge gap on the target CECs. The primary aim of this study is to generate new information on the prevalence and magnitude of PPCPs in fish tissue. The spatial extent of this study is Lake Union and the King County waters of Puget Sound. Target species will include recreationally fished and consumed species: brown, quillback, or copper rockfish, and/or English sole, plus largemouth and/or smallmouth bass.

2.2 Study Area and Surroundings

The study areas are within King County with a focus on subareas in Elliott Bay and Lake Union already within King County's tissue monitoring program. Within the existing monitoring program, fish will be collected in waterways influenced by local wastewater and stormwater discharges (Figure 1). Target sampling locations will include Pier 62, Myrtle Edwards, and Alki stations in Elliott Bay (marine) and the Lake Union and ship canal area (freshwater) on Figure 2.

2.2.1 General Description of Study Area

Elliott Bay, Lake Union and the Ship Canal provide fish and wildlife habitat, important cultural resources, recreational boating and fishing opportunities, and commercially important fisheries and aquaculture to King County residents and visitors. The surrounding area includes a mix of residential, commercial, and industrial lands, along with parks and other natural areas. All of these waters serve the needs of Seattle's urban residents, industry, and transportation, and are the receiving environment for related municipal wastewater and stormwater. In addition, the Ship Canal receives water from Lake Washington and Elliott Bay receives water from the Duwamish River, which includes the Lower Duwamish Waterway Superfund Site located adjacent to Elliott Bay.

Human activities have contributed to the loading of chemicals in these waters through wastewater and stormwater effluents and combined sewer overflows (CSOs). Human use of medicines and products for personal care such as soaps, cosmetics, and insect repellents leads to discharges of these chemicals to King County waterways. Figure 1 shows the location of CSOs in Elliott Bay, Lake Union, and the Ship Canal, and wastewater treatment plants/facilities in Elliott Bay. In King County waters and more generally, there is relatively little information on the extent to which the large number of human-made chemicals are encountered by fish (and other aquatic life), whether the chemicals accumulate and persist in tissues, and what the possible toxicity of these chemicals may be to fish and other aquatic life.

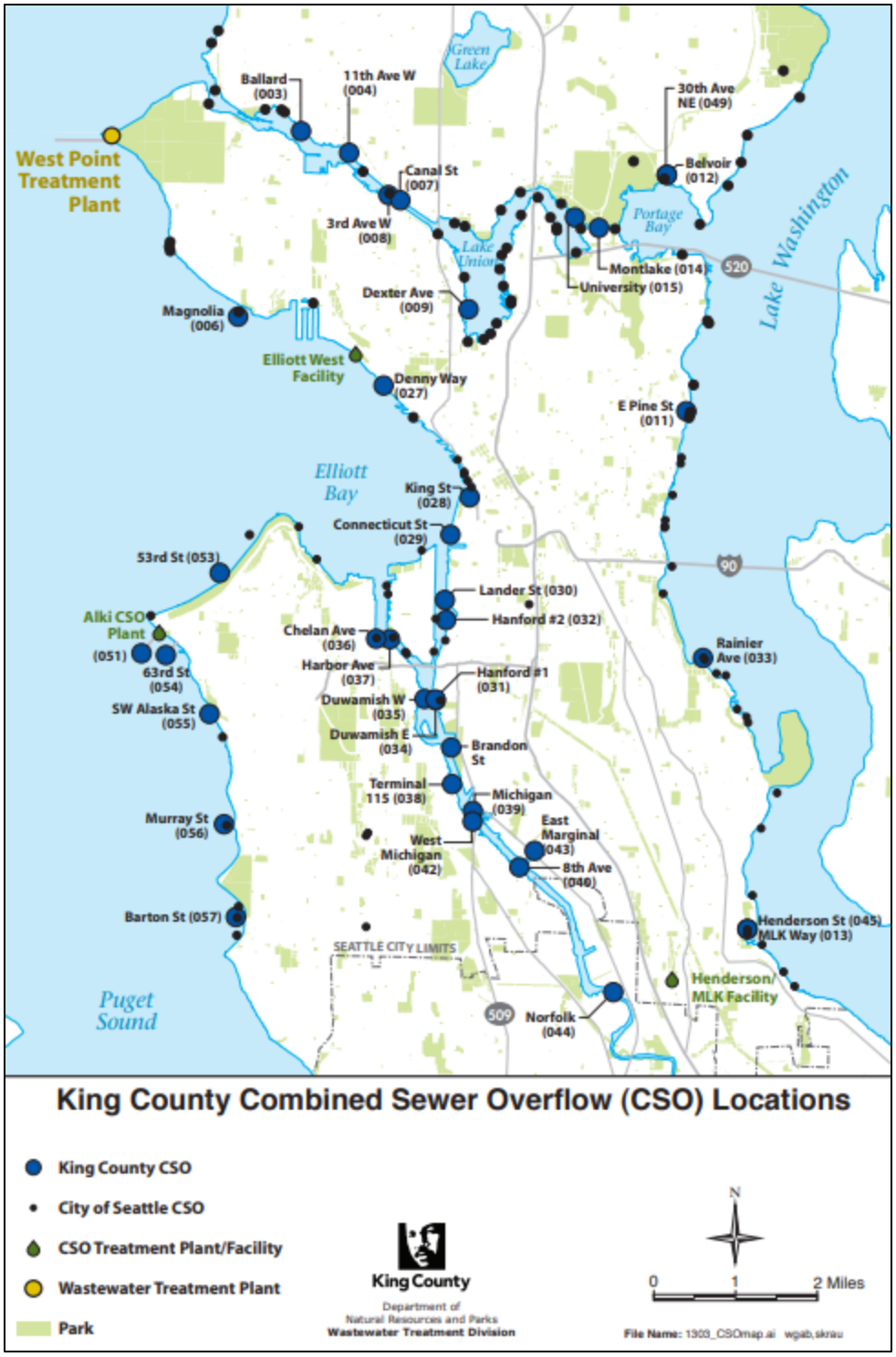

Figure 1. King County Combined Sewer Overflow (CSO) Locations.

2.2.2 Summary of Previous Studies and Existing Data

There have been a few studies on PPCPs and cleansers in fish tissue that provide information relevant to this study:

- Ramirez et al. (2009) conducted a survey of fish tissue nationwide, targeting rivers that receive direct discharge from urban WWTPs from Texas, New Mexico, Illinois, Arizona, Florida and Pennsylvania. Ramirez et al. (2009) collected composites of fillet and liver tissue from a variety of freshwater fishes, including largemouth bass, a species used in King County's freshwater fish tissue monitoring program. The authors analyzed for 24 pharmaceuticals and of these five were detected in fillets and seven were detected in livers. Of 12 personal care products (PCPs) analyzed, two (the musks galaxolide and tonalide) were detected in fillets (PCPs were not analyzed in livers). These two PCPs were detected in every effluent-dominated river sampled, but not detected in the reference site. Chemicals detected were in one of the following classes: antidepressant, antihistamine, antihypertension, antilipemics and antiseizure drugs. Chemicals never detected were of the following types: antispazmodic, analgesic, stimulant, antibiotic, antifungal and anticoagulant (Table 1). Some chemicals not detected by Ramirez et al. (2009) have been reported in fish tissues by others. None of the detected pharmaceuticals correlated significantly with lipid content, regardless of tissue, but the two PCP musks did correlate with lipid. The absence of a correlation with lipids is likely because medicines are designed to contain ionizable functional groups to facilitate excretion, and are not analogous to the persistent, lipophilic chemicals already considered by King County's tissue monitoring programs. Ramirez et al. (2009) also reported matrix interference with the high-performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) for some pharmaceuticals, particularly for liver samples. Method improvements have since reduced uncertainties associated with this issue.
- Long et al. (2013) presents an early effort to establish a baseline description of 119 PPCPs and 13 per- and poly-fluoroalkylated substances (PFAS) in Puget Sound sediments. Results are presented for 10 long-term sediment monitoring locations in the region. For 40 composite sediment samples of 2 to 3 cm depth, Long et al. (2013) report detection of 14 PPCPs (Table 1). Diphenhydramine was the most commonly detected PPCP (an antihistamine), with the maximum concentration below those detected in other urban areas in the United States. Long et al. (2013) conclude that detection frequency and concentrations of PPCPs in sediment were relatively low, and that future studies to describe PPCPs and PFAS in environmental media will support evaluation of trends and identification of potential problems.
- Meador et al. (2016) conducted a survey of CECs that included PPCPs in several Puget Sound locations in WWTP effluent, ambient water, whole juvenile Chinook and whole staghorn sculpin collected within 4,000 m of an effluent source. Meador et al. (2016) reported 81 analytes detected in effluent, and only 25 analytes detected in ambient water. They also reported analytes present in whole fish samples that were not detected in effluent or ambient water. Thirty-two pharmaceuticals were never detected in fish tissue, and only one-third of constituents detected were

detected in both fish species (Table 1). Meador et al. (2016) demonstrated that chemicals not detected in WWTP effluent or estuary waters were present in whole fish, illustrating that tissue chemistry provides a unique perspective on fish exposure to PPCPs, as well as the challenges of understanding and controlling sources of these chemicals in fish habitat.

- O'Neill et al. (2016) collected data for seven endocrine disrupting compounds (EDCs) in bile of English sole (estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethinylestradiol, Bisphenol-A (BPA), nonylphenol (NP), and octylphenol (OP)), and three selective serotonin reuptake inhibitors (SSRIs, or antidepressants) in the livers of English sole: fluoxetine, citalopram and sertraline (findings not presented in Table 1, which is a summary of literature results for only those analytes to be included in the present study). Vitellogenin was also measured in blood plasma and gonads were evaluated to determine the stage of development. In addition, 19 English sole captured along the Seattle waterfront were maintained in the laboratory and dosed with the SSRIs targeted by the study at levels indexed to the median of these SSRIs' concentrations in WWTP effluents studied previously (Schultz, unpublished) at 1X and 10X. The study reports differences in exposure of English sole to EDCs according to the extent of human development in the surrounding lands, though not statistically significant in many cases. EDC exposure (as concentrations of EDCs in bile) also did not correlate positively with development metrics. Common estrogenics (E2 and E1) were detected in 100% of samples, regardless of location, while detection of others (BPA, E3 and tOP) was variable. The laboratory study demonstrated that at high concentrations, SSRIs accumulate in liver, brain, and trunk kidney tissue, though exposure-response (accumulation) relationships were not clear nor consistent among chemicals. However, SSRIs were not detected in any of the liver samples collected. This is in contrast to results of Meador et al. (2016) which reported SSRIs fluoxetine and sertraline in whole juvenile Chinook salmon and sertraline in whole staghorn sculpin collected near (within 3,000-4,000 m of) WWTPs. Whether this difference in findings is related to the tissues sampled, the method precision, or biological differences is unknown.
- James et al. (2020) conducted a survey of over 200 CECs in transplanted marine mussels at 18 locations in the Puget Sound nearshore. They found a wide range of chronic exposures to trace levels of organic contaminants (Table 1), including the opioid oxycodone, generally below levels associated with biological impacts. However, concentrations of select compounds (e.g. alkylphenol ethoxylates and the chemotherapy drug melphalan) occurred at levels that may be of biological concern. Alkylphenols, antibiotics, central nervous system agents, and melphalan were the most frequently detected chemical classes, with three compounds (4-nonylphenol, virginiamycin M1, and sertraline) detected at all 18 locations. Other pharmaceuticals and metabolic regulators were less frequently detected, as were some current-use pesticides and N,N-diethyl-meta-toluamide (DEET). In this study the reporting limits varied for different chemicals and chemical classes, ranging from <1 to 83 ng/g wet weight, which likely affected the relative detection frequencies of the CECs. As a result, they suggest that detection frequency alone are

not a sufficient metric by which to prioritize CECs. In addition, the reporting limits for individual compounds varied between samples due to small variations in sample mass and specific matrix effects. This affected the reporting limit for selected chemicals and may have resulted in under-reporting of detection frequencies, especially in cases where the reporting limit for samples at one location(s) was higher than the reported concentration found at other locations (e.g., melphalan or metformin).

Collectively, this literature demonstrates the following considerations for the study described herein:

- Fish tissue chemistry is useful as a straightforward metric of chronic exposure of fish to targeted CECs and provides information that is not obtained through collection of effluents and surface waters within fish habitat. General surveys are still needed to support understanding of CEC exposures in aquatic and marine habitats and species-specific susceptibility to CEC exposures within King County waters.
- A comprehensive literature of toxicological studies to support assessment of risk to fish does not exist, but toxicological data may be available to support risk evaluation for some of the target CECs.
- The literature reports variation in the detection frequency of target CECs in fish liver, fish muscle, in whole fish, and among species. To date, pharmaceutical concentrations in fish tissues have not been found to correlate with tissue lipid content. General rules and patterns to support the design of a targeted study of CECs have not yet been established. Broadly descriptive data, including data to characterize uncertainty associated with potential matrix effects in complex tissue samples for some PPCP analytes, are valuable.

A general description of targeted CECs in fish tissues will be valuable to King County and the technical community in describing existing conditions.

Table 1. Summary of prior findings* for fish fillet and livers nationally, and whole fish, mussels and sediments in Puget Sound.

	Ramirez et al. 2009		Meador et al. 2016		James et al. 2020		Long et al. 2013		
	Fish tissue		Fish tissue		Mussel tissue		Sediments		
Analytes in Current Study	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Use**
Octylphenol, Nonylphenol & Nonylphenol Ethoxylates[a]									
4-Nonylphenol (4-NP)	--	--	--	--	X[a]		--	--	
4-n-Octylphenol (4N-OP)	--	--	--	--	X		--	--	
Nonylphenol diethoxylate (NP2EO)	--	--	--	--	X[a]		--	--	
Nonylphenol monoethoxylate (NP1EO)	--	--	--	--	X[a]		--	--	
Triclosan and Methyl Triclosan									
Methyl triclosan	--	--	--	--	--	--	--	--	Antibiotic (metabolite)
Triclosan	F		C			X		X	Antibiotic
Pharmaceuticals and Personal Care Products, Lists 1 - 6									
1,7-Dimethylxanthine		X		X		X		X	Antispasmodic
10-hydroxy-amitriptyline	--	--	C, S			X	--	--	Antidepressant (metabolite)
2-Hydroxy-ibuprofen	--	--		X		X		X	Anti-inflammatory (metabolite)
4-Epianhydrochlortetracycline [EACTC]	--	--	--	--	X			X	Antibiotic (metabolite)
4-Epianhydrotetracycline [EATC]	--	--	--	--		X		X	Antibiotic (metabolite)
4-Epichlortetracycline [ECTC]	--	--	--	--		X		X	Antibiotic (metabolite)
4-Epioxytetracycline [EOTC]	--	--	--	--		X		X	Antibiotic (metabolite)
4-Epitetracycline [ETC]	--	--	--	--		X	X		Antibiotic (metabolite)
Acetaminophen		X	--	--		X		X	Analgesic
Albuterol	--	--		X		X		X	Bronchodilator
Alprazolam	--	--	--	--		X		X	Antianxiety
Amitriptyline	--	--	C		X		X		Antidepressant
Amlodipine	--	--	S			X		X	Antihypertension
Amphetamine	--	--	C, S			X	X		CNS Stimulant
Amsacrine	--	--	--	--		X	--	--	Antineoplastic agent
Anhydrochlortetracycline [ACTC]	--	--	--	--		X	X		Antibiotic
Anhydrotetracycline [ATC]	--	--				X		X	Antibiotic
Atenolol		X		X		X		X	Antihypertension
Atorvastatin	--	--		X		X		X	Antihyperlipidemic
Azathioprine	--	--	--	--		X	--	--	Immunosuppressant
Azithromycin	--	--	C		X		X		Antibiotic
Benzoylecgonine	--	--		X		X		X	Cocaine metabolite
Benztropine	--	--	C			X		X	Anticholinergic

	Ramirez et al. 2009		Meador et al. 2016		James et al. 2020		Long et al. 2013		
	Fish tissue		Fish tissue		Mussel tissue		Sediments		
Analytes in Current Study	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Use**
Betamethasone	--	--	--	--		X		X	Corticosteroid anti-inflammatory
Bisphenol A	--	--	C, S			X		X	Plastics ingredient
Busulfan	--	--	--	--		X	--	--	Antineoplastic agent
Caffeine		X	C, S			X		X	Stimulant
Carbadox	--	--	--	--		X		X	Antibiotic
Carbamazepine	F, L			X		X		X	Anti-seizure
Cefotaxime	--	--	--	--		X		X	Antibiotic
Chlortetracycline [CTC]	--	--	--	--		X		X	Antibiotic
Cimetidine		X		X		X	--	--	Antacid reflux
Ciprofloxacin	--	--	S		X			X	Antibiotic
Citalopram	--	--	--	--	X		--	--	Antidepressant
Clarithromycin	--	--		X		X		X	Antibiotic
Clinafloxacin	--	--	--	--		X		X	Antibiotic
Clonidine	--	--	--	--		X		X	Antihypertension
Clotrimazole	--	--	--	--		X	--	--	Antifungal
Cloxacillin	--	--	--	--		X		X	Antibiotic
Cocaine	--	--		X		X		X	Stimulant
Codeine		X		X		X		X	Analgesic
Colchicine	--	--	--	--		X	--	--	Anti-inflammatory
Cotinine	--	--		X		X		X	Nicotine metabolite
Cyclophosphamide	--	--	--	--		X	--	--	Immunosuppressant
Daunorubicin	--	--	--	--		X	--	--	Antineoplastic agent
DEET (N,N-Diethyl-meta-toluamide)	--	--	C, S		X			X	Insect repellant
Dehydronifedipine	--	--		X		X		X	Calcium channel blocker metabolite
Demeclocycline	--	--	--	--		X		X	Antibiotic
Desmethyldiltiazem	--	--	--	--		X		X	Calcium channel blocker metabolite
Diatrizoic acid	--	--	--	--		X	--	--	X-Ray contrast medium
Diazepam	--	--	C, S			X		X	Antianxiety
Digoxigenin	--	--	--	--		X		X	Plant steroid in medical use
Digoxin	--	--	--	--		X		X	Antiarrhythmic
Diltiazem	F, L		C		X			X	Antihypertension
Diphenhydramine	F, L		C,S		X		X		Antihistamine

	Ramirez et al. 2009		Meador et al. 2016		James et al. 2020		Long et al. 2013		
	Fish tissue		Fish tissue		Mussel tissue		Sediments		
Analytes in Current Study	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Use**
Doxorubicin	--	--	--	--		X	--	--	Chemotherapy
Doxycycline	--	--	--	--		X		X	Antibiotic
Drospirenone	--	--	--	--	X		--	--	Synthetic progesterone
Enalapril	--	--	C			X		X	Blood pressure medication
Enrofloxacin	--	--	--	--	X			X	Antibiotic
Erythromycin-H2O		X	C			X		X	Antibiotic
Etoposide	--	--	--	--		X	--	--	Chemotherapy
Flumequine	--	--	--	--		X		X	Antibiotic
Fluocinonide	--	--	C			X		X	Anti-inflammatory
Fluoxetine	L		C		X			X	Antidepressant
Fluticasone propionate	--	--	--	--		X		X	Anti-inflammatory
Furosemide	--	--	--	--		X	--	--	Diuretic
Gemfibrozil	L		C			X		X	Antilipemic
Glipizide	--	--		X		X		X	Sulfonylurea
Glyburide	--	--		X		X		X	Sulfonylurea
Hydrochlorothiazide	--	--		X		X		X	Diuretic
Hydrocodone	--	--		X		X		X	Opioid
Hydrocortisone	--	--	--	--		X		X	Corticosteroid anti-inflammatory
Ibuprofen		X		X		X	X		Analgesic
Iopamidol	--	--	--	--	X		--	--	X-Ray contrast medium
Isochlortetracycline [ICTC]	--	--	--	--		X		X	Antibiotic metabolite
Lincomycin		X		X		X		X	Antibiotic
Lomefloxacin	--	--	--	--	X			X	Antibiotic
Medroxyprogesterone Acetate	--	--	--	--		X	--	--	Hormonal medication
Melphalan	--	--	--	--	X		--	--	Chemotherapy
Meprobamate	--	--		X		X		X	Antianxiety
Metformin	--	--	S		X			X	Diabetes treatment
Methylprednisolone	--	--	--	--		X		X	Anti-inflammatory
Metoprolol		X	--	X		X		X	Antihypertension
Metronidazole	--	--	--	--		X	--	--	Antibiotic
Miconazole		X	C		X		X		Antifungal
Minocycline	--	--	--	--		X		X	Antibiotic
Moxifloxacin	--	--	--	--		X	--	--	Antibiotic

	Ramirez et al. 2009		Meador et al. 2016		James et al. 2020		Long et al. 2013		
	Fish tissue		Fish tissue		Mussel tissue		Sediments		
Analytes in Current Study	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Use**
Naproxen	--	--		X		X		X	Anti-inflammatory
Norfloxacin	--	--	--	--		X		X	Antibiotic
Norfluoxetine	F, L		C			X		X	Antidepressant
Norgestimate	--	--	--	--		X		X	Hormonal medication
Norverapamil	--	--	C, S			X	X		Calcium channel blocker
Ofloxacin	--	--		X		X		X	Antibiotic
Ormetoprim	--	--	C			X		X	Antibiotic co-treatment
Oxacillin	--	--	--	--		X		X	Antibiotic
Oxazepam	--	--	--	--		X	--	--	Antianxiety
Oxolinic Acid	--	--	--	--		X		X	Antibiotic
Oxycodone	--	--		X	X			X	Opioid
Oxytetracycline [OTC]	--	--	--	--		X	X		Antibiotic
Paroxetine	--	--		X		X		X	Antidepressant
Penicillin G	--	--	--	--		X		X	Antibiotic
Penicillin V	--	--	--	--		X		X	Antibiotic
Prednisolone	--	--	--	--		X		X	Anti-inflammatory
Prednisone	--	--	--	--		X		X	Anti-inflammatory
Promethazine	--	--		X		X		X	Antihistamine
Propoxyphene	--	--		X		X	X		Pain reliever
Propranolol		X		X		X		X	Antihypertension
Ranitidine	--	--	C, S			X		X	Antihistamine, Antacid
Rosuvastatin	--	--	--	--		X	--	--	HMG-CoA reductase inhibitor (Statin)
Roxithromycin	--	--		X		X		X	Antibiotic
Sarafloxacin	--	--	--	--		X		X	Antibiotic
Sertraline	F, L		C, S		X			X	Antidepressant
Simvastatin	--	--		X		X		X	HMG-CoA reductase inhibitor (Statin)
Sulfachloropyridazine	--	--	--	--		X		X	Antibiotic
Sulfadiazine	--	--	C			X		X	Antibiotic
Sulfadimethoxine	--	--	C			X		X	Antimicrobial
Sulfamerazine	--	--	C			X		X	Antimicrobial
Sulfamethazine	--	--	--	--	X			X	Antimicrobial
Sulfamethizole	--	--	--	--		X		X	Antimicrobial
Sulfamethoxazole		X		X		X		X	Antimicrobial

	Ramirez et al. 2009		Meador et al. 2016		James et al. 2020		Long et al. 2013		
	Fish tissue		Fish tissue		Mussel tissue		Sediments		
Analytes in Current Study	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Use**
Sulfanilamide	--	--	--	--		X		X	Antimicrobial
Sulfathiazole	--	--	--	--		X		X	Antimicrobial
Tamoxifen	--	--	--	--		X	--	--	Hormonal medication
Teniposide	--	--	--	--		X	--	--	Chemotherapy
Tetracycline [TC]	--	--	--	--		X		X	Antibiotic
Theophylline	--	--	--	--		X		X	Bronchodilator (Xanthine)
Thiabendazole		X		X		X		X	Antifungal
Trenbolone	--	--	--	--		X		X	Androgenic steroid
Trenbolone acetate	--	--	--	--		X		X	Androgenic steroid
Triamterene	--	--		X		X	X		Diuretic
Triclocarban	--	--	C			X	X		Antibacterial
Triclosan	--	--	C			X		X	Antibacterial
Trimethoprim		X		X		X		X	Antibiotic
Tylosin		X	--	--		X		X	Antibiotic
Valsartan	--	--		X		X		X	Antihypertension
Venlafaxine	--	--	--	--	X[a]			X	Antidepressant
Verapamil	--	--	C, S		X		X		Calcium channel blocker
Virginiamycin M1	--	--	C, S		X			X	Antibiotic
Warfarin		X		X		X		X	Anticoagulant
Zidovudine	--	--	--	--		X	--	--	HIV Antiviral

* - Results from O'Neill et al. 2016 not included in this table.
[a] - Compounds detected were also present in laboratory blanks.
F - Fillet
L - Liver
C - Juvenile Chinook (whole body)
S - Pacific staghorn sculpin (whole body)
X - Detected or not detected, as shown
-- - Not analyzed

2.3 Parameters of Interest and Potential Sources

Pollutants of interest to this project are 142 PPCPs, triclosan, OP, NP and NPEs (Table 10). Concentrations of these chemicals will be characterized in individual whole fish because this study is exploratory: whole-fish samples present the best opportunity to detect the target CECs, if they are present, and individual samples will provide data to understand within-population variability of detected compounds.

There are a number of concerns regarding the presence of the target CECs in fish. Recent studies of aquatic species have shown drugs can bioaccumulate in fish to concentrations that may affect fish health (Brown et al. 2014; Corcoran et al. 2010; Meador et al. 2016; 2017; Bossé and Peterson 2017). Some CECs, including bisphenol A (BPA), NP, and OP, have endocrine disrupting properties that can adversely affect physiological functions supporting growth, development, behavior, and reproduction. These compounds are widely detected in water and sediments and can disrupt hormonal and metabolic processes even at relatively low concentrations, but data on exposure concentrations and toxic effects in marine and freshwater ecosystems are limited (Scott et al. 2006; Scott et al. 2007). Opiates such as oxycodone and chemotherapy drugs have been found in invertebrates from Puget Sound (James et al. 2020). Both bivalves and fish have opiate receptors and have been shown to respond both physiologically and behaviorally to opioids (Mantione et al. 2006; Bossé and Peterson 2017). The presence of pharmaceutical antibiotics in aquatic environments is of concern because they can lead to the development of antibiotic resistance genes, decreasing the effectiveness of antibiotics (Carlson et al. 2015). Other studies have shown widespread antibiotic resistance in shellfish, which can lead to antibiotic-resistant infections in the humans who consume them (Allen et al. 2010; Marshall and Levy 2011; Martínez 2008). In addition, continual exposure to multiple drugs with similar modes of action (e.g., SSRIs or antibiotics) may lead to additive or synergistic effects in aquatic organisms (Henry and Black 2007).

Sources of many of the target CECs in this study are associated with anthropogenic activity, including human use of pharmaceuticals, personal care products, flame retardants, plasticizers, and other chemicals. These chemicals are often transported via wastewater discharges into receiving waters and from urban or suburban environments in stormwater runoff. James et al. (2020) found exposures to alkylphenol ethoxylates (e.g., NP, OP, NPE) in mussels increased with increases in the extent of impervious surfaces in nearby watersheds. Meador et al. (2016) reported a range of CECs in effluent from three Puget Sound WWTPs, suggesting there may be a localized risk of health effects to fish near wastewater outfalls. A recent study of SSRIs detected in Puget Sound WWTP effluent found concentrations associated with alterations to reproduction, growth, and development (Harding et al. 2016). Several other studies, including one in Puget Sound, have concluded that although advanced nutrient removal systems can remove a number of CECs, others make it through treatment into effluent (Lubliner et al. 2010; Phillips et al. 2012).

Thus, exposures of aquatic life to CECs may involve complex mixtures with unknown effects. Scientists in Puget Sound are only beginning to define and describe the potential

problems associated with CECs in fish habitats. Additional descriptive information on both the magnitude and the inter- and intra-habitat variability of exposures is needed. A better description of the region-specific issues will support more focused investigation and development of water quality management priorities.

2.4 Regulatory Criteria or Standards

There are no tissue-based regulatory standards for PPCPs, triclosan and OP, NP and NPEs in fish tissue that are applicable in King County.

2.5 Water Quality Impairment Studies

Not applicable.

2.6 Effectiveness Monitoring Studies

Not applicable.

3.0 PROJECT DESCRIPTION

This project will be conducted to provide baseline information on the presence/absence and concentrations of PPCPs in fish tissues. It will be performed using species and locations already monitored for persistent organic chemicals (e.g. polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethanes (DDTs), PFAS, chlordanes) and metals in King County freshwater and marine fish habitats, as described in the King County Fish Tissue Monitoring Work Plan (King County 2015a); sampling and analysis plans (King County 2015b; King County 2017) and the 2021 SAP addendum (in progress).

3.1 Project Goals

The goal of this project is to fill a knowledge gap regarding the extent to which fish may be exposed to PPCPs in King County waters. Results will 1) inform whether future monitoring or more detailed assessment may be required, 2) generate baseline information that may be used to inform the public, 3) enable documentation of time trends (if subsequent studies are conducted), and 4) provide context for qualitative comparisons with similar data from other regions and jurisdictions.

The purpose and goals of conducting this project are as follows:

- Characterize the level of selected PPCPs in individual whole fish with species and locations typically used in King County's tissue monitoring programs.
- Establish general information on the presence/absence of PPCPs in fish tissue, and the concentrations of detected chemicals for use in planning future studies, performing simple qualitative comparisons with results of other studies, and with results of future monitoring, if conducted.
- Provide information to assess whether adverse effects of PPCPs on the fish themselves should be considered further by King County.

3.2 Project Objectives

The project objectives include sampling fish from both marine and freshwater systems. In both environments, this project targets individual whole fish, with stomach or stomach contents removed, for analysis of triclosan, OP, NP, NPEs and 142 PPCPs (Table 10). The program is summarized below.

3.2.1 Freshwater Fish

Largemouth bass (*Micropterus salmoides*) are the focus of this sampling effort and are abundant in Lake Union and the Ship Canal, although smallmouth bass (*Micropterus dolomieu*) are a second priority species if insufficient largemouth are collected. These species were selected because they are popular with recreational fishers and are high on the food web with a diet that consists of fish (including smaller bass), crayfish, insects, snakes, frogs, mice, ducklings, and even small turtles on rare occasions. They prefer slow

moving, clear waters and reside in rivers, lakes and ponds with an abundance of aquatic vegetation. Largemouth bass generally maintain relatively small home ranges (0.5 – 12.8 acres) in lakes (Fish & Savitz, 1983; Mesing & Wicker, 1986; Wildhaber & Neill, 1992). Fish are provided to King County staff by WDFW staff who will be obtaining them as part of a non-native predator removal project conducted periodically in Lake Union. Objectives are as follows:

- Sample number – Up to nine samples of individual whole largemouth bass or smallmouth bass
- Fish size – sexually mature bass are targeted, with a minimum fork length of at least 200 mm
- Sampling locations – Locations within 2,500 m of combined sewer overflows in Lake Union and/or the Ship Canal (Figure 2; Zones A, B, and C)
- Fish stomach contents will be removed via gastric lavage by WDFW staff in the field prior to handing off the fish to King County staff.
- King County staff will weigh and measure the length of each freshwater fish received in the field, wrap them in foil, place them into labeled bags and take them to the King County Environmental Lab (KCEL) where they will be packaged and shipped to the SGS AXYS laboratory for analysis.

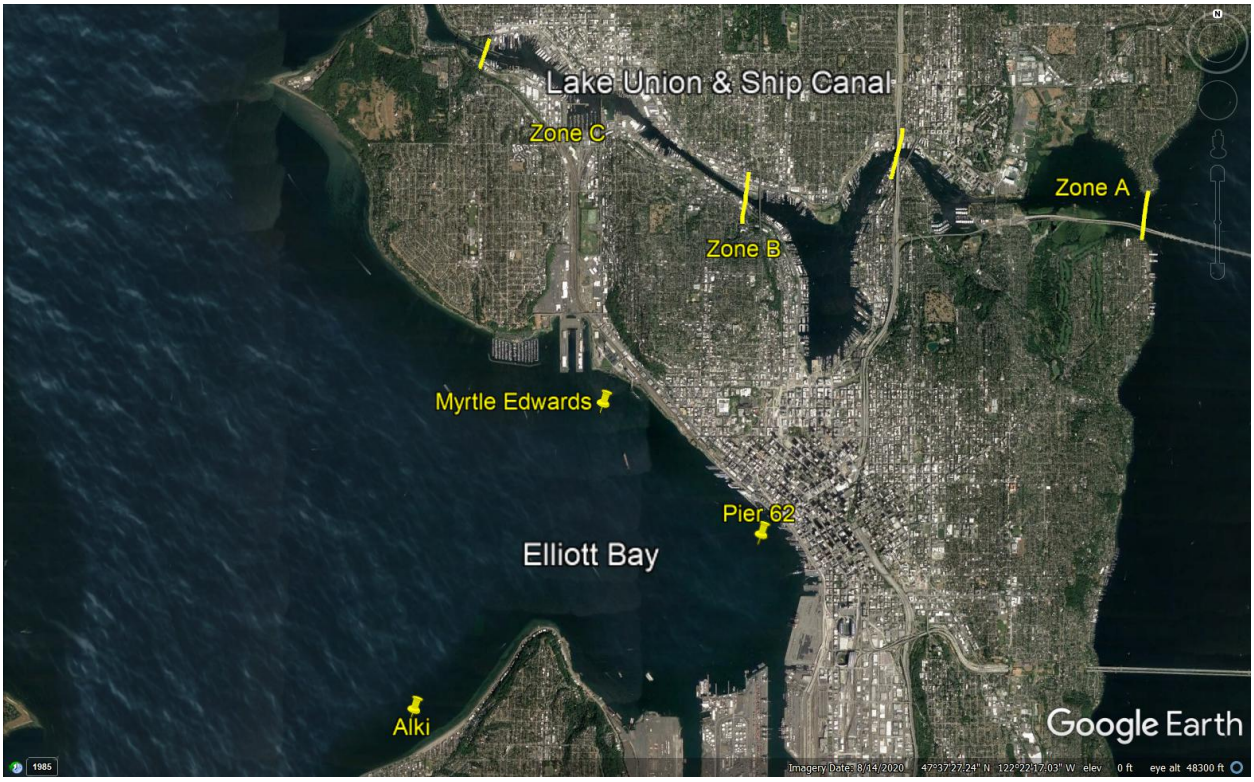

Figure 2. Target sampling areas for this study include up to three zones in Lake Union and the Ship Canal and up to three stations in Elliott Bay.

3.2.2 Marine Fish

King County's marine fish tissue monitoring program targets English sole and common rockfish species, particularly brown rockfish, at specific locations in King County marine waters. Sampling for this program is performed in coordination with Washington Department of Fish and Wildlife (WDFW). Timing and execution of the monitoring relies on vessel availability, which is less predictable in 2021 due to unusual sampling protocols and limitations associated with managing COVID-19 risk.

Brown (*Sebastes auriculatus*), quillback (*S. maliger*), or copper rockfish (*S. caurinus*) are the preferred species for this study and English sole (*Parophrys vetulus*) are a secondary target. These rockfish species are bottom dwellers that are relatively high on the food web, are long lived (>50 years) and are fished recreationally in Puget Sound. Adult brown rockfish aggregate near rocks, sewer pipes, old tires and other hard structures in Puget Sound. They display strong homing tendencies to their natural and artificial reefs, and rarely move more than three kilometers (Matthews 1990a; Matthews 1990b). Their prey includes Pacific herring, crabs, shrimp, surfperch, greenlings, and benthic invertebrates such as amphipods (Palsson et al. 2009). Quillback and copper rockfish share similar traits to the brown rockfish. English sole are one of the most abundant bottom-dwelling flatfish in Puget Sound. They feed on organisms that live in the sediments, including marine worms, mollusks, crustaceans, and echinoderms and have relatively high site fidelity to their feeding grounds (Moser et al. 2013).

- Sample number – Up to nine samples of individual whole rockfish are targeted for this project. However, availability of this number of the target fish species at the target locations is uncertain. Whole English sole will be used to supplement the target number of samples.
- Fish size – sexually mature brown, quillback or copper rockfish are targeted, with a minimum total length of 100 mm; sexually mature English sole that are 230 mm total length and longer are targeted.
- Sampling locations - King County's team will target sample collection from Pier 62, Myrtle Edwards, and Alki (Figure 2). However, whether these locations can be successfully sampled for the target species and sample number is uncertain. All marine fish will be collected from Elliott Bay.
- WDFW or King County staff will measure the length of each fish in the field, wrap them in foil, place them into labeled bags and take them to the KCEL where they will be packaged and shipped to the SGS AXYS Analytical Services LTD laboratory in Canada (SGS AXYS) for analysis.
- SGS AXYS staff will weigh each fish and remove their stomachs prior to analysis.

3.3 Information Needed and Sources

Data developed by this project will be evaluated in consideration of results from studies already conducted in Washington State and elsewhere in the United States (US) (Section 2.2.2). Of interest in such comparisons are: (1) whether chemicals detected in

tissues sampled in this study are consistent with those detected in fish sampled by others from the same or similar locations and/or other regions, regardless of tissue type and species, {2) whether more or fewer analytes are detected in this study than in other studies, and {3) individual-level variability of tissue concentrations within sampled populations.

Sources of existing data for these evaluations include the papers cited in Section 2.2.2 and any tissue chemistry data for the targeted CECs available on the Washington Department of Ecology's environmental information management (EIM) system.

Information to be generated by this project is summarized in the previous section. All project activities will be performed within the context of the King County fish tissue monitoring program, described in the program work plan (King County 2015a).

3.4 Tasks Required

Execution of this project requires tasks associated with planning, sampling and delivery of samples, data management, analysis, and reporting, and data presentation.

Planning

- Obtaining fish sampling permits including the Federal Endangered Species Act (ESA) Section 10 permit from the National Marine Fisheries Service (because ESA-protected species may be encountered, though they are not targeted); and WDFW scientific collections permit.
- Scheduling vessel with WDFW staff for sampling, use and establishing safety protocols to manage on-board COVID-19 risk, in addition to communicating typical health and safety protocols.
- Coordination of sample collection with King County's partner agency (WDFW).
- Preparing a laboratory scope of work and work agreement with SGS AXYS under existing contract with King County.
- Coordinating with the analytical laboratories (KCEL and SGS AXYS) to determine the schedule and logistics of sample transport and delivery. King County will retain SGS AXYS for processing and analysis of tissue samples, and therefore, planning requires including a process and logistics for sample transport through customs at the US-Canada border.

Sampling and Sample Delivery

- Performing fish sample collection
- Performing appropriate sample packaging for shipment within seven day hold time, including 5 kg of dry ice per 40-liter cooler per day of shipping.
- Sample shipping to the laboratory, shipment tracking, and confirmation of receipt

Data Management and Analysis

- Data entry of field observations and ancillary data for fish captured and field document management; field data validation.
- Receiving and managing analytical data, performing data verification as described in Section 12.
- Assembly of all project data, including field data sheets with trawling locations (sampling coordinates in decimal degrees), fish identification and measurements and other ancillary data generated by the project.
- Compilation of analytical and ancillary data into one database in MS Access or Excel, submit compiled dataset with appropriate quarterly progress report to Ecology's Administration of Grants and Loans (EAGL) (see reporting task below).
- Preparation of analyses as required by the Agreement (SOW Task 4.2): summary statistics, comparisons to data from other studies, and to relevant effects thresholds available from the PSEMP Toxics Work Group's CEC Prioritization effort. Analysis will include qualitative evaluation of potential source areas and chemical pathways to fish exposures.

Reporting

- Preparation of a project fact sheet and submittal with the first quarterly progress report.
- Preparation of a Detailed Project Plan (DPP).
- Reporting to the US Environmental Protection Agency (EPA), Ecology and Puget Sound Partnership as required in the Agreement:
    - Quarterly progress report and payment request (PRPR) reporting to EAGL website, including a complete set of analysis results and an interim evaluation of data in a memorandum (SOW Task 4.2)
    - Semi-annual progress reporting and a final report to the EPA's Financial and Ecosystem Accounting Tracking System (FEATS)
    - Semi-annual progress reporting to the Puget Sound Partnership on project implementation status; and annual reporting on project financial status
    - NTA reporting to the Puget Sound Partnership semi-annually on implementation status and annually on financial status
    - Report uploaded to Ecology's Environmental Information Management (EIM) system and EPA's Water Quality Exchange (WQX)
- Data evaluation, analysis and reporting to the Supervisor for the Project Manager (Table 2). This will be a technical report that describes methods, results, lessons learned and recommendations for future work. It will serve as the final report for both King County and for Ecology.
- Updating project fact sheet following completion of reports

Meetings

- Meeting with the Stormwater SI Grant Program Representative during the planning process to discuss project goals, tasks timeline and shared workload (Section 1.1 of the scope of work (SOW) in the Agreement)
- Schedule and attend a 30-minute Effectiveness Consultation meeting by teleconference with the Puget Sound Partnership once each year of the project or once total for this project (per Section 1.3 of the SOW). This meeting will likely take place following laboratory reporting and during initial data review in Fall of 2021, at the discretion of the SI Grant Program Representative.

3.5 Systematic Planning Process

For this discrete, targeted reconnaissance survey, preparing this QAPP is adequate systematic planning. Summaries of the King County field staff and KCEL and SGS AXYS laboratory staff roles and responsibilities for the logistics of sample collection, transport, and processing is available in Appendices B and C.

4.0 ORGANIZATION AND SCHEDULE

This project will utilize the existing project team and team structure in place for the existing fish tissue monitoring program.

4.1 Key Individuals and Responsibilities

Table 2 shows the responsibilities of those who will be involved in this project.

Table 2. Organization of Project Staff and Responsibilities

Staff	Title	Responsibilities
Justin Donahue Water Quality Program, Dept. of Ecology Phone: (360) 407-7671	Project/Financial Manager	Clarifies scope of the project. Provides internal review of the QAPP and approves the final QAPP.
Jennifer Lanksbury King County, Dept. of Natural Resources & Parks, Water & Land Division Phone: (206) 263-3674	Principal Investigator	Plans project design; oversees project implementation, drafts and finalizes QAPP.
Jennifer White King County, Dept. of Natural Resources & Parks, Water & Land Division Phone: (206) 477-5411	Co-Investigator	Helps draft QAPP and collect samples and records field information.
Jenée Colton King County, Dept. of Natural Resources & Parks, Water & Land Division Phone: (206) 477-4075	Supervisor for the Principal Investigator	Provides internal review of the QAPP, approves the budget, and approves the final QAPP.
Richard Jack King County, Dept. of Natural Resources & Parks, Water & Land Division Phone: (206) 477-4715	Coordinator for freshwater fish sampling	Receives freshwater fish from WDFW predator removal program and provides them to Principal Investigator.
Jim West Washington Department of Fish and Wildlife, Toxics Biological Observation System (TBiOS) Phone: (360) 870-8303	Coordinator for WDFW sampling of marine fish	Collects marine fish for this project and gives them to Principal Investigator.
Rhonda Stoddard SGS AXYS Analytical Services, LTD Phone: (250) 655-5801	Laboratory QA Manager	Coordinates with King County Project Manager.
Britta Voss Department of Ecology Phone: (360) 407-6070	NEP Quality Coordinator	Reviews the draft QAPP and recommends the final QAPP for approval.
Arati Kaza Department of Ecology Phone: (360) 407-6964	Quality Assurance Officer	Reviews and approves the draft QAPP and the final QAPP.

4.2 Special Training and Certifications

There are no special training requirements or certification requirements for this project.

Jennifer Lanksbury is the Principal Investigator for this project. She has worked on contaminant monitoring programs in Puget Sound fisheries for 12 years and has been engaged specifically in King County's tissue monitoring program since 2019.

Jim West is the coordinator for WDFW sampling of marine fish for this project. He is a senior research scientist and lead for the Washington Department of Fish and Wildlife's TBiOS team. His primary research is focused on toxic contaminants in Puget Sound biota, including rockfish, English sole, and other species. He has been sampling and identifying rockfish for research purposes at WDFW since 1990.

Execution of laboratory methods for PPCPs is a specialized laboratory service. The selected laboratory, SGS AXYS, pioneered the standard method for use of high-performance liquid chromatography, tandem mass spectrometry (HPLC-MS/MS) to be used in measurement of the targeted CECs for this project (EPA Method 1694). Laboratory personnel performing the analyses described in this QAPP will be qualified to perform the analysis according to the requirements specified in EPA's standard Method 1694; and according to internal requirements for competency with each of the methods.

4.3 Organization Chart

Not applicable - See Table 2.

4.4 Proposed Project Schedule

Sampling is anticipated to be performed in late May and in June of 2021, pending approval of the QAPP; analyses of samples are expected to be completed in August 2021 (Table 3) and data management will be completed by October 31, 2021 (Table 4). Other schedule and reporting milestones are detailed in Table 5. The final report will be sent to Ecology within the first quarter of 2022.

Table 3. Schedule for Completing Field and Laboratory Work

Task	Performance Period	Lead staff
Field work	May - June 2021	Jennifer Lanksbury
Laboratory analyses	August 2021	
Receive results from contract lab	September 2021	

Table 4. Schedule for Data Entry

Task	Performance Period	Lead staff
WQX data loaded	October, 2021	Jennifer Lanksbury
WQX QA	November, 2021	
WQX complete	December, 2021	

Table 5. Schedule for Report Preparation

Task	Performance Period	Lead staff
Draft to supervisor	November, 2021	Jennifer Lanksbury
Draft to client/peer reviewer	January 5, 2022	
Draft to external reviewers	NA	
Final draft to Strategic Initiative	Within 30 calendar days of receiving input from client reviewer, or by the final due date below, whichever is sooner	
Final report due on web	February 27, 2022	

The schedule of interim reports required by the project sponsors is as follows:

- Quarterly reports to EAGL: no later than April 15, July 15, September 15, 2021; and January 15, 2022
- Semi-annual reports to FEATS: April 1, 2021; October 15, 2021; and April 1, 2022 with final report.
- NTA reports: April 1, 2021; October 15, 2021

This schedule reflects the targeted schedule for the project, and assumes all partners perform their functions in a timely manner. The schedule is controlled to a large extent by the availability of vessels for sampling. Changes in the vessel schedule may affect the actual timing of activities listed in Table 3.

4.5 Budget and Funding

The project described herein is partly funded by a grant under the NEP Stormwater Strategic Initiative, effective March 30, 2020 to March 30, 2022. The project is also supported through in-kind contributions of labor and equipment already committed to fish sampling through King County's fish tissue monitoring program (King County 2015b; King County 2017). A summary of the project's grant budget by major expense categories and funding sources is provided in Table 6. The analytical budget is presented in Table 7. SGS AXYS will return tissue splits to KCEL for additional chemical analyses (metals, mercury, etc.), which are not included in this study.

Table 6. Project Budget and Funding

Cost Category	Grant Funded Cost
Project Development	$15,395
Project Administration	$26,274
Laboratory (See Table 7 for details.)	$62,834
Data Analysis	$7,395
Broader impacts and communications	$3,102
Total	**$115,000**

Table 7. Laboratory Budget Detailed Estimate

Parameter/Method	Number of Fish Tissue Samples	Number of QA Samples	Total Number of Samples	Cost Per Sample	Lab Subtotal
Nonylphenols / SGS AXYS Method MLA080 (LC-MS/MS)	18		18	$638	$11,484
Triclosan / SGS AXYS Method MLA115 (GC/HRMS)	18		18	$533	$9,594
PPCPs / SGS AXYS Method MLA075 (LC-MS/MS) Revised EPA Method 1694	16	2*	18	$2,195	$39,510
Stomach removal, homogenization, splits for metals analysis (marine fish)	9		9	$65	$585
Homogenization (freshwater fish)	9		9	$35	$315
Shipping - packaging (all)					$900
Budget variance - contingency					$446
				Total	**$62,834**

*Cost for additional pair of matrix spike/matrix spike duplicate (MS/MSD) samples to be analyzed for List 1 & 2 PPCPs

5.0 QUALITY OBJECTIVES

This project is to be conducted as an exploratory survey and does not have specified analytical requirements for demonstration of compliance with regulatory or other standards. Quality objectives for this project are defined by those of the laboratory and requirements of its standard methods (Appendix A), as summarized in this section.

5.1 Data Quality Objectives

The DQOs described for this project are consistent with EPA's seven step DQO process (EPA QA/G-4, Publication EPA/240/B-06/001; USEPA 2006) presented in the Abstract (Section 1). They are to collect up to 18 whole fish samples for analysis, including nine freshwater fish (bass) from Lake Union or Ship Canal and nine marine fish (rockfish or English sole) from Elliott Bay during the second quarter of 2021, and analyze them for the presence and concentrations of 142 PPCPs, triclosan, and OP, NP and NPEs.

5.2 Measurement Quality Objectives

The measurement quality objectives (MQOs) for chemical and non-chemical measurements are described below.

5.2.1 Chemistry

The MQOs for the analytical data to be collected include quantitative indicators of precision, bias, and sensitivity, according to the analytical method descriptions (Appendix A) summarized in Table 8. Reporting limits are available for the majority of chemicals to be analyzed at SGS AXYS, except for List 2 PPCPs (Table 9). The reader is referred to the following sections of Appendix A, by analyte group for these details:

- PPCPs - Method SGS AXYS MLA-075 (PPCPs). Each batch consists of a maximum of 20 samples, one procedural blank and one spiked matrix sample for assessment of ongoing precision and recovery (OPR). For analyte-specific precision and bias, see Section 5.0 "Quality Acceptance Criteria" of MLA-075 in Appendix A; sensitivity is set at a signal to noise ratio of greater than or equal to 3. Four analytes that will not be quantitated, but for which the analytical result is estimated and of "information value" only, are identified with footnotes in the table of PPCP analytes on page 1.
- OP, NP, NPEs - Method SGS AXYS MLA-080. For precision, bias and sensitivity, see method sections entitled "Quantification and Data Reporting Procedures" and "Quality Assurance/Quality Control" of MLA-080 in Appendix A.
- Triclosan, methyl triclosan - Method: SGS AXYS MLA-115. For precision and bias, see method Section 4.0, "Quantification Procedures" of MLA-115 in Appendix A; sensitivity is set at a signal-to-noise ratio of greater than or equal to 5.
- Total solids – King County Environmental Laboratory Standard Operating Procedure for Total Solids and Total Volatile Solids, SOP #307v4. This standard operating procedure is based on Method 2540B E & G (2012), EPA Method 160.3 (1983), and

EPA method 160.4 - see references section in Appendix A. A method blank is analyzed at a rate of at least 1 in 20 samples, with at least one per analytical batch. Laboratory duplicates and triplicates are analyzed at a rate of one per 20 samples, per matrix. The control limit for the relative percent difference is 20%. For method QC see section 12 of SOP in Appendix A.

Table 8. Measurement Quality Objectives for SGS AXYS Laboratory Analyses of Tissue Samples[1].

Parameter	Method[1]	Lab Duplicates Relative Percent Difference (RPD)	MS/MSD[2] (RPD)	OPR (%Recovery)	Internal Standard Recovery (% Recovery)
Pharmaceuticals and Personal Care Products	SGS AXYS Method MLA-075 REV 09 VER 01	5% of the test samples within a batch (containing 7 or more test samples) are analyzed in duplicate RPD < 40%	If conc. >5x reporting limit (RL), RPD ≤40% for all analytes; If conc. <5x RL, RPD ≤40% for 60% of analytes	See Appendix A, Method MLA-075, p.33 for analyte specific OPR and surrogate recoveries	
Octylphenol, Nonylphenol & Nonylphenol Ethoxylates	SGS AXYS Method MLA080		NA	See Appendix A, Method MLA-080, p.5 for analyte specific OPR	30- 130%
Triclosan and Methyl Triclosan	SGS AXYS Method MLA115		NA	70 - 130%	30 - 130% for d_3-triclosan; 50 - 130% for $^{13}C_{12}$-methyl triclosan

[1] There are no field duplicates planned for this study, and there are no specific lower analytical limits of interest (Section 2.4); the respective columns from Ecology's template table have been removed.
[2] One MS/MSD pair will be performed for Lists 1 and 2 PPCPs only, to evaluate matrix bias. For the rest of the planned analyses, surrogate recoveries are considered sufficient to assess method performance. Specifications shown for the MS/MSD are guidelines.

MQOs also include consideration of data representativeness, comparability, and completeness, addressed below.

5.2.2 Field Measurements

Species will be identified and recorded in the field, and fish length will be measured to the nearest mm with a fish measuring board. Freshwater fish will be weighed in the field to the gram (wet weight) with a digital scale, prior to gastric lavage. Marine fish will be weighed by laboratory staff with a digital scale at SGS AXYS prior to stomach removal and homogenization. None of the field measurements will be performed in duplicate.

5.2.3 Targets for Comparability, Representativeness, and Completeness

These categories of MQO – comparability, representativeness, and completeness – inform whether the project will generate data that can be interpreted as planned.

5.2.3.1 Comparability

Analytical results of this project may be used in comparisons to 1) each other, among samples collected for this program, 2) results of similar surveys reported in the past or future by other local agencies or in the peer reviewed literature, 3) results of future surveys by King County, and 4) results of studies that document whole-body critical tissue residue (CTR) concentrations that define thresholds of potential toxicity to fish. To ensure comparability for these purposes, King County has:

- Retained a commercial analytical laboratory with knowledge and experience in development and application of the analytical methods conducted consistent with industry practice.
- Established sample handling and processing protocols. Field personnel will consistently follow required sample handling and processing protocols for the target analytes (Table 10).
- Assembled an experienced project team to plan and execute the program in a manner that minimizes interference from exogenous contamination, chemical degradation during handling, and various other errors that can occur during execution of fish sampling projects.

To ensure consistency within the resulting analytical dataset, King County will target species, use capture and handling protocols defined by the existing fish tissue monitoring program, and follow standard operating procedures (SOPs) in use, as well as target fish within specified size ranges (King County 2015b; King County 2017).

5.2.3.2 Representativeness

The sampling to be conducted for this project will generate descriptive data for target CECs in long-lived marine fish and upper trophic level freshwater fish inhabiting waters within 2,500 m of King County CSOs. Results will be generally representative of areas where CSO effluents may have introduced the target CECs to the habitats of these fishes. Results are expected to be representative of the species and locations sampled.

Because this is an exploratory project, data interpretation will involve qualitative comparisons of results among individuals (e.g., characterization of within-site variability), among species and with results from other locations. The degree to which results are considered "representative" of the locations or species may rely to some extent on the outcomes of these comparisons. However, because limited funding provides for analysis of only 18 fish samples (roughly split between freshwater and marine fish), results are unlikely to provide a statistically robust representation of CEC concentrations within species, area sampled, or habitat and population sampled.

The results will provide the basis for evaluating within-species or within-location variability of each analyte. This information could be used in a statistical power analysis to support future study designs, providing valuable information on the appropriate level of representative sampling necessary for future freshwater or marine fish studies. The results for all samples as a group will be representative of whole fish samples for this type of study, and those compounds common across habitats and species will inform which compounds, or types of compounds, should be prioritized in future studies, including studies of toxicity to fish.

5.2.3.3 Completeness

For this study to be successful, up to eight marine fish and nine freshwater fish should be collected and analyzed for target CECs. The most significant foreseeable impediment to this success is mishandling of samples during shipping (by the shipping vendor) that results in samples not arriving at the laboratory at the appropriate temperature.

Therefore, any of the target species in excess of the target number will be stored frozen. In the event of a significant shipping error that leads to sample warming above 4°C, the frozen whole fish samples kept in storage will be sent to the laboratory in a second shipment and analyzed instead of the compromised samples.

5.3 Acceptance Criteria for Quality of Existing Data

Interpretation of results of this study will include qualitative comparisons to results of other studies of biological tissue (e.g., those described in Section 2.2; Section 5.2.3.1). Results of studies published in the peer reviewed literature or in the gray literature by government agencies or academic institutions working locally will be used for interpretation of data resulting from this study.

Gray literature prepared under defined programs that include complete QAPPs that are publicly accessible will also be used in evaluation of the results of this study. Examples of studies considered applicable for this purpose include those summarized in Section 2.2. Examples of QAPPs supporting the subset of these studies performed by government entities include Yeh et al. (2013) and O'Neill et al. (2014).

5.4 Model Quality Objectives

Not applicable.

6.0 STUDY DESIGN

This study builds on the existing King County tissue monitoring program, with a focus on fish collection within 2,500 m of King County CSOs. The study targets one freshwater (largemouth bass) and one marine (brown rockfish) species within specific size ranges, though substitute species will be collected to supplement the target species as needed (Section 3.2). Study implementation relies on King County's cooperative relationship with WDFW in collecting fish, and on the performance of the County's typical fish tissue monitoring programs. King County conducts its monitoring programs with flexibility, because the County does not always control where and when sampling takes place, and the species and numbers of fish caught varies depending on catch success. The study design summarized below reflects the flexibility necessary for King County to successfully perform the work.

6.1 Study Boundaries

The spatial boundaries of the study are defined by the existing King County (2016) fish tissue monitoring work plan in Lake Union and Elliott Bay, with a preference for locations within 2,500 m of King County CSOs (Figure 2). The temporal boundary of the study is the second quarter of 2021, when regular monitoring sample collections will be conducted. Samples will therefore represent a 2021 snapshot of the targeted CEC analytes in fish tissue at locations where the target analytes are most likely to be found in tissues (Section 4).

6.2 Field Data Collection

Sampling will be performed in King County waters, within 2,500m of King County CSO outfalls, though specific locations within Lake Union will be determined within a few weeks of sampling, based on the sampling schedule for WDFW's predator control program. Sampling will be conducted over a period of days in late May or June, 2021, and will be performed opportunistically within the constraints of the existing monitoring program.

6.2.1 Sampling Locations and Frequency

King County and its partners will sample opportunistically during the performance of King County's routine monitoring program in 2021. Trawl locations are determined by the objectives of the monitoring program. King County's partner in monitoring, WDFW, may provide King County with samples, as surplus from their own target catch. Sampling locations (Figure 2) will be sampled once over a period of one or two days in the marine habitat and over multiple days in the freshwater habitat in late spring/early summer of 2021.

6.2.2 Field Parameters and Laboratory Analytes to be Measured

Analytes for tissue chemistry are listed in Table 9. In addition to tissue chemistry, the following parameters will be measured for each fish:

- Species,
- Fish fork length and total length (to mm),
- Total fish wet weight (to gram),
- Location of capture (GPS coordinates for marine fish; one of three Zones in Lake Union or Ship Canal for freshwater fish, Figure 2).

After collection, each individual fish will be frozen whole and then packaged in dry ice (25 lbs of dry ice per 40 qt cooler, as recommended by SGS AXYS) and shipped by KCEL to SGS AXYS for processing into whole-fish tissue homogenates.

Table 9. Laboratory Measurement Methods

Analyte Class	Analyte	Reporting Limit (RL) or Method Detection Limit (MDL) (ng/g)		Analytical (Instrumental) Method	Lab
Sample Matrix: Tissue					
Conventionals	Total solids	RL = 0.01%	MDL = 0.005%	KCEL SOP # 307v4	KCEL
Octylphenol, Nonylphenol & Nonylphenol Ethoxylates	***ESI Positive***			LC-MS/MS; Method SGS AXYS MLA-080	SGS AXYS
	4-Nonylphenol monoethoxylate (NP1EO)		0.5		
	4-Nonylphenol diethoxylate (NP2EO)		0.5		
	ESI Negative				
	Nonylphenol (NP)		0.5		
	Octylphenol (OP)		0.5		
Triclosan and Methyl Triclosan	Triclosan	0.2		High Resolution GC/MS, Method SGS AXYS MLA-115	
	Methyl Triclosan	0.02			
Pharmaceuticals and Personal Care Products	***List 1 - Acid Extraction in Positive Ionization***	**Acid extractions**	**Basic extraction**	LC-MS/MS; Method SGS AXYS MLA-075	
	Acetaminophen	6			
	Azithromycin	0.6			
	Caffeine	6			
	Carbadox	0.6			
	Carbamazepine	0.6			
	Cefotaxime [1]	2.4			
	Ciprofloxacin	2.4			
	Clarithromycin	0.6			

	Clinafloxacin	2.4			
	Cloxacillin [2]	1.2			
	Dehydronifedipine	0.24			
	Digoxigenin	2.4			
	Digoxin	2.4			
	Diltiazem	0.12			
	1,7-Dimethylxanthine	24			
	Diphenhydramine	0.24			
	Enrofloxacin	1.2			
	Erythromycin-H20 [3]	0.12			
	Flumequine	0.6			
	Fluoxetine	0.6			
	Lincomycin	1.2			
	Lomefloxacin	1.2			
	Miconazole	0.6			
	Norfloxacin	6			
	Norgestimate	1.2			
	Ofloxacin	0.6			
	Ormetoprim	0.24			
	Oxacillin [2]	1.2			
	Oxolinic acid	0.24			
	Penicillin G [2]	1.2			
	Penicillin V	1.2			
	Roxithromycin	0.12			
	Sarafloxacin	6			
	Sulfachloropyridazine	0.6			
	Sulfadiazine	0.6			
	Sulfadimethoxine	0.12			
	Sulfamerazine	0.24			
	Sulfamethazine	0.24			
	Sulfamethizole	0.24			
	Sulfamethoxazole	0.24			
	Sulfanilamide	6			
	Sulfathiazole	0.6			
	Thiabendazole	0.6			
	Trimethoprim	0.6			
	Tylosin	2.4			
	Virginiamycin	1.2			
	List 2 - Tetracyclines in Positive Ionization [4]				
	Anhydrochlortetracycline	AXYS has not established			
	Anhydrotetracycline				
	Chlortetracycline				

	Demeclocycline	RLs for these analytes			
	Doxycycline				
	4-Epianhydrochlortetracycline				
	4-Epianhydrotetracycline				
	4-Epichlortetracycline				
	4-Epioxytetracycline				
	4-Epitetracycline				
	Isochlortetracycline [5]				
	Minocycline				
	Oxytetracycline				
	Tetracycline				
	List 3 - Acid Extraction in Negative Ionization				
	Bisphenol A	2.4			
	Furosemide	1.6			
	Gemfibrozil	0.32			
	Glipizide	0.32			
	Glyburide	0.32			
	Hydrochlorothiazide [6]	6.4			
	2-hydroxy-ibuprofen	1.6			
	Ibuprofen	1.6			
	Naproxen	0.8			
	Triclocarban	0.16			
	Triclosan	2.4			
	Warfarin	0.16			
	List 4 - Basic Extraction in Positive Ionization				
	Albuterol		0.3		
	Amphetamine		1.5		
	Atenolol		0.6		
	Atorvastatin		1.5		
	Cimetidine		0.6		
	Clonidine		1.5		
	Codeine		3		
	Cotinine		1.5		
	Enalapril		0.3		
	Hydrocodone		1.5		
	Metformin		3		
	Oxycodone		0.6		
	Ranitidine		0.6		
	Triamterene		0.3		
	List 5 - Acid Extraction in Positive Ionization				
	Alprazolam	0.12			

	Amitriptyline	0.12			
	Amlodipine	0.4			
	Benzoylecgonine	0.06			
	Benztropine	0.12			
	Betamethasone	0.6			
	Cocaine	0.06			
	DEET	0.12			
	Desmethyldiltiazem	0.06			
	Diazepam	0.2			
	Fluocinonide	0.8			
	Fluticasone propionate	0.8			
	Hydrocortisone	2.4			
	10-hydroxy-amitriptyline	0.06			
	Meprobamate	0.6			
	Methylprednisolone	1.6			
	Metoprolol	0.2			
	Norfluoxetine	0.2			
	Norverapamil	0.06			
	Paroxetine	0.4			
	Prednisolone	1.6			
	Prednisone	2.4			
	Promethazine	0.12			
	Propoxyphene	0.12			
	Propranolol	0.12			
	Sertraline	0.12			
	Simvastatin	0.8			
	Theophylline	2.4			
	Trenbolone	0.8			
	Trenbolone acetate	0.12			
	Valsartan	1.6			
	Verapamil	0.06			
	List 6 - Acid Extraction in Positive Ionization				
	Amsacrine	0.016			
	Azathioprine	0.4			
	Busulfan	0.8			
	Citalopram	0.16			
	Clotrimazole	0.16			
	Colchicine	0.32			
	Cyclophosphamide	0.16			
	Daunorubicin	0.8			
	Diatrizoic acid	4.8			
	Doxorubicin	2.4			

	Drospirenone	3.2			
	Etoposide	0.4			
	Iopamidol	32			
	Medroxyprogesterone acetate	1.6			
	Melphalan	9.6			
	Metronidazole	0.8			
	Moxifloxacin [7]	1.6			
	Oxazepam	1.6			
	Rosuvastatin	1.6			
	Tamoxifen	0.16			
	Teniposide	1.6			
	Venlafaxine	0.16			
	Zidovudine	2.4			

Notes:
[1] The typical reporting limits for cetofaxime are approximate.
[2] Data for compound is for information only.
[3] The typical reporting limits may be elevated by labelled surrogate purity limitation contributing to background Erythromycin-H20 levels.
[4] Analysis of List 2 analytes in tissues is not routinely offered and the tissue method is not validated for List 2.
[5] Concentration of compound is an estimated maximum value.
[6] Lowest method calibration limits for hydrochlorothiazide based on Calibration B due to native contamination in the labeled standard.
[7] Data for Moxifloxacin in solid samples is for information only; concentration is estimated.

6.3 Modeling and Analysis Design

Not applicable.

6.4 Assumptions of Study Design

The assumptions built into the study design include:

- That fish in the areas to be sampled will be exposed to the target CECs and will accumulate the target CECs into their tissues.
- That proximity to potential sources of CECs is an important driver of exposure to the target CECs.

Logistical assumptions include that the target species will be successfully collected. However, as an exploratory study, capture of substitute species (English sole in marine waters; smallmouth bass in freshwaters) will be equally informative for this basic study.

6.5 Possible Challenges and Contingencies

There are three inter-related challenges to completion of this study: COVID-19 safety requirements, reliance on a partner agency, and very brief sample hold times for target CECs. Logistics of sampling for this project prioritize worker health and safety, which creates scheduling and shipping challenges.

6.5.1 Logistical and Schedule Challenges

For its marine monitoring program, King County coordinates with WDFW, sharing a marine fish sampling vessel and conducting sampling according to a certain schedule during each sampling event. Because of the COVID-19 pandemic, logistics of sampling in 2021 are somewhat uncertain. Safety practices to prevent transmission to or by King County personnel and partner agency personnel will affect the availability of the vessel for King County's work. Usage of vessels, scheduling of staff, and managing logistics of sampling freshwaters are also affected by the ongoing pandemic and required safety considerations and staffing challenges. Further, these scheduling constraints are added to the more common uncertainty regarding the number, species, and sizes of fish that King County's team will successfully collect during its available sampling window.

The other important logistical constraint is that the samples must be shipped to the SGS AXYS laboratory as quickly as possible, and ideally within seven days of capture (Table 10). The seven days is considered a precautionary hold time, because actual degradation rates for each of the 142 PPCP analytes have not been established. External entities (i.e., FedEx) perform the shipping duties, and the available laboratory is in Canada, so shipping is complicated by the necessity of crossing an international border. Shipping delays may occur, partly due to the COVID-19 pandemic, which could result in thawing of samples en route to the laboratory. If an error occurs in shipping, samples may arrive at the laboratory above specified temperatures.

6.5.2 Logistical and Schedule Contingency Plans

The contingency plan to manage logistical challenges includes remaining flexible about which species are acceptable and when sampling is to be performed. In King County's marine monitoring program, fish samples analyzed by King County are collected by both WDFW and King County on different dates. WDFW collects fish from Pier 62 and shares them with King County staff at the end of their sampling day, while King County staff sample fish from the Myrtle Edwards and Alki stations on a separate date. For this study, King County will analyze rockfish from one of the following stations, in the following order or preference: (1) Pier 62, (2) Myrtle Edwards, (3) Alki. If none of the target fish are collected by WDFW at the Pier 62 station, or if fewer than nine rockfish are collected, then King County will collect the remaining fish required for this study at either Myrtle Edwards or Alki. The specific timing and sequence of events and final locations of the sampling goal of nine whole rockfish samples is unknown beyond this general plan. If nine rockfish are not obtained across all three locations, King County will substitute whole English sole to complement the rockfish catch.

King County's freshwater sampling involves collection of fish that result from WDFW's predator control program. Under King County's typical monitoring effort, King County coordinates with WDFW to accept a set of specimens that are later analyzed in King County's analytical laboratory. The sampling for target CECs is expected to be performed in a similar manner. Specific logistical issues related to fish capture are not anticipated. However, similar to the marine program, the final sample schedule, timing, and species and

individuals collected are not under King County's direct control and rely on actions and planning by WDFW.

To mitigate the risk of a possible shipping error, a second set of whole fish, if available as surplus from the sampling events, will be maintained frozen at -20° C until the analytical laboratory can confirm that samples have arrived according to specifications of this QAPP. King County will maintain up to nine frozen, whole fish collected in excess from each habitat type (marine and freshwater). If samples arrive at the laboratory at a temperature above 4°C, they will be discarded, and the stored fish will be shipped to the laboratory as substitutes. Even though this will result in an exceedance of the seven-day holding time, it will not compromise results as much as a significant exceedance of temperature requirements would compromise them.

The logic of prioritizing rockfish but using English sole as a substitute applies to the nine-fish surplus to be maintained to mitigate against possible shipping errors. For example, if only five rockfish are captured, King County will retain four whole English sole for use in CEC analysis to make eight fish in total, and an additional nine English sole will be held frozen until the lab confirms that the original samples are received at 4°C or below. If 13 rockfish are captured, nine that are closest in size, will be shipped and four will be held with five English sole frozen as backup, in case of shipping error. Similarly, King County will prioritize largemouth bass for CEC analysis, and smallmouth bass as a substitute, for both the initial and the backup samples.

6.5.3 Practical Constraints

Although the descriptive information on the presence/absence information and variability of individual analytes within the sampled populations is valuable to King County, interpretation of the results of sampling to address risk to fish and to people is expected to be limited. There is a paucity of toxicity reference values for whole fish or other means that can be used for comparison to the results for the target CECs. Whole fish in an uncooked state are not representative of what most humans will eat. Data interpretation to address potential risks to any human or ecological receptor may be limited, at least in the near term.

The contingency for this limitation is to evaluate data in comparison to results of other studies and to any relevant effects thresholds available from the PSEMP Toxics Work Group's CEC Prioritization effort. King County will use the resulting information to frame more focused study questions and establish priorities for future studies.

7.0 FIELD PROCEDURES

7.1 Invasive Species Evaluation

Washington law prohibits the transportation of all aquatic plants, animals, and many noxious weeds. The possible contamination with invasive species of both protective gear and sampling equipment, including boats, rafts, waders, nets, and other devices used in the water will be managed by vessel operators at WDFW for the freshwater program. Ecology's SOP EAP070 addresses how to minimize invasive species transport and contamination and is available here: https://fortress.wa.gov/ecy/publications/SummaryPages/1803201.html.

For the marine sampling program, invasives are not a concern because the vessel travels only by water and only regionally.

7.2 Measurement and Sampling Procedures

Measurement and sampling procedures will follow those described in King County's 2015 Marine Fish Tissue Monitoring and Sampling Plan (King County 2015b) and 2016 Lake Union Tissue Monitoring Sampling and Analysis Plan (King County 2017). These include standard methods for sample collection and sample delivery and storage.

7.3 Containers, Preservation Methods, Holding times

Table 10 summarizes the containers, preservation techniques, and holding times necessary to maintain sample integrity following capture and during shipping to the laboratory. Each fish sample will be individually wrapped in foil prior to being packaged as individuals in labeled plastic bags. Wrapping in foil will be performed to prevent fin rays from puncturing plastic bags during transport.

Table 10. Sample Containers, Preservation, and Holding Times for Tissue Sample Matrix

Method/Parameter	Minimum Quantity Required	Container	Sample Condition Upon Receipt	Storage Condition	Sample Holding Time	Extract Holding Time	Preservative
Total Solids and Total Volatile Solids	5 g wet	4 oz glass	≤4°C	-20°C,	14 days @ 4°C; 180 days @ -20°C	n/a	None required
SGS AXYS Method MLA-075: PPCPs Lists 1 - 6	**Sample Size Required for each of the 2 extractions (acidic and basic).**						
	Up to 1.25 g (wet) for acid extraction	Fish will be wrapped in foil and sealed in a Ziploc bag prior to freezing	<4°C, dark	-20°C, dark	7 days	40 days	None required
	Up to 0.5 g (wet) for basic extraction						
AXYS Method MLA-080: OP, NP, NPEs	2 g, wet		<4°C	<-10°C, dark	1 year	40 days	None required
SGS AXYS Method MLA-115: Triclosan, methyl triclosan	5 g wet		≤ 4°C, dark	-20°C, dark	1 year	To be determined	None required

7.4 Equipment Decontamination

Not applicable.

7.5 Fish ID

Each fish used for this study will be assigned an individual identification (ID); this fish ID[2] will be based on the King County Environmental Lab's Laboratory Information Management System (LIMS) requirements. Each individually bagged fish will be labeled with the sample location, sample date, species, and fish ID before being sent to SGS AXYS. SGS AXYS will assign a laboratory-specific identifier to each of the samples analyzed, according to its laboratory protocol, which will be cross-referenced with the KCEL fish ID.

7.6 Chain of Custody

The data to be generated for this project are not for regulatory use. However, chain-of-custody (COC) records (Appendix D) will be maintained when samples are transferred

[2] For the purposes of this study the term fish ID is synonymous with sample ID since each fish collected will be processed into an individual homogenate sample; for simplicity we use the term Fish ID throughout.

from King County staff to KCEL staff and when samples are transferred from KCEL to SGS AXYS.

The date and time of sample delivery will be recorded and both parties will then sign off in the appropriate sections on the COC forms at that time. COC seals will not be necessary.

Completed SGS AXYS COCs will be included in coolers used to ship samples to SGS AXYS, protected in a sealed Ziploc bag. Prior to securing the cooler for shipment, the King County personnel signing the COC form will scan or photograph the completed form and deliver the scan or photograph to the Project Manager, who will file it in the appropriate project folder. Similarly, when SGS AXYS ships whole fish homogenates back to KCEL, SGS AXYS personnel will sign and enclose the COC; the KCEL personnel receiving the homogenates will sign the COC, scan or photograph it, and provide the electronic and hardcopy record to the Project Manager who will file the final records.

7.7 Field Log Requirements

A field notebook will not be maintained for the sample collections that will be performed by WDFW and provided to King County. However, sample records will be maintained on sample collection forms by King County staff. Sampling information and sample metadata including the fishing location will be documented using the forms included in Appendix D.

Field datasheet forms will be used to record field measurements at all stations and will include the following information:

1. station name (locator)
2. equipment used
3. location of sampling
4. fish species collected
5. Fish ID assignment
6. individual fish total length and/or fork length and fish weight
7. date and time of sample collection
8. counts of fish taken by species
9. notes on fish condition
10. initials of all sampling personnel

COC documentation is described in Section 7.6 and Appendix D.

8.0 LABORATORY PROCEDURES

Narrative laboratory procedures for analysis of the target CECs (PPCPs, OP, NP, NPE, and triclosan) at SGS AXYS and total solids analysis at KCEL are provided in Appendix A.

8.1 Lab Procedures Table

A summary of laboratory procedures and QA specifications for analysis of the targeted CECs in tissue is provided in Table 9, including:

- Analyte/parameter names,
- Reporting Limits (RLs) for each analyte,
- Analytical methods developed by SGS AXYS for the parameters of interest,
- Analytical method for total solids used by the KCEL.

More detailed information is provided in the laboratory method narratives (Appendix A).

8.2 Sample Preparation Method(s)

Stomachs or stomach contents must be removed prior to homogenization. Rockfish will be frozen whole and shipped to the SGS AXYS laboratory, where their stomachs will be removed prior to homogenization. Gastric lavage is difficult to perform on rockfish because as these bottom fish are brought to the surface the rapid drop in atmospheric pressure induces barotrauma, which causes their swim bladders to protrude out of their mouths, blocking access to their stomachs. Stomachs will be resected from rockfish by the analytical laboratory.

Barotrauma does not occur in the freshwater fish to be captured for this program, so they will have their stomach contents removed via lavage. Upon capture an elongated hose will be inserted into the stomach of the fish orally. Once in place, water pressure is pumped into the subject fish, emptying the stomach contents. Water from Lake Union will be used for this purpose.

Once at the laboratory, sample preparation of the freshwater fish and rockfish (with stomachs removed) will consist of homogenization of each whole fish. Homogenization will be performed by SGS AXYS according to its Method SLA-013. At a minimum, homogenization procedures will:

- Minimize repetition of thawing and freezing (e.g., for dissection)
- Conserve all tissue mass (for rockfish, following stomach removal) for preparation of the final whole fish homogenate
- Thoroughly homogenize tissue samples, eliminating any large particles in the homogenization process, such that the entire sample has a uniform quality and texture and any aliquot is fully representative of the whole body sample

- Thoroughly decontaminate the homogenization tools and equipment between samples. Minimize scraping of metal instruments during the cleaning process as sample aliquots may be needed for metals analysis (King County 2021).
- Control temperature during homogenization to the lowest practicable level, minimize time at room temperature
- Maintain written documentation of any deviations from SLA-013 for each individual fish tissue sample, with appropriate sample identifier (Fish ID).

Fish scales will not be removed prior to homogenization.

8.3 Special Method Requirements

There are no special requirements for the methods to be used.

8.4 Laboratory Accredited for Methods

SGS AXYS Analytical Services LTD, located in Sidney, British Columbia, Canada, will perform all laboratory-based chemical analyses related to this project and is an Ecology-accredited laboratory[3]. SGS AXYS is compliant with and accredited to ISO/IEC 17025:2017 standards, through the Canadian Association of Laboratory Accreditation (CALA) and maintains a policy and practice of continuous improvement through its laboratory Quality Manager and trained staff. Ecology has a reciprocity agreement with CALA which allows acceptance of CALA accreditation to Ecology accreditation.

SGS AXYS does not hold accreditation for the tests required for this program because there is no accreditation program for the analytes of interest to this study. A lab accreditation waiver will be obtained from Ecology for the non-accredited analyses performed by SGS AXYS. Further information on and documentation of SGS AXYS quality systems supporting the scope of this study, including the laboratory's QA Manual and individual SOPs is available on request.

[3] https://apps.ecology.wa.gov/laboratorysearch/Default.aspx

9.0 QUALITY CONTROL PROCEDURES

Quality control for King County fish tissue monitoring programs is maintained by the use of trained and experienced personnel in all aspects of the program. King County accepts fish samples that are collected by WDFW for other purposes (freshwater) and for purposes similar to King County's purposes (marine). WDFW personnel are asked to maintain the samples that will be donated to King County for chemical analysis in pre-cleaned coolers provided by King County, which limits potential for extraneous contamination while samples are not within King County's control. King County relies on the professionalism and care of WDFW personnel in maintaining sample integrity when samples are not in King County's custody.

9.1 Table of Field and Laboratory Quality Control

The QA sample regime is summarized in Table 11. This program will not include field duplicates because of limited funding, and the limited information to be gained from the expense of duplicates, given the overall small sample sizes. The nine specimens each from the freshwater and marine habitats will provide a description of variation within the populations sampled; an MS/MSD pair will be run on one marine fish to evaluate matrix bias.

Table 11. Summary of QA Sample Program

Parameter	Field Blanks	Field Replicates	Laboratory Check (OPR) Standards	Laboratory Method Blanks	Analytical Duplicates	Laboratory Matrix Spike and Matrix Spike Duplicates
Octylphenol, Nonylphenol & Nonylphenol Ethoxylates	0	0	1 per batch	1 per batch	1 per batch	0
Triclosan and Methyl Triclosan	0	0	1 per batch	1 per batch	1 per batch	0
Pharmaceuticals and Personal Care Products	0	0	1 per batch	1 per batch	1 per batch	1*

*A matrix spike and matrix spike duplicate (MS/MSD) pair will be performed on one marine fish sample only for List 1 and 2 PPCPs (see Table 9).

Analytical methods for PPCPs have been under development for more than 10 years. Early literature reported matrix interference for some analytes with certain fish tissues. Although the mature and established laboratory methods (Appendix A) provide a batch duplicate and other method performance checks sufficient for this program (Table 8), the complexity of the fish tissue matrix suggests adding a matrix spike and matrix spike duplicate (MS/MSD) to the sample analyses would yield valuable information on the precision of the analyses on the whole fish matrix for PPCP Lists 1 and 2. Results will be used to characterize matrix-specific analytical uncertainty. The analytical laboratory's

assessment of analyte recoveries is considered sufficient for describing analytical uncertainty for all other PPCPs, NPEs and triclosan.

9.2 Corrective Action Processes

Corrective actions for anticipated logistical problems are described in Section 6.5.2. If analysis does not meet the laboratory's internal QA requirements, the laboratory will be asked to repeat analyses with archived samples. King County does not anticipate a need for further planning of corrective actions.

10.0 DATA MANAGEMENT PROCEDURES

10.1 Data Recording and Reporting Requirements

Field data will be recorded on field sheets at the time of collection and data will be entered into an Excel spreadsheet by the project lead or other King County staff afterward. Laboratory data will be recorded by SGS AXYS and KCEL according to their protocols.

10.2 Laboratory Data Package Requirements

The SGS AXYS lab and KCEL will provide findings to the project manager in electronic form when the work has been completed. This will include detailed results presented in a standard Level 2 data package (Excel spreadsheets with defined fields) that includes a general narrative and reports on the analysis of the contracted chemicals. The labs will provide all relevant quality control data as well, such as reports on matrix spike analyses, precision and recovery, calibration percent recovery, calibration retention times, and calibration verification.

10.3 Electronic Transfer Requirements

The SGS AXYS and KCEL labs will submit the analytical data resulting from this project to King County in the form of electronic data deliverables (EDDs) in Microsoft Excel file format, to minimize data entry problems and facilitate data analysis. SGS AXYS will send King County one Excel spreadsheet per batch, per method, and one per sub-method, as specified in the contract.

10.4 Data Upload Procedures

The project manager will compile analytical data from SGS AXYS and KCEL into one MS Access database. The project manager will upload the analytical data to Ecology's Administration of Grants & Loans (EAGL) database as outlined in the project grant contract. The project manager will also report any physical, chemical, and environmental data gathered as part of this project to Ecology's Environmental Information Management (EIM) system and EPA's Water Quality Exchange (WQX) at the end of the project term. To assist in WQX tracking, the project will be named as follows: NEP_2021_KingCountyCECsInFish.

10.5 Model Information Management

Not applicable.

11.0 AUDITS AND REPORTS

There are no audits planned for this project, but audits may be performed by Ecology at its discretion.

11.1 Frequency and Distribution of Reports

One report will be prepared to describe the results of this project, consistent with requirements of the Agreement, which is summarized in Section 3.4.

11.2 Responsibility for Reports

Reporting is the responsibility of King County's Principal Investigator (Table 2).

12.0 DATA VERIFICATION

Data verification is "the process of evaluating the completeness, correctness, and conformance/compliance of a specific data set against the method, procedural, or contractual requirements" (EPA, 2002). https://www.epa.gov/quality/guidance-environmental-data-verification-and-data-validation.

12.1 Field Data Verification, Requirements, and Responsibilities

Field data for this project are limited (Section 6). Fish species identifications are performed by field staff and verified with a second opinion in the field, often from the expertise of the WDFW staff and/or operators of the sampling vessel. Fish lengths and weights (freshwater fish only) will be measured in the field. The location of sampling (i.e. sampling coordinates) is verified by vessel staff via their on-board positioning system and/or cellular phone GPS, depending on availability.

12.2 Laboratory Data Validation and Verification

King County will perform Stage 2A laboratory data validation and verification (USEPA 2009), including:

- Check of the analytical data package for completeness and verification that all data requested is actually present in the data deliverables.
- Review all analytical quality assurance and quality control data for acceptance using the data quality objectives defined in this QAPP.
- Laboratory compliance with QAPP requirements for conditions of samples upon receipt, and the comparison of quality control results in the analytical data package to specified acceptance criteria (Table 8), guidelines and requirements according to the analytical methods (Appendix A; USEPA Method 1694). Attainment of both sample-related and instrument-related quality criteria will be verified.

King County will not use a third party to perform data validation.

12.3 Validation Requirements, if Necessary

Not applicable.

12.4 Model Quality Assessment

Not applicable.

13.0 DATA QUALITY (USABILITY) ASSESSMENT

13.1 Process for Determining Project Objectives Were Met

Project outcomes will be considered to have met the original objectives if the field and analytical data are generated according to the specifications of this QAPP and consistent with the study design, methods, and procedures described herein. Rejection of analytical data is not anticipated; if King County is unable to get samples to the laboratory at specified temperatures, then the laboratory will be instructed to dispose of samples and not analyze them. Data for analytes that SGS AXYS flags as "analysis result is classified as 'information value' of estimated concentration" will be flagged with a J-qualifier to indicate the analyte was positively identified but the reported result is an estimate. Data that do not meet MQOs according to the assessment by the laboratory will be qualified by the laboratory (e.g., with an R-qualifier, to indicate the data are rejected).

13.2 Treatment of Non-Detects

Censored analytical results (i.e., non-detects) for those analytes never detected will be considered to indicate that the chemical was absent in the population sampled. Censored results will be used in the establishment of study designs for future studies, by either indicating a need for a lower reporting limit or by not including never-detected analytes in future studies. Censored analytical results for analytes will be substituted at half the reporting limit for computation of summary statistics. Non-detects will not be included in in the interpretation of risk to individual fish or fish consumers. For the presence/absence evaluation, each non-detect will be considered to indicate the analyte is "absent" from the individual fish sample.

13.3 Data Analysis and Presentation Methods

Data analysis for this project will include a presence/absence evaluation for individual samples and an overall detection frequency count for each analyte within each habitat type (freshwater and marine). It will also include qualitative comparisons with other reported results (e.g., Meador et al. 2016; O'Neill et al. 2016) for whole fish samples and with each other. Statistical characterization of within-species variability and within-habitat variability will be included in the final report. Presentation of results will include maps illustrating the locations of detects and summary information on detected analytes by species.

13.4 Documentation of Assessment

The assessment of data usability will be documented in an appendix of the final report.

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15.0 APPENDICES

Appendix A: Analytical Method Summaries

STANDARD OPERATING PROCEDURE

for

Total Solids and Total Volatile Solids

SOP # 307v4

Date of Implementation: January 1, 2016

Supersedes SOP # 307v3

Approved by:

Author: _____ Date: _____

Supervisor: _____ Date: _____

QA Officer: _____ Date: _____

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Contents

1 SCOPE AND APPLICATION

1.1 This Standard Operating Procedure applies to the determination of total solids (TOTS) and total volatile solids (TVS) in aqueous and non-aqueous matrices.

1.2 The method detection limit (MDL) for TOTS and TVS is 10 mg/L in aqueous samples and 0.005% in non-aqueous samples. The reporting detection limit (RDL) for TOTS and TVS is 20 mg/L in aqueous samples and 0.01% in non-aqueous samples. The type of sample is defined by the Laboratory Information Management System (LIMS). There are three types of samples: liquid, solid and tissue.

1.3 The practical range of determination for liquid samples is 10 mg/L to 20,000 mg/L. The practical range of determination for solid samples is 0.005% to 100%. Samples are not to be diluted to extend the analytical range.

2 ASSOCIATED STANDARD OPERATING PROCEDURES*

(*Latest editions of the following SOPs)

2.1 Conventionals Unit Balance Calibration SOP 343.

2.2 Conventionals Unit Temperature Monitoring and Calibration Verification SOP 342.

2.3 Settleable Solids – Gravimetric and Volumetric SOP 305.

2.4 Suspended Solids - Total, 0.45µm and Volatile SOP 309.

2.5 Total Dissolved Solids SOP 308.

3 METHOD SUMMARY

3.1 Aqueous Samples

3.1.1 A measured volume of a well-mixed sample is poured into a tared porcelain evaporating dish and dried to a constant weight at 104°C. The resulting net weight represents the TOTS of the sample. The dried sample is then ignited at 550°C to constant weight. The resulting weight loss represents the TVS of the sample.

3.2 Non-aqueous Samples

3.2.1 A well-mixed sample is weighed in a tared aluminum evaporating dish and dried at 104°C to constant weight. The resulting net weight represents the TOTS of the sample. The dried sample is then ignited at 550°C to constant weight. The resulting weight loss represents the TVS of the sample.

4 INTERFERENCES

4.1 Multi-phasic samples are subject to sub sampling and sample preparation errors. Difficulty with sample homogenizing, volume measuring and drying to constant weight due to the presence of oil and grease in the matrix may yield questionable results. The analyst must document all observations and anomalies related to these types of phenomena in the Comments box on the Excel spreadsheet (13.5.1).

4.2 Highly mineralized waters may be hygroscopic and require prolonged drying, proper desiccation and rapid weighing.

4.3 Exclude large floating particles or submerged agglomerates of non-homogenous materials from the sample, if it is determined that their inclusion is not representative. Removal or exclusion of any non-homogenous material must be documented by the analyst in the Excel spreadsheet (13.5.1).

4.4 For aqueous samples, too much residue in the dish can crust over and entrap water that will not be driven off during drying. Total residue should be limited to 200mg. If it is suspected that a sample has crusted over, the analysis should be repeated with a smaller volume of sample.

5 DEFINITIONS

5.1 TOTS – The residue retained in an evaporating dish dried to a constant weight at 104°C.

5.2 TVS – The portion of TOTS lost after ignition at 550°C (obtained by difference).

5.3 Aqueous Samples – All "L%" matrices in LIMS, where "%" is a wildcard representing a letter. If a liquid sample is too viscous to measure volumetrically, then the sample can be analyzed like a solid sample and the sample aliquot is weighed.

5.4 Non-Aqueous Samples – All "S%" and "T%" matrices in LIMS.

5.5 . The preparation date and time are defined as the date and time the sample crucibles go into the drying oven for the initial TOTS drying cycle. The analysis date and time are defined as the date and time that the sample crucibles are weighed for the final TOTS and TVS determinations.

5.6 A batch consists of all samples that are prepared and analyzed at the same time using the same procedure. Because liquids and solids are processed differently, separate into different batches even if prepared on the same day. Matrix QC samples are analyzed for each matrix. Typically a batch is assigned as a single workgroup. TOTS and TVS can be included in the same workgroup.

6 SAFETY

6.1 General Safety – Comply with all general laboratory safety practices including wearing a lab coat, safety glasses and gloves. Treat samples with regard to possible toxicity and microbiological potential.

6.2 Method safety

6.2.1 Use thermal protection gloves and long metal tongs when using the 104°C oven and the 550°C muffle furnace.

6.2.2 After the test is completed and the samples are deemed non-hazardous, discard the aluminum dishes and solids in the garbage.

6.2.3 For hazardous samples, follow the laboratory's Hazardous Waste Disposal SOP 1108.

7 SAMPLE COLLECTION, PRESERVATION AND STORAGE

7.1 Sample Containers

7.1.1 The recommended sample container for aqueous samples is a 500mL clear wide mouth HDPE bottle.

7.1.2 The recommended sample container for non-aqueous samples is a 4oz clear polypropylene or glass jar. Solid samples requiring Total Organic Carbon in addition to TOTS are analyzed from the same jar.

7.2 Sample Holding Times

7.2.1 Aqueous samples may be stored at <6°C for up to 7 days.

7.2.2 Non Aqueous Samples

7.2.2.1 Solid samples may be stored at 4°C for 14 days or may be frozen at -18°C for up to 6 months.

7.2.2.2 Sludge samples may be stored at 4°C for up to 7 days.

7.2.2.3 Tissues are frozen at -18°C for up to 6 months. Storage at 4°C for 7 days is acceptable but storage outside of the freezer should be minimized to the extent possible.

7.3 Minimum Sample Volumes

7.3.1 Aqueous Samples - For a full set of QC sample analyses to be performed, a minimum of 350mL of sample is required. Smaller volumes may be used depending on the matrix and sample characteristics.

7.3.2 Non-Aqueous Samples - For a full set of QC sample analyses to be performed, a minimum of 60g of sample is required. Smaller amounts may be used depending on the sample characteristics.

8 APPARATUS AND EQUIPMENT

8.1 Drying oven with suitable exhaust, set at 104 ± 1°C

8.2 Muffle furnace with suitable exhaust, set at 550 ± 50°C

8.3 Desiccator

8.4 Analytical balance (capable of ±0.1mg) – MT XP 205. Balance includes ionizer for static control.

8.5 Graduated cylinders

8.6 Long metal tongs

8.7 Win Wedge Balance Software

8.8 250mL porcelain crucible, high form (VWR #89038-002)

9 STANDARDS, REAGENTS AND CONSUMABLES

9.1 Reverse Osmosis (RO) Water (ASTM Type I)

9.2 70mm Aluminum dishes (Fisher #08734C)

9.3 110mm Aluminum Dishes (Fisher #08-732-10D)

9.4 Disposable plastic spoons for weighing solid samples

9.5 Indicating Desiccant (Fisher #07-577-3B)

9.6 Non-Indicating Desiccant (Fisher #07-577-3B)

9.7 Laboratory Control Sample (ERA Mineral Quality Control Sample, #506)

10 MAINTENANCE

10.1 Desiccator – A mixture of indicating and non-indicating desiccant is utilized. Monitor indicator desiccant for moisture content. When the blue indicator desiccant turns purple, replace desiccant mixture with a fresh batch of three parts non-indicating to one part indicating desiccant. Spent desiccant can be disposed of in a garbage can. Alternately, the desiccant can be dried up to three times by placing in a 180°C oven for two hours.

10.2 Analytical Balance – The analyst should keep the surfaces of the analytical balance free of any debris.

10.3 Drying Ovens – The analyst should keep the interior surface of the oven free of any debris. The inside of the muffle furnace should be swept clean of any debris before each use.

10.4 Work Area - Counter tops should be wiped down after each use and the area should be free of clutter.

11 PROCEDURE

11.1 Calibration

11.1.1 Analytical Balance – Refer to SOP 343 Balance Calibration. Check that the internal balance calibration has been performed prior to weighing. Perform the daily calibration if necessary.

11.1.2 Drying oven temperatures are monitored daily as outlined in SOP 342, Temperature Monitoring and Calibration Verification.

11.1.3 Monitor indicator desiccant for moisture content. If the blue indicator desiccant turns purple, see 10.1.

11.2 Aqueous Sample Preparation and Analysis

11.2.1 For TOTS, heat clean porcelain crucibles in the 104°C oven for one hour. If TVS is requested, heat the porcelain crucibles at 550 ± 50°C for one hour. Prepare one crucible per sample and separate crucibles for the method blank (MB) and Lab Control Sample (LCS) per 20 samples. Prepare one lab duplicate (LD) per 20 samples per matrix. Remove the crucibles from the oven or muffle furnace and place them in the desiccator for at least one hour. Crucibles must be at room temperature before balance readings can be taken.

11.2.2 Open Excel and from the **Add-Ins** tab locate the **Custom Toolbars** menu. Click **Data Entry/Reduction** followed by **Solids** then **Total**. From the list of templates select **TOTS (L%)** or **TOTS_TVS (L%)** to open the applicable analytical template.

11.2.3 WinWedge software is utilized to securely manage the transfer of balance readings to the analytical template. This software opens and activates the appropriate configuration automatically when the template is opened under the following conditions.

11.2.3.1 The PC being utilized is linked directly to the balance used for data collection.

11.2.3.2 A current version of the WinWedge software is not already active on the PC linked to the balance.

11.2.4 Enter the Workgroup number, LCS Prep ID and the analyst's initials. Enter the crucible IDs under the column labeled Crucible ID. Save the file to the following location: I:\InstrumentData\Solids. Files are further organized into folders by month and year analyzed. Use the following naming convention for files: TOTS(L)_YYMMDD or TVS_YYMMDD, where YYMMDD is the year, month and day of analysis. The Prep/Date Time is defined as time the sample crucibles go into the drying oven for the initial TOTS drying cycle. The QC Prep ID corresponds to the assigned ID for the current LCS.

11.2.5 Immediately before use, weigh the porcelain evaporating dishes individually from the desiccator. To open or close the balance shield, press the **Select 1** or **Select 2** key. With the shield closed, tare the empty balance by pressing the **Re-Zero** key. Open the shield and place a dish onto the tared balance using forceps or tongs. Close the shield and weigh the empty dish. Record the empty dish weight onto the Excel spreadsheet under the **Crucible Initial (g)** column next to the corresponding crucible ID by pressing the **Print** key. The WinWedge software will automatically transfer the weight displayed on the balance into the active cell. Repeat the above steps to weigh all the dishes.

11.2.6 To end the working session close the analytical template. The WinWedge session will then close and program will terminate automatically. Save the file before closing it.

11.2.7 Measure 150mL of a well-mixed sample in a graduated cylinder and transfer the measured volume into a labeled porcelain crucible. Smaller volumes may be required when analyzing liquid samples with high solids contents (i.e., liquid digested sludge samples; see 4.4.). Rinse the graduated cylinder with three aliquots (<5mL) of RO water, transferring the rinsate to the crucible. Record the sample IDs with the corresponding crucible IDs, volumes used and any sample observations or anomalies in the spreadsheet. For each QC sample enter its workgroup ID in the corresponding **LIMS QC ID** field of the spreadsheet.

11.2.8 Carefully transfer the crucibles to the 104°C oven, verify the temperature is within ± 1°C of the target temperature and dry for a minimum of four hours to overnight. The oven temperature, date, and time are recorded at the beginning and end of the drying cycle on the Excel spreadsheet. Avoid drying times that exceed one day unless necessary to completely dry the sample or to reach a constant weight. Transfer the crucibles into the desiccator and allow them to cool for at least one hour and no more than 5 days past drying

11.2.9 Open the previously saved Excel analytical file containing the initial crucible weights. WinWedge will again automatically open and activate the appropriate configuration for data collection. Weigh the crucibles to the nearest 0.1mg and record the weight under the Crucible Final (g) column of the Excel analytical file.

11.2.10 Samples with residue amount greater than 200mg should be reanalyzed using smaller sample volume. If this is not possible, then check with your supervisor.

11.2.11 To end the working data collection session close the analytical template. The WinWedge session will then close and program will terminate automatically.

11.2.12 At this point, analysis for TOTS is complete. If TVS is requested, continue to the next step with the porcelain crucibles used for the TOTS analysis. Typically the cycle of drying, desiccating and reweighing is not repeated because sample residues usually dry quickly and they generally do not quickly absorb atmospheric water vapor.

11.2.13 For samples requiring TVS, place the crucibles previously used for TOTS in the muffle furnace and turn it on to slowly bring the samples to the ignition temperature of 550 ± 50°C. Leave the crucibles in the muffle furnace for at least 30 minutes at temperature to allow for complete combustion of all volatile material. The oven temperature, date, and time are recorded at the beginning and end of the drying cycle on the Excel analytical template. Remove (use metal tongs and oven gloves) the crucibles from the muffle furnace, transfer them into the desiccator and allow them to cool to room temperature for at least one hour.

11.2.14 Open the previously saved Excel analytical file containing the initial crucible weights. WinWedge will again automatically open and activate the appropriate configuration for data collection. Weigh the crucibles to the nearest 0.1mg and record the weight under the Crucible Post Ignition (g) column of the Excel analytical file.

11.2.15 To end the working data collection session close the analytical template. The WinWedge session will then close and program will terminate automatically. Save the file before closing it.

11.3 Non-aqueous Sample Preparation and Analysis

11.3.1 If the samples are frozen, remove them from the freezer and allow them to thaw in the hood or a 4°C cooler. Record on the Excel analytical template the time and date the samples were removed from the freezer. After analysis, return the samples to the freezer and record the time and date on the Excel analytical template.

11.3.2 Number a set of aluminum dishes, place them on a tray, transfer the tray with the dishes into the 104°C oven and heat them for at least one hour. If TVS is requested, heat the aluminum dishes at 550 ± 50°C for one hour in the muffle furnace. Do not place the metal tray in the muffle furnace. Remove the dishes from the oven or muffle furnace and place them in the desiccator for at least one hour. Dishes must be at room temperature before balance readings can be taken.

11.3.3 Open Excel and from the Add-Ins tab locate the Custom Toolbars menu. Click Data Entry/Reduction followed by Solids then Total. From the list of templates select TOTS (S%) or TOTS_TVS (S%) to open the applicable analytical template.

11.3.4 WinWedge software is utilized to securely manage the transfer of balance readings to the analytical template. This software opens and activates the appropriate configuration automatically when the template is opened under the following conditions.

11.3.4.1 The PC being utilized is linked directly to the balance used for data collection.

11.3.4.2 A current version of the WinWedge software is not already active on the PC linked to the balance.

11.3.5 Enter the Workgroup number and the analyst's initials. Enter the crucible IDs under the column labeled Crucible ID. Save the file to the following location: I:\InstrumentData\Solids. Files are further organized into folders by month and year analyzed. Use the following naming convention for files: TOTS(S)_YYMMDD or TVS_YYMMDD, where YYMMDD is the year, month and day of analysis. The Prep/Date Time is defined as time the sample crucibles go into the drying oven for the initial TOTS drying cycle.

11.3.6 Remove the tray containing the aluminum dishes from the desiccator. To open or close the balance shield, press the Select 1 or Select 2 key. With the shield closed, tare the empty balance by pressing the Re-Zero key. Open the shield and place a dish onto the tared balance using forceps or tongs. Close the shield and weigh the empty dish. Record the empty dish weight onto the Excel spreadsheet under the Crucible Initial (g) column next to the corresponding crucible ID by pressing the Print key. The WinWedge software will automatically transfer the weight displayed on the balance into the active cell. Repeat the above steps to weigh all the dishes.

11.3.7 To end the working session close the analytical template. The WinWedge session will then close and program will terminate automatically. Save the file before closing it

11.3.8 Mix the sample well with a plastic spoon and transfer ~20-25g of the mixed sample into a **tared** and numbered aluminum dish. For a solid matrix with high water content, use a larger sample aliquot (~30-40g). Record the sample weights in the spreadsheet under the **Sample Mass (g)** column. Record the sample IDs with the corresponding dish IDs and any sample observations or anomalies into the spreadsheet. For each QC sample enter its workgroup ID in the corresponding **LIMS QC ID** field of the analytical template.

11.3.9 Place the tray with the aluminum dishes in the 104°C oven and heat them for at least four hours to overnight. Avoid drying times exceeding one day unless necessary to completely dry the samples or to reach constant weight. Remove the tray with the dishes from the oven, transfer them into the desiccator and allow them to cool for at least one hour, but not exceeding 5 days.

11.3.10 Open the previously saved Excel analytical file containing the initial dish weights. WinWedge will again automatically open and activate the appropriate configuration for data collection. Weigh the dishes to the nearest 0.1mg and record the weight under the **Crucible Final (g)** column of the Excel analytical file.

11.3.11 To end the working data collection session close the analytical template. The WinWedge session will then close and program will terminate automatically.

11.3.12 At this point, analysis for TOTS is complete. If TVS is requested, continue to the next step with the aluminum dishes used for the TOTS analysis. Typically the cycle of drying, desiccating and reweighing is not repeated because sample residues usually dry quickly and they generally do not quickly absorb atmospheric water vapor.

11.3.13 For TVS analysis, carefully transfer the aluminum dishes previously used for TOTS to the muffle furnace and turn it on to slowly bring the samples to the ignition temperature of 550 ± 50°C. Do not place the metal tray in the muffle furnace. Leave the samples in the muffle furnace for at least one hour after the oven reaches 550°C to allow for complete combustion of volatile material. The oven temperature, date, and time are recorded at the beginning and end of the drying cycle on the Excel spreadsheet. Remove the dishes from the muffle furnace, transfer them to the desiccator and allow them to cool to room temperature for at least one hour.

11.3.14 Open the previously saved Excel analytical file containing the initial crucible weights. WinWedge will again automatically open and activate the appropriate configuration for data collection. Weigh the dishes to the nearest 0.1mg and record the weight under the **Crucible Post Ignition (g)** column of the Excel analytical file.

11.3.15 To end the working data collection session close the analytical template. The WinWedge session will then close and program will terminate automatically. Save the file before closing it.

12 QA/QC REQUIREMENTS

12.1 Data Security

12.1.1 Data collection is performed using protected, formatted Excel templates in conjunction with WinWedge software to facilitate data transfer in a secure environment. The following considerations and procedures are in place to assist in minimizing undocumented data changes or deletions.

12.1.1.1 Cells designated for initial & final crucible / dish weights, analysis dates/times, calculated solids values, calculated MDLs, and calculated RDLs are protected from direct modification or deletion by the analyst.

12.1.1.2 Data entry to the above fields can only occur if the analyst is (1) at the specific PC designated for data collection and (2) utilizing a recognized, protected WinWedge configuration.

12.1.1.3 Data entry can only be performed by clicking the **Transfer** or **Print** button on the balance being utilized for data collection. Direct data entry via a PC keyboard is not allowed.

12.1.1.4 Individual worksheets within an Excel template workbook cannot be added or removed by the analyst. As with protected cells within each worksheet, this serves to protect the integrity of the overall workbook structure against loss of data or alteration of the standardized data collection environment.

12.1.1.5 Situations are anticipated where an analyst may be required to replace an existing value obtained from a balance. This could include the initial reading being recorded in a wrong cell designated for a different sample. The following process is utilized to perform and document any data changes performed by the analyst.

12.1.1.5.1 Data entry attempted for a protected cell already containing data will trigger a message prompting the analyst to recognize this situation.

12.1.1.5.2 If the analyst decides to proceed in changing the existing data, a reason must be provided by the analyst indicating the nature of this change.

12.1.1.5.3 Upon providing a reason, the data are replaced and an entry is recorded in the protected Audit Log worksheet within the existing workbook. This entry includes: date/time of change, sample ID, crucible / dish ID, initial dish / crucible weight, sample volume, final dish / crucible weight, calculated MDL, and calculated RDL.

12.1.1.5.4 An entry is also made to the **Audit Log** field of the analytical template for the affected sample. This entry is an identifier that a data change was performed and that the data present before the change have been documented in the Audit Log Report.

12.1.1.5.5 Any occurrence of an **Audit Log** field entry on the analytical worksheet requires inclusion of a copy of the Audit Log Report in the hardcopy data package submitted for peer review.

12.2 Method QC

12.2.1 MB for TOTS and TVS – MBs are analyzed at a rate of at least 1 in 20 samples, with at least one per analytical batch. The MB should be <MDL. If a MB is >MDL, then any sample results >MDL and <10 times the detected MB may be affected. The MBs and affected samples should be redried, cooled, desiccated and reweighed. If the MB is still >MDL, check with a lead chemist or your supervisor.

12.2.2 LCS - LCSs are aqueous QC samples with certified values and control limits which are purchased from a vendor (see 9.7).

12.2.2.1 Aqueous TOTS Samples – A LCS is analyzed with each batch of aqueous samples.

12.2.2.2 Non-Aqueous TOTS Samples – A LCS is not available for non-aqueous samples for TOTS.

12.2.2.3 TVS Samples – A LCS is not available for aqueous or non-aqueous TVS samples.

12.3 Matrix QC

12.3.1 LD for TOTS and TVS

12.3.1.1 Non-Aqueous Samples - LDs are analyzed at a rate of one per 20 samples, per matrix. Analyze at least one LD for each batch unless there is insufficient sample. The control limit for the relative percent difference (RPD) is 20%. If the RPD is >20%, check first for a dish and sample ID mix-up. If sample and dish ID is not the source of error, dry, cool and reweigh the sample and LD. If the RPD is still >20%, repeat the analysis if sample volume is available and the holding time can be met. If not resolved, check with a senior chemist or your supervisor.

12.3.1.2 Aqueous Samples - LDs are analyzed at a rate of one per 20 samples (5% frequency), per matrix. Standard Methods 2540B states LDs are to be analyzed at a rate of one per 10 samples (10% frequency). Because most sample batch sizes are less than 20 samples and often consist of multiple matrices, the actual LD frequency routinely exceeds the recommended 10%. The control limit for the RPD is 25%. Though Standard Methods 2540B recommends an RPD limit of ±5%, historical performance-based data suggests that ≤25% is more a more realistic and statistically meaningful limit. See 12.2.1.1 for the corrective action steps for a LD failure.

12.3.2 Laboratory Triplicates (LT) for TOTS and TVS

12.3.2.1 Non-Aqueous Samples - LTs are analyzed for all non-aqueous samples at a rate of one per 20 samples, per matrix. If there is insufficient sample to perform a LT, the analyst must attach a "TA" flag to the affected data and submit a Data Anomaly Form indicating "Insufficient sample amount". The control limit for the relative standard deviation (RSD) in sediments is 20%. The control limit for RSD in non-sediment solids (soils, sludges, and tissues) is 25%. If the RSD exceeds these control limits, check first for a dish and sample ID mix-up. If the sample and dish ID is not the source of error, dry, cool and reweigh the sample, LD and LT. If the RSD is still >20%, repeat the analysis if sample volume is available and the holding time can be met. If the problem is not resolved, check with a senior chemist or your supervisor.

12.3.2.2 Aqueous Samples – LTs are not analyzed for aqueous samples.

12.4 Corrective Actions – Any action taken that corrects a QC failure should typically be applied to all samples in the batch unless it is clear that the problem was isolated to the QC sample itself.

13 DATA REDUCTION, REPORTING AND DOCUMENTATION

13.1 Aqueous Sample Calculations

13.1.1 TOTS, mg/L = [(A-B)*1,000,000]/V

Where:

A = the final crucible weight (104°C dried residue + evaporating dish) in g.
B = the initial crucible weight (weight of the dried evaporating dish) in g.
V = volume of sample, mL

13.1.2 TVS, mg/L = [(A-B)*1,000,000]/V

Where:

A = the final crucible weight (104°C dried residue + evaporating dish weight) in g.
B = the post-ignition weight (550°C ignited residue + evaporating dish weight) in g.
V = volume of sample, mL

13.2 Non-aqueous Sample Calculations, reported on a wet weight basis in LIMS.

13.2.1 TOTS, % = [(A-B)*100]/C

Where:

A = the final crucible weight (104°C dried residue + aluminum dish weight) in g.
B = the initial crucible weight (weight of the dried aluminum dish) in g.
C = the sample weight (initial wet sample weight) in g.

13.2.2 TVS, % = [(A-B)*100]/C

Where:

A = the final crucible weight (104°C residue + aluminum dish weight) in g.
B = the post-ignition weight (550°C ignited residue + aluminum dish) in g.
C = the sample weight (initial wet sample weight) in g

13.3 Reporting

13.3.1 Workgrouping in LIMS - Prior to data entry to the LIMS, all samples and associated QC are assigned a unique workgroup number in LIMS.

13.3.1.1 Within LIMS select "Sample Management" followed by "Work Group Management".

13.3.1.2 Select "Create New", enter a description for the workgroup in the "Description" field, and enter your initials in the "Operator" field. Note that the "Prep Date" field is left blank. Conventionals prep date data is entered via the CSV file during data entry.

13.3.1.3 Select "OK" to return to the "Work Group Management" form. Enter the "Department", "New Status" and "Product" fields to query and add samples to the newly created workgroup. Matrix, login numbers, sample numbers, etc., also can be entered to assist the query.

13.3.1.3.1 The "Department" is entered as the number 3 which indicates the Conventionals unit.

13.3.1.3.2 The "Product" code is the LIMS product name; i.e., TOTS or TVS.

13.3.1.3.3 The "New Status" field should be updated from "NEED" to "WKGP", "PREP" or "ANAL" to identify samples as workgrouped, prepared or analyzed, respectively. The status code used is at the discretion of the analyst; however, the code should reflect the current conditions of the samples. The status of a workgroup should be updated as changes are made (i.e., updated from "PREP" to "ANAL" once analysis has been completed).

13.3.1.4 Once the query information has been entered, select "OK" at the bottom of the "Work Group Management" form. On the "Work Group / Status Editor" form that appears, select the samples to be added to the workgroup by placing an "X" in the far left field. Select "Save" at the bottom of the screen then "Cancel" to return to the "Work Group Management" form.

13.3.1.5 To enter associated QC, select "ADD QC" at the bottom of the "Workgroup Management" form. On the "QC Samples" form that appears, the following information is added for each QC sample.

13.3.1.5.1 Matrix – Two character LIMS matrix code (e.g., LN, LE, LK). Method QC including MBs and LCSs are entered with the matrix code "LN" to identify these as blank waters. Matrix QC; i.e., LDs and LTs, are entered with the matrix code of their associated samples.

13.3.1.5.2 Product - The "Product" code is the LIMS product name; i.e., TOTS or TVS.

13.3.1.5.3 QC Type – LIMS code identifying the QC type associated with the specific sample. QC codes for TOTS and TVS analysis include MB, LCS, LD and LT.

13.3.1.5.4 Reference – This field is used to reference associated sample IDs or look-up table references such that QC calculations can be performed automatically by the LIMS QC module.

13.3.1.5.4.1 MB – No look-up table is available and therefore no unique entry is required. The MB sample ID is entered so that multiple MBs in a workgroup can be differentiated by their reference on the workgroup report (i.e., MB1 150402 to identify the first MB from April 2, 2015).

13.3.1.5.4.2 LCS – Each LCS references the List Function ID from the associated look-up table. The TOTS List Function is LEVEL1.

13.3.1.5.4.3 LD – Each LD references its associated LIMS sample number (i.e. L12345-1).

13.3.1.5.4.4 LT – Each LT references its associated LD (WG-) and sample number (i.e. L12345-1).

1.1.1.1 Select "Save" at the bottom of the screen then "Cancel" to return to the "Work Group Management" form. The workgroup creation is completed and the workgroup is now ready for data entry.

13.4 LIMS Data Entry – Data to be sent to the LIMS can be prepared by using macros (13.4).

13.4.1 Data is submitted to LIMS as a .csv file using the format displayed below. The .csv file is prepared by using macros during data reduction.

WG12345 Sample Number	CVTOTS mg/L	Prep DateTime	AnalDateTime	DilFactor	MDL	RDL
WG12345-1	0.0000	6/23/05 12:30	6/24/05 9:30	1	10	20
WG12345-2	95.0000	6/23/05 12:30	6/24/05 9:33	1	10	20
L12345-1	0.4000	6/23/05 12:30	6/24/05 9:40	1	10	20
L12345-2	3.8000	6/23/05 12:30	6/24/05 9:50	1	10	20
L12345-3	7.8000	6/23/05 12:30	6/24/05 10:00	1	10	20

13.4.1.1 Workgroup Number - LIMS identification defining a batch of analytical data and QC.

13.4.1.2 Sample Number - LIMS identification of each sample. Includes LIMS QC sample numbers (i.e., WG12345-1) used for QC samples in the workgroup.

13.4.1.3 Date Analyzed - The analysis date/time is the date/time stamp provided by the software interface at the time of the final balance reading following sample drying. A unique analysis date/time entry is defined on a per sample basis.

13.4.1.4 Preparation Date/Time – The prep date/time applicable to TOTS and TVS is the date/time when the batch of samples are placed in a drying oven.

13.4.1.5 Dilution Factor – Dilution factor is calculated on a per sample basis as the ratio of the adjusted MDL to the default LIMS MDL.

13.4.1.6 Units - Analytical units associated with the data. Units are mg/L for liquids and % for solids.

13.4.1.7 List Type - The parameter name associated with the product. The listtype CVTOTS is used for TOTS; CVTVS for TVS.

13.4.1.8 MDL - The MDL column is optional, but its use allows MDL values to be updated if needed.

13.4.1.9 RDL - The RDL column is optional, but its use allows RDL values to be updated if needed.

13.5 Data Reduction Tools

13.5.1 Excel macros, using Visual Basic for Applications (VBA), are utilized to streamline the data reduction process. The macros provide an automated means of report formatting and CSV file creation that are consistent between analysts.

13.5.2 Open the data file (*.xls) previously used for data collection.

13.5.3 Enter workgroup information pertaining to assigned QC identifiers in LIMS. This should go in the LIMS QC ID column. The shorthand version of the LIMS identifier is entered (i.e. WG12345-1 = WG-1).

13.5.4 Print this sheet for the final data package.

13.5.5 Select the samples for which a formatted CSV file will be generated.

13.5.6 Create a formatted CSV file

13.5.6.1 Once all QC samples have been identified in the **LIMS QC ID** field, generate the CSV file(s) by clicking the command button in the header of this field. A LIMS compatible CSV file will be generated for each listtype. CSV files are created and stored as separate worksheets within the existing workbook.

13.5.6.2 Adjust the listtype if needed. The listtype CVOTS is used for Total Solids and the listtype TVS is used for Total Volatile Solids.

13.5.7 Load the CSV file to the LIMS

13.5.7.1 With the CSV file active select the **ADD-IN** and click EnvLab followed by **CNV Data Entry**.

13.5.7.2 From the **Select Product(s):** form that appears, click the radial button next to **Open existing file** then click **OK**.

13.5.7.3 Close out the file browser form that opens returning the Excel workbook containing the CSV file. A new toolbar will be available within the **Custom Toolbars** section of the **ADD-Ins** tab. Click the **Load LIMS** button from this toolbar to triggering the routine that transfer the data to the LIMS.

13.5.7.4 Print the CSV file for the final data package

13.6 LIMS Association of Samples and QC – All samples in a workgroup are associated to all QC samples of the same parameter in the workgroup. This can result in an over association of samples to QC. "B" qualifiers are automatically assigned by LIMS, indicating blank contamination. The analyst needs to review any LIMS "B" qualified data to confirm appropriateness and manually remove qualifiers if necessary.

13.7 Data Package - The data package includes the workgroup review sheet, workgroup report, lab review report, the Excel data file created by WinWedge, CSV files and LIMS QC report. A data anomaly form must be included in the event that any data were qualified. In addition, a sample receipt record (from sample login) is included for when anomalies were observed upon sample receipt at the laboratory and corrective action steps taken prior to sample analysis. An analyte comparison chart should be included when TDS, aqueous TOTS, and/or TSS are requested on the same sample. The values for TDS and TSS should roughly sum to the aqueous TOTS value. If a difference of greater than 25% is found between these values, consult your supervisor for corrective action.

13.8 Documentation

13.8.1 Excel Analytical Template File – The Excel analytical template file will contain the initial and final crucible weights in grams, TOTS in mg/L or % as appropriate and TVS, if applicable, in mg/L or % as appropriate, time, temperature of the oven/furnace, sample storage info and comments.

14 REFERENCES

14.1 Method 2540B, E & G, Standard Methods for the Examination of Water and Wastewater, 22nd ed., 2012.

14.2 Residue, Non-Filterable (Gravimetric dried at 104°C), EPA Method 160.3, (1983).

14.3 Residue, Volatile, Gravimetric, Ignition at 550°C, EPA Method 160.4, (1983).

14.4 PSEP 1997a. Recommended guidelines for sampling marine sediment, water column, and tissue in Puget Sound. U.S. EPA, Region 10, Seattle, WA and Puget Sound Water Quality Authority, Olympia, WA.

14.5 King County Environmental Laboratory QA Manual.

15 TRAINING OUTLINE

15.1 Training a new analyst is to demonstrate and explain the steps outlined in the SOP and to show how to use the SOP as a reference aid for analysis. The trainer will first show how to properly perform the procedure. The trainee then performs the procedure, while being observed by the trainer, who answers questions, provides feedback on technique and further explains the steps in the procedure. The trainer continues to observe the trainee until the trainee successfully demonstrates competence with the procedure.

15.2 Training is documented on the Conventionals Unit Cross-Training Guidelines Check Sheet, which outlines the major topics to cover during the training. This sheet documents who was involved in the training and when each topic of the training was completed. The completed check sheet is kept on file by the supervisor.

15.3 To complete training, the trainee has to successfully analyze a blind sample provided by the trainer. The blind sample can be prepared by the trainer or purchased as a commercially prepared sample. Upon successful analysis of the blind sample, a complete data package including the instrument report and a copy of the analysis logbook are provided to the supervisor. These are filed along with the cross-training check sheet (15.2).

SGS AXYS Analytical Services Ltd.

SUMMARY OF SGS AXYS METHOD MLA-075 REV 08 VER 01:

SGS AXYS METHOD MLA-075: ANALYSIS OF PHARMACEUTICAL AND PERSONAL CARE PRODUCTS AND HORMONES IN SOLID, AQUEOUS, TISSUE AND POCIS SAMPLES BY LC-MS/MS

This method is suitable for the determination of a suite of hormones and pharmaceutical and personal care compounds in biosolid, aqueous and POCIS samples (Lists 1, 2, 3, 4, 5, 6 and hormones), soil/sediment samples (Lists 1, 3, 4, 5, 6 and hormones) and in tissue samples (Lists 1, 3, 4, 5, 6 and hormones). The analysis requires extraction at two different pH conditions: Basic extraction for analysis of List 4 analytes, and acidic extraction for the analysis of List 1, 2, 3, 5 and 6 analytes and hormones.

Target Analytes

List 1 - Aqueous, solid, tissue and POCIS samples (Acid extraction, positive ESI)	
Acetaminophen	Norfloxacin
Azithromycin	Norgestimate
Caffeine	Ofloxacin
Carbadox	Ormetoprim
Carbamazepine	Oxacillin [1]
Cefotaxime	Oxolinic acid
Ciprofloxacin	Penicillin G [1]
Clarithromycin	Penicillin V
Clinafloxacin	Roxithromycin
Cloxacillin [1]	Sarafloxacin
Dehydronifedipine	Sulfachloropyridazine
Digoxigenin	Sulfadiazine
Digoxin	Sulfadimethoxine
Diltiazem	Sulfamerazine
1,7-Dimethylxanthine	Sulfamethazine
Diphenhydramine	Sulfamethizole
Enrofloxacin	Sulfamethoxazole
Erythromycin	Sulfanilamide
Flumequine	Sulfathiazole
Fluoxetine	Thiabendazole
Lincomycin	Trimethoprim
Lomefloxacin	Tylosin
Miconazole	Virginiamycin M1

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List 2 – Aqueous, biosolid (only) and POCIS samples (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 - Aqueous, solid, tissue and POCIS samples (Acid extraction, negative ESI)	
Bisphenol A	2-hydroxy-ibuprofen
Furosemide	Ibuprofen
Gemfibrozil	Naproxen
Glipizide	Triclocarban
Glyburide	Triclosan
Hydrochlorothiazide	Warfarin
List 4 - Aqueous, solid, tissue and POCIS samples (Base extraction, positive ESI)	
Albuterol	Cotinine
Amphetamine	Enalapril
Atenolol	Hydrocodone
Atorvastatin	Metformin
Cimetidine	Oxycodone
Clonidine	Ranitidine
Codeine	Triamterene
List 5 - Aqueous, solid, tissue and POCIS samples (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
List 6 - Aqueous, solid, tissue and POCIS samples (Acid Extraction, positive ESI)	
Amsacrine	Iopamidol
Azathioprine	Medroxyprogesterone acetate
Busulfan	Melphalan
Citalopram	Metronidazole
Clotrimazole	Moxifloxacin [2]
Colchicine	Oxazepam
Cyclophosphamide	Rosuvastatin
Daunorubicin	Tamoxifen
Diatrizoic acid	Teniposide
Doxorubicin	Venlafaxine
Drospirenone	Zidovudine
Etoposide	
HM-APOS - Aqueous, solid, tissue and POCIS samples (Acid Extraction, positive ESI)	
Allyl trenbolone	Mestranol
Androstenedione	Norethindrone
Androsterone	Norgestrel
Desogestrel	Progestrone
17beta-Estradiol 3-Benzoate (not tissues)	Testosterone
HM-ANEG - Aqueous, solid, tissue and POCIS samples (Acid Extraction, negative ESI)	
17alpha-Dihydroequilin	17beta-Estradiol
Equilenin	Estriol
Equilin	Estrone
17alpha-Estradiol	17alpha-Ethinyl estradiol

[1] Analysis result is classified as ‘information value’ of estimated concentration.

[2] Moxifloxacin in solid samples is classified as ‘information value’ of estimated concentration.

1.0 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 for the analysis of the other analytes (Lists 1, 2, 3, 5 and 6 and hormones). Prior to extraction and/or clean-up, samples are adjusted to the required pH and spiked with surrogates.

Surrogate standards are added to all samples before extraction.

Solid and tissue samples are extracted by sonication with aqueous buffered acetonitrile and with 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 filtered, cleaned up by solid phase extraction (SPE), and analyzed by LC/ESI-MS/MS in positive and negative ionization modes.

POCIS samplers are solvent extracted before analysis by LC/ESI-MS/MS.

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 0.5 L 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.

Before analysis by LC/ESI-MS/MS all extracts are spiked with recovery standards.

2.0 INSTRUMENTATION

Analysis of the sample extract is performed on a high-performance liquid chromatograph (for Lists 1 and 2,) or on an ultra-performance liquid chromatograph (for Lists 3, 4, 5, 6 and hormones) coupled to a triple quadrupole mass spectrometer. 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. Analysis of the complete list of analytes requires 8 different LC-MS/MS runs.

3.0 CALIBRATION

Initial calibration is performed using a series of calibration solutions that encompass the working concentration range. Initial calibration solutions contain the suite of labelled surrogate and recovery standards and authentic targets. The concentration of the native analytes in the solutions varies to

encompass the working range of the instrument, while the concentrations of the surrogates and recovery standards remain constant. A mid-level solution is analyzed every 12 hours or every 20 samples, whichever occurs first. The List 1, List 3, List 5, List 6 and hormones calibration standards are prepared in 75:25 methanol:0.1% formic acid buffer, the List 2 calibration standards in methanol and the List 4 calibration standards in 1:1 methanol:acetonitrile.

Initial calibration for any native compound requires by defaults at least 5 consecutive calibration levels, although in some cases it may be necessary to use only 4, 5 or 6 of the calibration points to establish a linear quantification range (constant RRF method for List 3, 4, 5, 6 and hormones) or a calibration curve meeting residual specifications (Lists 1 and 2 use a linear regression calculation). All calibration solutions in the table below may be analyzed, but in certain cases only 4, 5 or 6 of the levels are used to establish the initial calibration. In the table below the calibration concentrations routinely included are printed in bold type. If the number of routinely included calibration points shown for a compound is less than five, concentrations below and/or above are added as necessary based on analyst judgement to achieve the minimum number of consecutive concentration levels. Note that reporting limits are adjusted as necessary to reflect the lowest calibration concentration included in the initial calibration.

Nominal Concentrations of Calibration Solutions

List 1 (Acid extraction, positive ESI)

Compound name	Calibration Standards List 1 (Acid extraction, positive ESI)						
	CS-1 Level A	CS-2 Level B	CS-3 Level C	CS-4 CAL VER Level D	CS-5 Level E	CS-6 Level F	CS-7 Level G
Acetaminophen	**3.75**	**12.5**	**37.5**	**187**	**625**	**2500**	**12500**
Azithromycin	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	*250*	*1250*
Caffeine	**3.75**	**12.5**	**37.5**	**187**	**625**	*2500*	*12500*
Carbadox	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	*250*	*1250*
Carbamazapine	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	**250**	*1250*
Cefotaxime	*1.5*	*5*	*15*	*75*	*250*	*1000*	*5000*
Ciprofloxacin	**1.5**	**5**	**15**	**75**	**250**	*1000*	*5000*
Clarithromycin	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	*250*	*1250*
Clinafloxacin	*1.5*	**5**	**15**	**75**	**250**	*1000*	*5000*
Cloxacillin	*0.75*	**2.5**	**7.5**	**37.5**	**125**	**500**	*2500*
Dehydronifedipine	**0.15**	**0.5**	**1.5**	**7.5**	**25**	**100**	*500*
Digoxigenin	*1.5*	**5**	**15**	**75**	**250**	**1000**	*5000*
Digoxin	**1.5**	**5**	**15**	**75**	**250**	*1000*	*5000*
Diltiazem	**0.075**	**0.25**	**0.75**	**3.75**	**12.5**	**50**	*250*
1,7-Dimethylxanthine	**15**	**50**	**150**	**750**	**2500**	**10000**	*50000*
Diphenhydramine	**0.15**	**0.5**	**1.5**	**7.5**	**25**	**100**	*500*
Enrofloxacin	**0.75**	**2.5**	**7.5**	**37.5**	**125**	*500*	*2500*
Erythromycin	**0.075**	**0.25**	**0.75**	**3.75**	**12.5**	**50**	*250*

Flumequine	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	*250*	*1250*
Fluoxetine	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	*250*	*1250*
Lincomycin	**0.75**	**2.5**	**7.5**	**37.5**	**125**	*500*	*2500*
Lomefloxacin	**0.75**	**2.5**	**7.5**	**37.5**	**125**	*500*	*2500*
Miconazole	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	*250*	*1250*
Norfloxacin	**3.75**	**12.5**	**37.5**	**187**	**625**	*2500*	*12500*
Norgestimate	**0.75**	**2.5**	**7.5**	**37.5**	**125**	*500*	*2500*
Ofloxacin	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	*250*	*1250*
Ormetoprim	**0.15**	**0.5**	**1.5**	**7.5**	**25**	*100*	*500*
Oxacillin	*0.75*	**2.5**	**7.5**	**37.5**	**125**	*500*	*2500*
Oxolinic acid	*0.15*	**0.5**	**1.5**	**7.5**	**25**	*100*	*500*
Penicillin G	**0.75**	**2.5**	**7.5**	**37.5**	**125**	*500*	*2500*
Penicillin V	*0.75*	**2.5**	**7.5**	**37.5**	**125**	**500**	*2500*
Roxithromycin	**0.075**	**0.25**	**0.75**	**3.75**	**12.5**	*50*	*250*
Sarafloxacin	**3.75**	**12.5**	**37.5**	**187**	**625**	*2500*	*12500*
Sulfachloropyridazine	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	*250*	*1250*
Sulfadiazine	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	**250**	**1250**
Sulfadimethoxine	**0.075**	**0.25**	**0.75**	**3.75**	**12.5**	**50**	*250*
Sulfamerazine	**0.15**	**0.5**	**1.5**	**7.5**	**25**	**100**	*500*
Sulfamethazine	*0.15*	**0.5**	**1.5**	**7.5**	**25**	*100*	*500*
Sulfamethizole	**0.15**	**0.5**	**1.5**	**7.5**	**25**	**100**	*500*
Sulfamethoxazole	*0.15*	**0.5**	**1.5**	**7.5**	**25**	**100**	**500**
Sulfanilamide	**3.75**	**12.5**	**37.5**	**187.5**	**625**	**2500**	*12500*
Sulfathiazole	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	*250*	*1250*
Thiabendazole	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	*250*	*1250*
Trimethoprim	**0.375**	**1.25**	**3.75**	**18.7**	**62.5**	**250**	*1250*
Tylosin	**1.5**	**5**	**15**	**75**	**250**	*1000*	*5000*
Virginiamycin M1	**0.75**	**2.5**	**7.5**	**37.5**	**125**	*500*	*2500*
Surrogate Standards							
$^{13}C_2$, ^{15}N-Acetaminophen	50	50	50	50	50	50	50
$^{13}C_3$-Caffeine	75	75	75	75	75	75	75
d_{10}-Carbamazepine	25	25	25	25	25	25	25
$^{13}C_3$, ^{15}N-Ciprofloxacin	100	100	100	100	100	100	100
$^{13}C_2$-Erythromycin	25	25	25	25	25	25	25
d_5-Fluoxetine	25	25	25	25	25	25	25
$^{13}C_6$-Sulfamethazine	25	25	25	25	25	25	25
$^{13}C_6$-Sulfamethoxazole	25	25	25	25	25	25	25
d_6-Thiabendazole	25	25	25	25	25	25	25
$^{13}C_3$-Trimethoprim	25	25	25	25	25	25	25
Recovery Standards							

$^{13}C_3$-Atrazine	50	50	50	50	50	50	50

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List 2 (Tetracyclines)

Compound name	Calibration Standards List 2 (ng/mL) (Tetracyclines)						
	CS-1 Level A	CS-2 Level B	CS-3 Level C	CS-4 CAL VER Level D	CS-5 Level E	CS-6 Level F	CS-7 Level G
Anhydrochlortetracycline (ACTC)	*3.75*	*12.5*	**31.25**	**62.5**	**125**	*375*	*1000*
Anhydrotetracycline (ATC)	*3.75*	**12.5**	**31.25**	**62.5**	**125**	*375*	*1000*
Chlortetracycline (CTC)	*1.5*	**5**	**12.5**	**25**	**50**	**150**	*400*
Demeclocycline	**3.75**	**12.5**	**31.2**	**62.5**	**125**	**375**	**1000**
Doxycycline	**1.5**	**5**	**12.5**	**25**	**50**	**150**	**400**
4-Epianhydrochlortetracycline (EACTC)	*15*	**50**	**125**	**250**	**500**	**1500**	*4000*
4-Epianhydrotetracycline (EATC)	**3.75**	**12.5**	**31.2**	**62.5**	**125**	**375**	*1000*
4-Epichlortetracycline (ECTC)	**3.75**	**12.5**	**31.2**	**62.5**	**125**	**375**	**1000**
4-Epioxytetracycline (EOTC)	**1.5**	**5**	**12.5**	**25**	**50**	**150**	*400*
4-Epitetracycline (ETC)	*1.5*	**5**	**12.5**	**25**	**50**	*150*	*400*
Isochlortetracycline (ICTC)	**1.5**	**5**	**12.5**	**25**	**50**	**150**	**400**
Minocycline	*15*	**50**	**125**	**250**	**500**	*1500*	*4000*
Oxytetracycline (OTC)	**1.5**	**5**	**12.5**	**25**	**50**	**150**	*400*
Tetracycline (TC)	**1.5**	**5**	**12.5**	**25**	**50**	**150**	*400*
Surrogate Standards							
d_6-Thiabendazole	25	25	25	25	25	25	25
Recovery Standards							
$^{13}C_3$-Atrazine	50	50	50	50	50	50	50

List 3 (Acid extraction, negative ESI)

Compound name	Calibration Standards List 3 (ng/mL) (Acid extraction, negative ESI)						
	CS-1 Level A	CS-2 Level B	CS-3 Level C	CS-4 CAL VER Level D	CS-5 Level E	CS-6 Level F	CS-7 Level G
Bisphenol A	1.5	5	15	75	225	1125	3750
Furosemide	1	3.00	10	50	150	750	2500
Gemfibrozil	0.2	0.60	2	10	30.0	150	500
Glipizide	0.2	0.60	2	10	30.0	150	500
Glyburide	0.2	0.60	2	10	30.0	150	500
Hydrochlorothiazide[1]	1	3.00	10	50	150	750	2500
2-hydroxy-ibuprofen	1	3.00	10	50	150	750	2500
Ibuprofen	1	3.00	10	50	150	750	2500
Naproxen	0.5	1.50	5	25	75.0	375	1250
Triclocarban	0.1	0.30	1	5	15.0	75	250

Triclosan	1.5	4.50	15	75	225	1125	3750
Warfarin	0.1	0.30	1	5	15.0	75	250
Surrogate Standards							
d_6-Bisphenol A	100	100	100	100	100	100	100
d_5- Furosemide	50	50	50	50	50	50	50
d_6-Gemfibrozil	5	5	5	5	5	5	5
d_{11}-Glipizide	25	25	25	25	25	25	25
d_3-Glyburide	10	10	10	10	10	10	10
$^{13}C_1$-d_2-Hydrochlorothiazide	50	50	50	50	50	50	50
d_6-2-hydroxy-ibuprofen	25	25	25	25	25	25	25
$^{13}C_6$-Ibuprofen	10	10	10	10	10	10	10
d_3-Naproxen	50	50	50	50	50	50	50
$^{13}C_6$-Triclocarban	5	5	5	5	5	5	5
$^{13}C_6$-Triclosan	100	100	100	100	100	100	100
d_5-Warfarin	10	10	10	10	10	10	10
Recovery Standard							
$^{13}C_3$-Ibuprofen	50	50	50	50	50	50	50
$^{13}C_1$-d_3-Naproxen	50	50	50	50	50	50	50
$^{13}C_6$-2,4,5-Trichlorophenoxy-acetic acid ($^{13}C_6$-2,4,5-T)	50	50	50	50	50	50	50

List 4 (Base extraction, positive ESI)

Compound Name	Calibration Standards List 4 (ng/mL) (Base extraction, positive ESI)						
	CS-1 *Level A*	**CS-2 Level B**	**CS-3 Level C**	**CS-4 CAL VER Level D**	**CS-5 Level E**	**CS-6 Level F**	**CS-7 Level G**
Albuterol	0.075	0.25	0.75	3.75	12.5	50	250
Amphetamine	0.075	0.25	0.75	3.75	12.5	50	250
Atenolol	0.075	0.25	0.75	3.75	12.5	50	250
Atorvastatin	0.30	1.00	3.00	15	50	200	1000
Cimetidine	0.15	0.50	1.5	7.5	25	100	500
Clonidine	0.30	1.00	3.00	15.0	50	200	1000
Codeine	0.30	1.00	3.00	15.0	50	200	1000
Cotinine	0.075	0.25	0.75	3.75	12.5	50	250
Enalapril	0.075	0.25	0.75	3.75	12.5	50	250
Hydrocodone	0.30	1.00	3.00	15.0	50	200	1000
Metformin	0.075	0.25	0.75	3.75	12.5	50	250
Oxycodone	0.15	0.50	1.50	7.50	25	100	500

Ranitidine	0.15	0.50	1.50	7.50	25	100	500
Triamterene	0.075	0.25	0.75	3.75	12.5	50	250
Surrogate Standards							
d_3-Albuterol	5.0	5.0	5.0	5.0	5.0	5.0	5.0
d_5-Amphetamine	5.0	5.0	5.0	5.0	5.0	5.0	5.0
d_7-Atenolol	15	15	15	15	15	15	15
d_5-Atorvastatin	50	50	50	50	50	50	50
d_3-Cimetidine	5.0	5.0	5.0	5.0	5.0	5.0	5.0
d_4-Clonidine	15	15	15	15	15	15	15
d_6-Codeine	50	50	50	50	50	50	50
d_3-Cotinine	5.0	5.0	5.0	5.0	5.0	5.0	5.0
d_5-Enalapril	5.0	5.0	5.0	5.0	5.0	5.0	5.0
d_3-Hydrocodone	15	15	15	15	15	15	15
d_6-Metformin	5.0	5.0	5.0	5.0	5.0	5.0	5.0
d_6-Oxycodone	15	15	15	15	15	15	15
d_6-Ranitidine	5.0	5.0	5.0	5.0	5.0	5.0	5.0
d_5-Triamterene	15	15	15	15	15	15	15
Recovery standards							
d_9-Albuterol	5.0	5.0	5.0	5.0	5.0	5.0	5.0
d_3-Amitriptyline	10.0	10.0	10.0	10.0	10.0	10.0	10.0

List 5 (Acid extraction, positive ESI)

Compound name	Calibration Standards List 5 (ng/mL) (Acid extraction, positive ESI)						
	CS-1 Level A	CS-2 Level B	CS-3 Level C	CS-4 CAL VER Level D	CS-5 Level E	CS-6 Level F	CS-7 Level G
Alprazolam	0.075	0.25	0.75	3.75	12.5	50	150
Amitriptyline	0.075	0.25	0.75	3.75	12.5	50	150
Amlodipine	0.25	0.833	2.5	12.5	41.7	167	500
Benzoylecgonine	0.0375	0.125	0.375	1.87	6.25	25	75
Benztropine	0.075	0.25	0.75	3.75	12.5	50	150
Betamethasone	0.375	1.25	3.75	18.7	62.5	250	750
Cocaine	0.0375	0.125	0.375	1.87	6.25	25	75
DEET	0.075	0.25	0.75	3.75	12.5	50	150
Desmethyldiltiazem	0.0375	0.125	0.375	1.87	6.25	25	75
Diazepam	0.125	0.417	1.25	6.25	20.83	83.3	250
Fluocinonide	0.50	1.67	5.0	25	83.3	333	1000

Fluticasone propionate	0.50	1.67	5.0	25	83.3	333	1000
Hydrocortisone	1.50	5.0	15	75	250	1000	3000
10-hydroxy-amitriptyline	0.0375	0.125	0.375	1.87	6.25	25	75
Meprobamate	0.375	1.25	3.75	18.7	62.5	250	750
Methylprednisolone	1.00	3.33	10.0	50	167	667	2000
Metoprolol	0.125	0.417	1.25	6.25	20.83	83.3	250
Norfluoxetine	0.125	0.417	1.25	6.25	20.83	83.3	250
Norverapamil	0.0375	0.125	0.375	1.87	6.25	25	75
Paroxetine	0.25	0.833	2.5	12.5	41.7	167	750
Prednisolone	1.00	3.33	10.0	50	167	667	2000
Prednisone	1.50	5.0	15	75	250	1000	3000
Promethazine	0.075	0.25	0.75	3.75	12.5	50	150
Propoxyphene	0.075	0.25	0.75	3.75	12.5	50	150
Propranolol	0.075	0.25	0.75	3.75	12.5	50	150
Sertraline	0.075	0.25	0.75	3.75	12.5	50	150
Simvastatin	0.50	1.67	5.0	25	83.3	333	1000
Theophylline	1.50	5.0	15	75	250	1000	3000
Trenbolone	0.50	1.67	5.0	25	83.3	333	1000
Trenbolone acetate	0.075	0.25	0.75	3.75	12.5	50	150
Valsartan	1.0	3.33	10.0	50	167	667	2000
Verapamil	0.0375	0.125	0.375	1.87	6.25	25	75

List 6 (Acid extraction, positive ESI)

Compound name	Calibration Standards List 6 (ng/mL) (Acid extraction, positive ESI)						
	CS-1 Level A	CS-2 Level B	CS-3 Level C	CS-4 Level D	CS-5 CAL VER Level E	CS-6 Level F	CS-7 Level G
Amsacrine	0.01 [1]	0.02	0.05	0.15	0.5	1.0	2.0
Azathioprine	0.25	0.5	1.25	3.75	12.5	25	50
Busulfan	0.5	1.0	2.5	7.5	25	50	100
Citalopram	0.1	0.2	0.5	1.5	5.0	10	20
Clotrimazole	0.1	0.2	0.5	1.5	5.0	10	20
Colchicine	0.2	0.4	1.0	3.0	10	20	40
Cyclophosphamide	0.1 [1]	0.2	0.5	1.5	5.0	10	20
Daunorubicin	0.5 [1]	1.0	2.5	7.5	25	50	100
Diatrizoic acid	3	6	15	45	150	300	600
Doxorubicin	1.5	3	7.5	22.5	75	150	300

Drospirenone	2	4	10	30	100	200	400
Etoposide	0.25 [1]	0.5	1.25	3.75	12.5	25	50
Iopamidol	20	40	100	300	1000	2000	4000
Medroxyprogesterone acetate	1	2	5	15	50	100	200
Melphalan	6	12	30	90	300	600	1200
Metronidazole	0.5	1.0	2.5	7.5	25	50	100
Moxifloxacin	1	2	5	15	50	100	200
Oxazepam	1	2	5	15	50	100	200
Rosuvastatin	1	2	5	15	50	100	200
Tamoxifen	0.1	0.2	0.5	1.5	5.0	10	20
Teniposide	1	2	5	15	50	100	200
Venlafaxine	0.1	0.2	0.5	1.5	5.0	10	20
Zidovudine	1.5	3	7.5	22.5	75	150	300
Surrogate Standards							
$^{13}C_4$-Azathioprine	15	15	15	15	15	15	15
d_8-Busulfan	75	75	75	75	75	75	75
d_6-Citalopram	1.25	1.25	1.25	1.25	1.25	1.25	1.25
d_5-Clotrimazole	10	10	10	10	10	10	10
d_6-Colchicine	10	10	10	10	10	10	10
d_4-Cyclophosphamide	5	5	5	5	5	5	5
$^{13}C_1$-d_3-Daunorubicin	50	50	50	50	50	50	50
d_6-Diatrizoic acid	250	250	250	250	250	250	250
$^{13}C_3$-Drospirenone	30	30	30	30	30	30	30
d_3-Etoposide	15	15	15	15	15	15	15
d_8-Iopamidol	500	500	500	500	500	500	500
d_6-Medroxyprogesterone acetate	30	30	30	30	30	30	30
d_8-Melphalan	250	250	250	250	250	250	250
d_4-Metronidazole	15	15	15	15	15	15	15
$^{13}C_1$-d_3-Moxifloxacin	30	30	30	30	30	30	30
d_5-Oxazepam	30	30	30	30	30	30	30
d_6-Rosuvastatin	125	125	125	125	125	125	125
d_5-Tamoxifen	2.5	2.5	2.5	2.5	2.5	2.5	2.5
d_6-Venlafaxine	2.5	2.5	2.5	2.5	2.5	2.5	2.5
d_3-Zidovudine	125	125	125	125	125	125	125
Recovery Standards							
$^{13}C_3$-Atrazine	50	50	50	50	50	50	50
d_4-Amlodipine (2-chlorophenyl-d_4)	50	50	50	50	50	50	50
d_7-Propranolol (1-methylethyl-d_7)	50	50	50	50	50	50	50

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Hormones (Acid extraction)

Compound name		Calibration Standards Hormones (ng/mL) (Acid extraction)					
	CS-1 Level A	CS-2 Level B	CS-3 Level C	CS-4 Level D	CS-5 CAL VER Level E	CS-6 Level F	CS-7 Level G
Allyl Trenbolone	0.1	0.2	0.4	1	2.5	6	15
Androstenedione	0.25	0.5	1	2.5	6.25	15	37.5
Androsterone	5	10	20	50	125	300	750
Desogestrel	10	20	40	100	250	600	1500
17alpha-Dihydroequilin	0.5	1	2	5	12.5	30	75
Equilenin	0.1	0.2	0.4	1	2.5	6	15
Equilin	0.5	1	2	5	12.5	30	75
17alpha-Estradiol	1	2	4	10	25	60	150
17beta-Estradiol	1	2	4	10	25	60	150
17beta-Estradiol 3-benzoate	0.2	0.4	0.8	2	5	12	30
Estriol	2	4	8	20	50	120	300
Estrone	0.5	1	2	5	12.5	30	75
17alpha-Ethinyl estradiol	1.25	2.5	5	12.5	31.25	75	187.5
Mestranol	5	10	20	50	125	300	750
Norethindrone	0.25	0.5	1	2.5	6.25	15	37.5
Norgestrel	0.25	0.5	1	2.5	6.25	15	37.5
Progesterone	0.1	0.2	0.4	1	2.5	6	15
Testosterone	0.1	0.2	0.4	1	2.5	6	15
Labelled Compounds (Surrogates)							
$^{13}C_3$-Androstenedione	12.5	12.5	12.5	12.5	12.5	12.5	12.5
d_4-Androsterone	125	125	125	125	125	125	125
$^{13}C_2,d_2$-Desogestrel	350	350	350	350	350	350	350
d_3-Equilenin	7.5	7.5	7.5	7.5	7.5	7.5	7.5
d_3-17alpha-estradiol	100	100	100	100	100	100	100
$^{13}C_2$-17beta-estradiol	25	25	25	25	25	25	25
d_4-17beta-estradiol	125	125	125	125	125	125	125
d_3-beta-Estradiol 3-benzoate	10	10	10	10	10	10	10
d_3-Estriol	100	100	100	100	100	100	100
$^{13}C_2$-Estrone	25	25	25	25	25	25	25
$^{13}C_2$-17alpha-Ethinylestradiol	100	100	100	100	100	100	100
d_4-Mestranol	125	125	125	125	125	125	125

d_6-Norethindrone	25	25	25	25	25	25	25
d_6-Norgestrel	25	25	25	25	25	25	25
d_9-Progesterone	5	5	5	5	5	5	5
$^{13}C_3$-Testosterone	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Labelled Injection Standards (Recovery standards)							
$^{13}C_3$-Atrazine	50	50	50	50	50	50	50
$^{13}C_6$-2,4,5-Trichlorophenoxy-acetic acid ($^{13}C_6$-2,4,5-T)	50	50	50	50	50	50	50
d_4-Estrone	25	25	25	25	25	25	25
$^{13}C_3$-17alpha-Hydroxy-progesterone	12.5	12.5	12.5	12.5	12.5	12.5	12.5

[1] May be excluded from the A-CAL

4.0 QUANTIFICATION PROCEDURES

Concentrations of the target compounds are calculated either by isotope dilution quantification against the surrogate standard or by internal standard quantification against the recovery standard.

The isotopically labeled analog of an analyte (surrogate) is used for quantitation (Isotope Dilution Quantitation). If a labeled analog is not commercially available, a surrogate with chemical similarity and close retention time is used for quantitation (internal standard quantitation). Final analyte concentrations are recovery corrected by this method of quantification

For Lists 1 and 2, calibration is done by linear regression calibration, using a 1/X weighting type, excluding origin.

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

Where: $Y = \text{Response ratio} = \left(\frac{\text{area Target}}{\text{area SUR}} \times \text{weight SUR spiked (ng)} \right)$

X = weight of target (ng)
SUR = the surrogate standard

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

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

Constant RRF calibration is used for Lists 3, 4, 5, 6 and Hormones (both ESI positive and ESI negative). Relative response factors (RRF) are determined from the initial calibration and are confirmed at least every 12 hours (CAL/VER).

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

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

and the Surr Std is the isotopically labeled quantitation reference

The recoveries of the surrogate standards are calculated by internal standard quantification against the recovery standards and monitored as an indication of overall data quality. Surrogate standard recovery is calculated as:

$$\%\,\text{surrogate recovery} = \left(\frac{\text{area of Surr}}{\text{area of Rec}} \right) \times \left(\frac{\text{weight of Rec}}{\text{weight of Surr}} \right) \times \frac{100}{\text{RRF}_S}\text{, in the sample}$$

where Rec = the recovery standard used, and

$$RRF_S = \left(\frac{\text{area of Surr}}{\text{area of Rec}}\right) \times \left(\frac{\text{weight of Rec}}{\text{weight of Surr}}\right)$$; from the calibration.

4.1 Reporting Limits

Results are reported as concentrations in the samples analyzed; measurement uncertainty estimates (K=2), reported as percentage ranges of the analysis results, are upon agreement included in the final analytical report.

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 by the same calculation used to convert target compounds responses to sample concentrations.

Concentrations and detection limits for the target analytes are reported. 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. Typical reporting units for all data are ng/g or ng/L. Concentrations for solids are reported on a dry weight basis. Concentrations in aqueous samples are reported on a volume basis. Concentrations for tissues are reported on a wet weight basis.

The following are commonly requested reporting limits:

Method Detection Limit (MDL) - determined as specified by EPA Fed. Reg. 40 CFR Part 136 Appendix B (no iteration option). The 99% confidence level MDL is determined based on analysis of a minimum of 7 replicate matrix spikes fortified at 1-10 times the estimated detection limit. MDL is determined as required based on accreditation, contract and workload requirements.

Lower Method Calibration Limit (LMCL) - determined by prorating the concentration of the lowest calibration limit for sample size and extract volume. The following equation is used. ((lowest level cal conc.) x (extract volume))/sample size. The typical extract volume for PPCP is 4 mL.

For the analysis of PPCP and hormones it is SGS AXYS standard to report sample concentrations using the LMCL as the lower reporting limit. In cases where the SDL is higher than the LMCL, the SDL will be used as the lower reporting limit.

The SDL is defined as follows: *Sample Specific Detection Limit or Sample Detection Limit (SDL)* – determined individually for every sample analysis run by converting the area equivalent of 3.0 times (2.5 times for EPA 1600 series methods) 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.

SGS AXYS Analytical Services Ltd.

Analytes, Ions and Quantification References

List 1 – Acid Extraction, Positive Electrospray Ionization (+)ESI

Target Analyte	Typical Retention Time (min)	Typical RRT	RRT Reference	Precursor Ion Mass	Product Ion Mass	Quantified against
Sulfanilamide	2.02	0.432	$^{13}C_2$,^{15}N-Acetaminophen	190.0	155.8	$^{13}C_6$-Sulfamethazine
Acetaminophen	4.68	1.000	$^{13}C_2$,^{15}N-Acetaminophen	152.2	110.0	$^{13}C_2$,^{15}N-Acetaminophen
Sulfadiazine	5.32	1.137	$^{13}C_2$,^{15}N-Acetaminophen	251.2	156.1	$^{13}C_6$-Sulfamethazine
1,7-Dimethylxanthine	7.02	0.753	$^{13}C_3$-Caffeine	181.2	124.0	$^{13}C_3$-Caffeine
Sulfathiazole	8.00	0.858	$^{13}C_3$-Caffeine	256.3	156.0	$^{13}C_6$-Sulfamethoxazole
Sulfamerazine	8.78	0.942	$^{13}C_3$-Caffeine	265.0	156.0	$^{13}C_6$-Sulfamethazine
Caffeine	9.32	1.000	$^{13}C_3$-Caffeine	195.0	138.0	$^{13}C_3$-Caffeine
Lincomycin	9.47	0.953	$^{13}C_3$-Trimethoprim	407.2	126.0	$^{13}C_3$-Trimethoprim
Trimethoprim	9.94	1.000	$^{13}C_3$-Trimethoprim	291.2	230.0	$^{13}C_3$-Trimethoprim
Sulfamethizole	10.09	0.983	$^{13}C_6$-Sulfamethazine	271.0	156.0	$^{13}C_6$-Sulfamethoxazole
Cefotaxime	10.09	1.015	$^{13}C_3$-Trimethoprim	456.4	396.1	$^{13}C_3$-Trimethoprim
Sulfamethazine	10.31	1.000	$^{13}C_6$-Sulfamethazine	279.0	156.0	$^{13}C_6$-Sulfamethazine
Ofloxacin	10.53	0.974	$^{13}C_3$,^{15}N-Ciprofloxacin	362.2	318.0	$^{13}C_3$,^{15}N-Ciprofloxacin
Carbadox	10.53	1.005	d_6-Thiabendazole	263.2	231.2	$^{13}C_3$-Trimethoprim
Ormetoprim	10.53	1.059	$^{13}C_3$-Trimethoprim	275.3	259.1	$^{13}C_3$-Trimethoprim
Norfloxacin	10.59	0.980	$^{13}C_3$,^{15}N-Ciprofloxacin	320.0	302.0	$^{13}C_3$,^{15}N-Ciprofloxacin
Thiabendazole	10.59	1.000	d_6-Thiabendazole	202.1	175.1	d_6-Thiabendazole
Ciprofloxacin	10.81	1.000	$^{13}C_3$,^{15}N-Ciprofloxacin	332.2	314.2	$^{13}C_3$,^{15}N-Ciprofloxacin
Sulfachloropyridazine	10.97	1.069	$^{13}C_6$-Sulfamethazine	285.0	156.0	$^{13}C_6$-Sulfamethazine
Lomefloxacin	11.14	1.031	$^{13}C_3$,^{15}N-Ciprofloxacin	352.2	308.1	$^{13}C_3$,^{15}N-Ciprofloxacin
Enrofloxacin	11.22	1.038	$^{13}C_3$,^{15}N-Ciprofloxacin	360.2	316.0	$^{13}C_3$,^{15}N-Ciprofloxacin
Sulfamethoxazole	11.33	1.000	$^{13}C_6$-Sulfamethoxazole	254.0	156.0	$^{13}C_6$-Sulfamethoxazole
Sarafloxacin	11.84	1.095	$^{13}C_3$,^{15}N-Ciprofloxacin	386.1	299.0	$^{13}C_3$,^{15}N-Ciprofloxacin
Clinafloxacin	12.04	1.059	$^{13}C_6$-Sulfamethoxazole	366.3	348.1	$^{13}C_3$,^{15}N-Ciprofloxacin
Digoxigenin	12.68	1.115	$^{13}C_6$-Sulfamethoxazole	391.2	355.2	$^{13}C_3$-Trimethoprim

Oxolinic Acid	13.11	0.819	$^{13}C_3$-Atrazine	262.1	244.0	$^{13}C_3$-Trimethoprim
Sulfadimethoxine	13.33	1.172	$^{13}C_6$-Sulfamethoxazole	311.0	156.0	$^{13}C_6$-Sulfamethoxazole
Azithromycin	13.55	0.846	$^{13}C_3$-Atrazine	749.9	591.6	$^{13}C_3$-Trimethoprim
Penicillin G	14.46	0.903	$^{13}C_3$-Atrazine	367.1	159.9	$^{13}C_3$-Trimethoprim
Diphenhydramine	14.57	0.910	$^{13}C_3$-Atrazine	256.2	167.0	$^{13}C_3$-Trimethoprim
Flumequine	15.25	0.953	$^{13}C_3$-Atrazine	262.0	173.7	$^{13}C_3$-Trimethoprim
Penicillin V	15.29	0.955	$^{13}C_3$-Atrazine	383.2	159.9	$^{13}C_3$-Trimethoprim
Diltiazem	15.34	0.958	$^{13}C_3$-Atrazine	415.5	178.0	$^{13}C_3$-Trimethoprim
Carbamazepine	15.38	1.007	d_{10}-Carbamazepine	237.4	194.2	d_{10}-Carbamazepine
Erythromycin [1]	15.94	1.000	$^{13}C_2$-Erythromycin	734.4	158	not quantified
Oxacillin	16.30	1.018	$^{13}C_3$-Atrazine	434.1	160.2	$^{13}C_3$-Trimethoprim
Tylosin	16.37	1.022	$^{13}C_3$-Atrazine	916.6	772.5	$^{13}C_6$-Sulfamethazine
Digoxin	16.58	1.036	$^{13}C_3$-Atrazine	798.5	651.3	$^{13}C_3$-Trimethoprim
Dehydronifedipine	16.65	0.981	d_5-Fluoxetine	345.1	284.1	$^{13}C_3$-Trimethoprim
Cloxacillin	16.82	0.991	d_5-Fluoxetine	468.1	160.1	$^{13}C_3$-Trimethoprim
Erythromycin anhydrate [1]	16.90	1.000	$^{13}C_2$-Erythromycin anhydrate	716.4	158	$^{13}C_2$-Erythromycin anhydrate
Fluoxetine	16.97	1.000	d_5-Fluoxetine	310.1	148.0	d_5-Fluoxetine
Virginiamycin M1	17.40	1.025	d_5-Fluoxetine	526.3	508.3	$^{13}C_3$-Trimethoprim
Clarithromycin	17.61	1.038	d_5-Fluoxetine	748.9	158.2	$^{13}C_6$-Sulfamethazine
Roxithromycin	17.83	1.051	d_5-Fluoxetine	837.6	679.0	$^{13}C_6$-Sulfamethazine
Miconazole	20.93	1.233	d_5-Fluoxetine	417.0	161.0	$^{13}C_3$-Trimethoprim
Norgestimate	21.80	1.285	d_5-Fluoxetine	370.5	124.0	$^{13}C_3$-Trimethoprim
Surrogate Standard						
$^{13}C_2$,^{15}N-Acetaminophen	4.68	0.292	$^{13}C_3$-Atrazine	155.2	111.0	$^{13}C_3$-Atrazine
$^{13}C_3$-Caffeine	9.32	0.582	$^{13}C_3$-Atrazine	198.0	140.0	$^{13}C_3$-Atrazine
$^{13}C_3$-Trimethoprim	9.94	0.621	$^{13}C_3$-Atrazine	294.2	233.0	$^{13}C_3$-Atrazine
$^{13}C_6$-Sulfamethazine	10.26	0.641	$^{13}C_3$-Atrazine	285.1	162.1	$^{13}C_3$-Atrazine
d_6-Thiabendazole	10.48	0.655	$^{13}C_3$-Atrazine	208.1	180.1	$^{13}C_3$-Atrazine
$^{13}C_3$,^{15}N-Ciprofloxacin	10.81	0.675	$^{13}C_3$-Atrazine	336.1	318.2	$^{13}C_3$-Atrazine
$^{13}C_6$-Sulfamethoxazole	11.37	0.710	$^{13}C_3$-Atrazine	260.0	162.0	$^{13}C_3$-Atrazine

d_{10}-Carbamazepine	15.28	0.954	$^{13}C_3$-Atrazine	247	204	$^{13}C_3$-Atrazine
$^{13}C_2$-Erythromycin [1]	15.86	0.991	$^{13}C_3$-Atrazine	736.4	160.0	monitor for less than 5%
$^{13}C_2$-Erythromycin anhydrate [1]	16.90	1.056	$^{13}C_3$-Atrazine	718.4	160.0	$^{13}C_3$-Atrazine
d_5-Fluoxetine	16.97	1.060	$^{13}C_3$-Atrazine	315.3	153.0	$^{13}C_3$-Atrazine
Recovery Standard						
$^{13}C_3$-Atrazine	16.01	1.000		219.1	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". 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

Target Analyte	Typical Retention Time (min)	Typical RRT	RRT Reference	Precursor Ion Mass	Product Ion Mass	Quantified against
Minocycline	3.45	0.739	d_6-Thiabendazole	458.0	441.0	d_6-Thiabendazole
Epitetracycline (ETC)	5.71	1.223	d_6-Thiabendazole	445.2	410.2	d_6-Thiabendazole
Epioxytetracycline (EOTC)	6.51	1.394	d_6-Thiabendazole	461.2	426.2	d_6-Thiabendazole
Oxytetracycline (OTC)	7.29	1.561	d_6-Thiabendazole	461.2	426.2	d_6-Thiabendazole
Tetracycline (TC)	7.74	1.657	d_6-Thiabendazole	445.2	410.2	d_6-Thiabendazole
Demeclocycline	9.63	0.470	$^{13}C_3$-Atrazine	465.0	430.0	d_6-Thiabendazole
Epichlortetracycline (ECTC)	9.92	0.485	$^{13}C_3$-Atrazine	479.0	444.0	d_6-Thiabendazole
Isochlortetracycline (ICTC) [1]	9.95	0.486	$^{13}C_3$-Atrazine	479.0	462.0	d_6-Thiabendazole
Chlortetracycline (CTC)	11.90	0.581	$^{13}C_3$-Atrazine	479.0	444.0	d_6-Thiabendazole
Doxycycline	14.40	0.703	$^{13}C_3$-Atrazine	445.2	428.2	d_6-Thiabendazole
Epianhydrotetracycline (EATC)	15.08	0.737	$^{13}C_3$-Atrazine	427.2	409.8	d_6-Thiabendazole
Anhydrotetracycline (ATC)	16.45	0.804	$^{13}C_3$-Atrazine	427.2	409.8	d_6-Thiabendazole
Epianhydrochlortetracycline (EACTC)	18.90	0.923	$^{13}C_3$-Atrazine	461.2	444.0	d_6-Thiabendazole
Anhydrochlortetracycline (ACTC)	20.63	1.008	$^{13}C_3$-Atrazine	461.2	444.0	d_6-Thiabendazole
Surrogate Standard						
d_6-Thiabendazole	4.67	0.228	$^{13}C_3$-Atrazine	208.0	180.0	$^{13}C_3$-Atrazine
Recovery Standard						
$^{13}C_3$-Atrazine	20.51	1.000		219.1	176.9 (134.0)	External Standard

[1] The presence of ECTC will create positive interference with ICTC due to use of a common transition ion.

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List 3 – Acid Extraction, Negative Electrospray Ionization (-)ESI

Target Analyte	Typical Retention Time (min)	Primary MRM Transition	Secondary MRM Transition	Typical Transition Ratio (Secondary Product Ion/Primary Product Ion)	Quantification and RRT Reference[1]
Bisphenol A	3.9	227.0 > 212.0	227.0 > 133.0	tbd	d_6-Bisphenol A
Furosemide	2.8	328.8 > 205.0	328.8 > 285.0	tbd	d_5-Furosemide
Gemfibrozil	7.6	249.0 > 121.0	249.0 > 126.9	tbd	d_6-Gemfibrozil
Glipizide	4.0	444.0 > 319.1	444.0 > 170.0	tbd	d_{11}-Glipizide
Glyburide	5.7	492.9 > 170.0	493.8 > 172.0	tbd	d_3-Glyburide
Hydrochlorothiazide	0.6	295.9 > 269.0	295.9 > 204.9	tbd	$^{13}C_1$-d_2-Hydrochlorothiazide
2-hydroxy-Ibuprofen	3.1	221.1 > 177.1	n.a.	tbd	d_6-2-hydroxy-Ibuprofen
Ibuprofen	6.1	205.0 > 161.1	n.a.	tbd	$^{13}C_6$-Ibuprofen
Naproxen	4.2	229.0 > 170.0	229.0 > 169.0	tbd	d_3-Naproxen
Triclocarban	7.7	312.8 > 160.0	314.8 > 162.0	tbd	$^{13}C_6$-Triclocarban
Triclosan	7.8	286.8 > 34.8	288.8 > 34.8	tbd	$^{13}C_{12}$-Triclosan
Warfarin	4.5	306.9 > 161.0	306.9 > 250.0	tbd	d_5-Warfarin
Surrogate Standard					
d_6-Bisphenol A	3.9	233.0 > 215.1	233.0 > 138.0	tbd	$^{13}C_1$-d_3-Naproxen
d_5-Furosemide	2.8	333.8 > 206.0	333.8 > 290.0	tbd	$^{13}C_1$-d_3-Naproxen
d_6-Gemfibrozil	7.6	255.0 > 121.0	255.0 > 133.0	tbd	$^{13}C_3$-Ibuprofen
d_{11}-Glipizide	4.0	455.0 > 319.0	455.0 > 170.0	tbd	$^{13}C_1$-d_3-Naproxen
d_3-Glyburide	5.7	494.9 > 170.0	496.7 > 171.9	tbd	$^{13}C_3$-Ibuprofen
$^{13}C_1$-d_2-Hydrochlorothiazide	0.6	298.8 > 270.1	298.8 > 206.1	tbd	$^{13}C_1$-d_3-Naproxen
d_6-2-hydroxy-Ibuprofen	3.1	227.1 > 183.1	n.a.	tbd	$^{13}C_3$-Ibuprofen
$^{13}C_6$-Ibuprofen	6.1	211.0 > 167.1	n.a.	n.a.	$^{13}C_3$-Ibuprofen
d_3-Naproxen	4.2	232.0 > 173.1	232.0 > 171.1	tbd	$^{13}C_1$-d_3-Naproxen
$^{13}C_6$-Triclocarban	7.7	318.9 > 159.9	320.9 > 161.9	tbd	$^{13}C_3$-Ibuprofen
$^{13}C_6$-Triclosan	7.8	298.9 > 34.8	294.9 > 34.8	tbd	$^{13}C_3$-Ibuprofen

d_5-Warfarin	4.5	312.0 > 161.0	312.0 > 255.1	tbd	$^{13}C_1$-d_3-Naproxen
Recovery Standard					
$^{13}C_3$-Ibuprofen	6.1	208.0 > 163.1	n.a.	n.a.	External Standard
$^{13}C_1$-d_3-Naproxen	4.2	233.0 > 170.0	233.0 > 169.0	n.a.	External Standard

[1] The primary transition of the quantification reference is used as the quantification reference for the primary transition of each analyte: the secondary transition of the quantification reference is used as the quantification reference for the secondary transition of each analyte.

* Third MRM transitions are available for some compounds and can be used if interferences are present in either the primary or secondary transition. 297.9 > 270.9 can be used for hydrochlorothiazide and 380.8 > 272.1 can be used for $^{13}C_1$-d_2-Hydrochlorothiazide.

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List 4 – Base Extraction, Positive Electrospray Ionization (+)ESI

Target Analyte	Typical Retention Time (min)	Primary MRM Transition	Secondary MRM Transition	Typical Transition Ratio (Secondary Product Ion/Primary Product Ion)	Quantification and RRT Reference[1]
Albuterol	4.47	240.1 > 148.0	240.1 > 222.2	tbd	d_3-Albuterol
Amphetamine	4.21	136.0 > 91.0	136.0 > 119.0	tbd	d5-Amphetamine
Atenolol	5.49	267.2 > 74.0	267.2 > 145.0	tbd	d7-Atenolol
Atorvastatin	1.14	559.5 > 440.3	559.5 > 250.1	tbd	d5-Enalapril
Cimetidine	2.57	252.6 > 159.1	252.6 > 117.0	tbd	d3-Cimetidine
Clonidine	2.26	230.1 > 44.0	231.5 > 44.0	tbd	d4-Clonidine
Codeine	4.85	300.2 > 215.1	300.2 > 58.0	tbd	d6-Codeine
Cotinine	1.16	177.0 > 80.0	177.0 > 98.0	tbd	d3-Cotinine
Enalapril	4.00	377.2 > 234.1	377.3 > 159.8	tbd	d5-Enalapril
Hydrocodone	4.37	300.2 > 199.1	300.2 > 127.9	tbd	d3-Hydrocodone
Metformin	5.26	130.0 > 60.0	130.0 > 71.0	tbd	d_6-Metformin
Oxycodone	3.34	316.2 > 241.1	316.2 > 298.2	tbd	d6-Oxycodone
Ranitidine	5.64	315.9 > 176.1	315.9 > 130.2	tbd	d_6-Ranitidine
Triamterene	2.59	254.2 > 104.0	254.2 > 237.1	tbd	d_5-Triamterene
Surrogate Standards					
d_3-Albuterol	4.62	243.3 > 151.1	243.3 > 225.2	tbd	d3-Amitriptyline
d_5-Amphetamine	4.21	141.0 > 92.8	141.0 > 124.0	tbd	d3-Amitriptyline
d_7-Atenolol	5.51	273.7 > 79.1	273.7 > 145.0	tbd	d3-Amitriptyline
d_5-Atorvastatin	1.14	564.4 > 440.4	564.4 > 250.1	tbd	d3-Amitriptyline
d_3-Cimetidine	2.65	255.6 > 162.1	255.6 > 120.0	tbd	d3-Amitriptyline
d_4-Clonidine	2.30	234.1 > 48.0	236.0 > 48.0	tbd	d3-Amitriptyline
d_6-Codeine	4.95	306.2 > 218.1	306.2 > 61.0	tbd	d3-Amitriptyline
d_3-Cotinine	1.17	180.1 > 80.0	180.1 > 101.0	tbd	d3-Amitriptyline
d_5-Enalapril	4.03	382.3 > 239.2	382.3 > 164.8	tbd	d3-Amitriptyline
d_3-Hydrocodone	4.47	303.3 > 199.1	303.3 > 127.9	tbd	d3-Amitriptyline
d_6-Metformin	5.28	136.2 > 59.9	136.2 > 77.1	tbd	d3-Amitriptyline
d_6-Oxycodone	3.59	322.3 > 247.2	322.3 > 304.2	tbd	d3-Amitriptyline
d_6-Ranitidine	5.74	321.2 > 176.0	321.2> 130.0	tbd	d3-Amitriptyline

d_5-Triamterene	2.59	259.2 > 108.0	259.2 > 242.0	tbd	d3-Amitriptyline
Recovery Standards					
d_9-Albuterol	4.60	249.3 > 148.0	249.3 > 231.2	tbd	External Standard
d_3-Amitriptyline	3.41	281.3 > 91.0	281.3 > 233.2	tbd	External Standard

List 5 – Acid Extraction, Positive Electrospray Ionization (+)ESI

Target Analyte	Typical Retention Time (min)	Primary MRM Transition	Secondary MRM Transition	Typical Transition Ratio (Secondary Product Ion/Primary Product Ion)	Quantification and RRT Reference[1]
Alprazolam	11.33	309.1 > 281.1	309.1 > 205.1	tbd	d_5-Alprazolam
Amitriptyline	12.01	278.2 > 105.1	278.2 > 91.1	tbd	d_6-Amitriptyline
Amlodipine	12.06	409.1 > 238.1	411.1 > 240.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4)
Benzoylecgonine	4.06	290.1 > 168.2	290.1 > 105.1	tbd	d_8-Benzoylecgonine
Benztropine	12.49	308.2 > 167.2	308.2 > 152.1	tbd	d_3-Benztropine
Betamethasone	9.89	393.2 > 373.2	393.2 > 355.2	tbd	d_5-Betamethasone
Cocaine	5.59	304.2 > 182.2	304.2 > 82.1	tbd	d_3-Cocaine
DEET	10.6	192.2 > 119.0	192.2 > 91.0	tbd	d_7-DEET
Desmethyldiltiazem	9.7	401.1 > 178.0	401.1 > 109.0	tbd	d_4-Desmethyldiltiazem
Diazepam	14.08	285.0 > 154.0	285.0 > 193.1	tbd	d_5-Diazepam
Fluocinonide	15.22	495.2 > 337.1	495.2 > 121.1	tbd	d_5-Fluticasone propionate
Fluticasone propionate	16.25	501.2 > 293.1	501.2 > 313.2	tbd	d_5-Fluticasone propionate
Hydrocortisone	8.03	363.2 > 121.1	363.2 > 91.1	tbd	d_4-Hydrocortisone
10-hydroxy-amitriptyline	6.28	294.2 > 276.2	294.2 > 58.0	tbd	d_6-Amitriptyline
Meprobamate	5.88	219.1 > 158.1	219.1 > 97.1	tbd	**d_5-Diazepam**
Methylprednisolone	9.75	375.2 > 357.1	375.2 > 161.1	tbd	d_3-Methylprednisolone
Metoprolol	4.94	268.2 > 116.1	268.2 > 72.1	tbd	d_7-Metoprolol
Norfluoxetine	12.68	296.1 > 134.1	296.1 > 30.0	tbd	d_5-Norfluoxetine
Norverapamil	11.6	441.3 > 165.1	441.3 > 150.1	tbd	d_7-Propranolol
Paroxetine	10.97	330.1 > 70.0	330.1 > 192.1	tbd	d_6-Paroxetine
Prednisolone	7.92	361.1 >147.1	361.1 >343.2	tbd	d_8-Prednisone
Prednisone	7.88	359.15 > 147.1	359.15 > 341.1	tbd	d_8-Prednisone
Promethazine	10.12	285.13 > 86.11	285.13 > 198.0	tbd	d_4-Promethazine

Propoxyphene	11.96	340.2 > 58.0	340.2 > 266.2	tbd	d_5-Propoxyphene
Propranolol	7.77	260.2 > 116.1	260.2 > 56.0	tbd	d_7-Propranolol (ring d_7)
Sertraline	13.28	306.07 > 159	308.07 > 161.0	tbd	d_3-Sertraline
Simvastatin	17.42	436.2 > 199.2	436.2 > 419.2	tbd	d_6-Simvastatin
Theophylline	3.32	181.01 > 124.0	181.1 > 42.0	tbd	$^{13}C,^{15}N_2$-Theophylline
Trenbolone	10.27	271.2 > 199.1	271.2 > 253.1	tbd	d_5-Trenbolone
Trenbolone acetate	15.8	313.2 > 253.2	313.2 > 107	tbd	**d_5-Trenbolone**
Valsartan	14.43	436.2 > 235.1	436.2 > 291.2	tbd	d_3-Valsartan
Verapamil	11.98	455.2 > 165.1	455.2 > 150.1	tbd	d_7-Verapamil
Surrogate Standards					
d_5-Alprazolam	11.25	314.1 > 286.1	314.1 > 210.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_6-Amitriptyline	12.01	284.2 > 105.0	284.2 > 90.9	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_4-Amlodipine (2-aminoethoxy-d_4)	12.04	413.1 > 239.0	414.9 > 240.0	tbd	d_4-Amlodipine (2-chlorophenyl-d_4)
d_8-Benzoylecgonine	4.04	298.1 > 171.1	298.1 > 110.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_3-Benztropine	12.48	311.2 > 167.1	311.2 > 152.0	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_5-Betamethasone	9.81	398.1 > 378.0	398.1 > 360.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_3-Cocaine	5.59	307.2 > 185.2	307.2 > 85.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_7-DEET	10.46	199.1 > 126.0	199.1 > 97.9	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_4-Desmethyldiltiazem	9.7	405.1 > 182.1	405.1 > 110.0	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_5-Diazepam	14	290.1 > 154.1	290.1 > 198.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_5-Fluticasone propionate	16.21	506.2 > 293.1	506.2 > 313.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)

d_4-Hydrocortisone	8.01	367.2 > 121.1	367.2 > 91.0	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_3-Methylprednisolone	9.72	378.2 > 360.2	378.2 > 342.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_7-Metoprolol	4.93	275.2 > 123.1	275.2 > 79.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_5-Norfluoxetine	12.67	301.1 > 139.1	301.1 > 32.0	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_6-Paroxetine	10.94	336.2 > 76.1	336.2 > 198.2	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_8-Prednisone	7.8	367.2 > 150.1	367.2 > 349.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_4-Promethazine	10.06	289.1 > 86.1	289.1 > 202.0	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_5-Propoxyphene	11.89	345.3 > 58.1	345.3 > 266.2	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_7-Propranolol (ring-d_7)	7.67	267.2 > 116.1	267.2 > 72.1	tbd	d_7-Propranolol (1-methylethyl-d_7)
d_3-Sertraline	13.27	309.1 > 159.0	311.1 > 160.9	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_6-Simvastatin	17.41	442.3 > 199.2	442.3 > 425.3	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
$^{13}C_1$,$^{15}N_2$-Theophylline	3.32	184.1 > 125.0	184.1 > 43.0	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_5-Trenbolone	10.21	276.2 > 204.1	276.2 > 258.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_3-Valsartan	14.41	439.1 > 235.1	439.1 > 294.2	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_7-Verapamil	11.89	462.2 > 165.1	462.2 > 150.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
Recovery Standards					
d_4-Amlodipine (2-chlorophenyl-d_4)	12.01	413.1 > 242.0	414.9 > 244.0	tbd	External Standard
d_7-Propranolol (1-methylethyl-d_7)	7.75	267.2 > 123.1	267.2 > 79.1	tbd	External Standard

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PPCP List 6 – Acid Extraction, Positive Electrospray Ionization (+)ESI

Target Analyte	Typical Retention Time (min)	Primary MRM Transition	Secondary MRM Transition	Typical Transition Ratio (Secondary Product Ion/Primary Product Ion)	Quantification and RRT Reference[1]
Amsacrine	8.3	394.3 > 315.2	394.3 > 179.2	tbd	d_6-Citalopram
Azathioprine	5.05	278.1 > 142.1	278.1 > 232.1	tbd	$^{13}C_4$-Azathioprine
Busulfan	6.09	264.2 > 151.1	264.2 > 247.1	tbd	d_8-Busulfan
Citalopram	10.04	325.3 > 109.1	325.3 > 262.2	tbd	d_6-Citalopram
Clotrimazole	14.61	277.3 > 165.2	277.3 > 241.2	tbd	d_5-Clotrimazole
Colchicine	8.96	400.3 > 358.2	400.3 > 310.2	tbd	d_6-Colchicine
Cyclophosphamide	8.06	261.2 > 140.1	261.2 > 106.0	tbd	d_4-Cyclophosphamide
Daunorubicin	10.44	528.3 > 321.2	528.3 > 381.1	tbd	$^{13}C,d_3$-Daunorubicin
Diatrizoic acid	3.61	631.8 > 361.1	631.8 > 233.1	tbd	d_6-Diatrizoic acid
Doxorubicin	8.89	544.2 > 397.1	544.2 > 130.1	tbd	$^{13}C,d_3$-Daunorubicin
Drospirenone	14.37	367.3 > 97.1	367.3 > 91.1	tbd	$^{13}C_3$-Drospirenone
Etoposide	9.53	606.3 > 229.2	606.3 > 185.1	tbd	d_3-Etoposide
Iopamidol	3.50	794.8 > 777.9	794.8 > 558.9	tbd	d8-Iopamidol
Medroxyprogesterone acetate	16.96	387.3 > 123.1	387.3 > 327.3	tbd	d_6-Medroxyprogesterone acetate
Melphalan	8.41	305.2 > 288.1	305.2 > 246.1	tbd	d_8-Melphalan
Metronidazole	4.31	172.2 > 128.1	172.2 > 82.0	tbd	d_4-Metronidazole
Moxifloxacin	7.47	402.3 > 384.2	402.3 > 96.1	tbd	$^{13}C,d_3$-Moxifloxacin
Oxazepam	11.26	287.1 > 241.1	287.1 > 269.1	tbd	d_5-Oxazepam
Rosuvastatin	12.77	482.3 > 258.2	482.3 > 300.2	tbd	d_6-Rosuvastatin
Tamoxifen	16.48	372.4 > 72.1	372.4 > 44.2	tbd	d_5-Tamoxifen
Teniposide	12.64	674.3 > 229.1	674.3 > 383.2	tbd	d_3-Etoposide
Venlafaxine	8.27	278.4 > 58.1	278.4 > 260.3	tbd	d_5-Venlafaxine
Zidovudine	5.50	268.3 > 127.1	268.3 > 109.9	tbd	d_3-Zidovidine
Surrogate Standards					
$^{13}C_4$-Azathioprine	5.05	282.1 > 146.1	282.1 > 236.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_8-Busulfan	6.03	272.2 > 159.1	272.2 > 262.2	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)

d_6-Citalopram	10.03	331.3 > 109.0	331.3 > 82.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4)
d_5-Clotrimazole	14.55	282.2 > 169.9	282.2 > 247.2	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_6-Colchicine	8.9	406.3 > 362.3	406.3 > 285.2	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_4-Cyclophosphamide	8.01	265.2 > 140.0	265.2 > 106.0	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
$^{13}C_1$-d_3-Daunorubicin	10.41	532.2 > 325.1	532.2 > 385.2	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_6-Diatrizoic acid	3.6	637.9 > 367.1	637.9 > 620.8	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
$^{13}C_3$-Drospirenone	14.37	370.2 > 97.1	370.2 > 91.2	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_3-Etoposide	9.49	609.2 > 229.1	609.2 > 185.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_8-Iopamidol	3.51	802.9 > 785.8	802.9 > 562.9	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_6-Medroxyprogesterone acetate	16.93	393.2 > 126.1	393.2 > 330.3	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_8-Melphalan	8.34	313.1 > 296.1	313.1 > 254.2	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_4-Metronidazole	4.29	176.2 > 128.1	176.2 > 87.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
$^{13}C_1$-d_3-Moxifloxacin	7.45	406.3 > 388.2	406.3 > 110.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_5-Oxazepam	11.18	292.1 > 246.1	292.1 > 274.1	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_6-Rosuvastatin	12.73	488.3 > 264.2	488.3 > 306.2	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_5-Tamoxifen	16.45	377.3 > 72.1	377.3 > 44.2	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)

d_6-Venlafaxine	8.26	284.4 > 64.1	284.4 > 266.3	tbd	d_4-Amlodipine (2-chlorophenyl-d_4) d_7-Propranolol (1-methylethyl-d_7)
d_3-Zidovudine	5.49	271.2 > 130.1	271.2 > 126.1	tbd	d_7-Propranolol (1-methylethyl-d_7)
Recovery Standards					
d_4-Amlodipine (2-chlorophenyl-d_4)	11.85	413.1 > 242.0	414.9 > 244.0	tbd	External Standard
d_7-Propranolol (1-methylethyl-d_7)	9.04	267.2 > 123.1	267.2 > 79.1	tbd	External Standard

* = Confirmation ions in instances of interference
** = Parent ion monitored from the breakdown product

Hormones (HM-APOS) – Positive Electrospray Ionization (+)ESI

Target Analyte	Typical Retention Time (min)	Primary MRM Transition	Secondary MRM Transition	Typical Ratio (Secondary Product Ion/Primary Product Ion)	Quantification and RT Reference[1]
Allyl trenbolone	6.2	311.4 > 227.3	311.4 > 159.2	tbd	d_6-Norethindrone
Androstenedione	6.0	287.1 > 97.0	287.1 > 109.0	tbd	$^{13}C_3$-Androstenedione
Androsterone*	6.4	291.2 > 255.2	291.2 > 273.3	tbd	d_4-Androsterone
Desogestrel	10.1	311.3 > 67.0	311.3 > 135.1	tbd	$^{13}C_2$,d_2-Desogestrel
17beta-Estradiol 3-benzoate*	7.5	377.2 > 105.0	377.2 > 77.0	tbd	d_3-17beta-Estradiol 3-benzoate
Mestranol*	7.0	311.2 > 121.1	293.0 > 146.7	tbd	d_4-Mestranol
Norethindrone	5.5	299.4 > 109.1	299.4 > 91.1	tbd	d_6-Norethindrone
Norgestrel	6.2	313.4 > 109.1	313.4 > 91.1	tbd	d_6-Norgestrel
Progesterone	6.4	315.1 > 97.0	315.1 > 109.0	tbd	d_9-Progestrone

Testosterone	5.5	289.1 > 97.0	289.1 > 109.0	tbd	$^{13}C_3$-Testosterone
Surrogate or Extracted Internal Standard					
$^{13}C_3$-Androstenedione	6.0	290.5 > 100.0	290.5 > 112.0	tbd	$^{13}C_3$-17alpha-Hydroxyprogesterone
d_4-Androsterone	6.4	295.4 > 259.3	295.4 > 277.3	tbd	$^{13}C_3$-17alpha-Hydroxyprogesterone
$^{13}C_2$,d_2-Desogestrel	10.1	315.5 > 135.0	315.5 > 93.0	tbd	$^{13}C_3$-17alpha-Hydroxyprogesterone
d_3-17beta-Estradiol 3-benzoate	7.5	380.4 > 105.5	380.4 > 77.0	tbd	$^{13}C_3$-17alpha-Hydroxyprogesterone
d_4-Mestranol	7.0	315.4 > 123.1	315.4 > 161.3	tbd	$^{13}C_3$-17alpha-Hydroxyprogesterone
d_6-Norethindrone	5.5	305.4 > 86.7	305.4 > 113.3	tbd	$^{13}C_3$-17alpha-Hydroxyprogesterone
d_6-Norgestrel	6.2	319.5 > 251.4	319.5 > 91.2	tbd	$^{13}C_3$-17alpha-Hydroxyprogesterone
d_9-Progestrone	6.4	324.3 > 100.0	324.3 > 113.0	tbd	$^{13}C_3$-17alpha-Hydroxyprogesterone
$^{13}C_3$-Testosterone	5.5	292.6 > 100.0	292.6 > 112.0	tbd	$^{13}C_3$-17alpha-Hydroxyprogesterone
Recovery or Injection Internal Standard					
$^{13}C_3$-17alpha-Hydroxyprogesterone	6.1	334.3 > 100.0	n.a.		External Standard

[1] The primary transition of the quantification reference is used as the quantification reference for the primary transition of each analyte: the secondary transition of the quantification reference is used as the quantification reference for the secondary transition of each analyte.

* Third MRM transitions are available for some compounds and can be used if interferences are present in either the primary or secondary transition. 255.0 > 198.8 can also be used for androsterone. 377.2 > 135.1 can also be used for 17beta-Estradiol 3-benzoate. 293.0 > 173.0 can also be used for mestranol. 380.4 > 138.0 can be used for d_3-17beta-Estradiol 3-benzoate.

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Hormones (HM-ANEG) – Negative Electrospray Ionization (-)ESI

Target Analyte	Typical Retention Time (min)	Primary MRM Transition	Secondary MRM Transition	Typical Ratio (Secondary Product Ion/Primary Product Ion)	Quantification Reference [1]
17alpha-Dihydroequilin	5.6	269.2 > 267.3	269.2 > 211.2	tbd	d_4-17beta-Estradiol
Equilenin	5.6	264.8 > 221.3	264.8 > 249.2	tbd	d_3-Equilenin
Equilin	6.1	267.2 > 265.1	267.2 > 143.0	tbd	d_4-17beta-Estradiol
17alpha-Estradiol	6.1	271.4 > 145.1	271.4 > 183.2	tbd	d_3-17alpha-Estradiol
17beta-Estradiol	5.5	271.4 > 145.1	271.4 > 183.2	tbd	d_4-17beta-Estradiol
Estriol	4.5	287.3 > 171.0	287.3 > 145.0	tbd	d_3-Estriol
Estrone	6.2	269.4 > 145.2	269.4 > 159.2	tbd	$^{13}C_3$-Estrone
17alpha-Ethinylestradiol	6.1	295.3 > 145.0	295.3 > 159.0	tbd	$^{13}C_2$-17alpha-Ethinylestradiol
Surrogate Standard				tbd	
d_3-Equilenin	5.6	268.2 > 222.2	268.2 > 252.1	tbd	d_4-Estrone
d_3-17alpha-Estradiol	6.1	274.4 > 145.0	274.4 > 185.0	tbd	d_4-Estrone
d_4-17beta-Estradiol	5.5	275.4 > 147.1	275.4 > 187.0	tbd	d_4-Estrone
d_3-Estriol	4.5	290.2 > 173.1	290.2 > 147.1	tbd	d_4-Estrone
$^{13}C_3$-Estrone	6.2	272.3 > 148.0	272.3 > 162.0	tbd	d_4-Estrone
$^{13}C_2$-17alpha-Ethinylestradiol	6.1	297.2 >145.1	297.2 > 199.1	tbd	d_4-Estrone
Recovery Standard					
d_4-Estrone	6.2	273.2 > 147.0	n.a.		External Standard

[1] The primary transition of the quantification reference is used as the quantification reference for the primary transition of each analyte: the secondary transition of the quantification reference is used as the quantification reference for the secondary transition of each analyte.

* Third MRM transitions are available for some compounds and can be used if interferences are present in either the primary or secondary transition. 267.2 > 239.1 can be used for equilin.269.4 > 183.3 can be used for estrone.

5.0 QUALITY ACCEPTANCE CRITERIA

Samples are analyzed in batches consisting of a maximum of twenty samples, one procedural blank and one spiked matrix (OPR) sample. A duplicate is analyzed, provided there is sufficient sample, with batches containing 7-20 samples. A Matrix Spike (MS) Sample prepared by spiking target analytes into a client sample, should be performed with every soil/sediment batch containing 7 or more test samples. 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.

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QC Acceptance Limits [1], Aqueous, POCIS [5], Solid [11] and Tissue [10] Samples

	Aqueous Samples (and POCIS [5])			Solid/Biosolid [10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
List 1 Compounds (APOS)									
Acetaminophen	70-140		≤15	70-130		≤15	70-130		≤15
Azithromycin	10-130		≤1.5	70-160		≤1.5	70-250		≤1.5
Caffeine	25-160		≤15	70-135		≤15	70-130		≤15
Carbadox	25-180		≤1.5	30-130		≤1.5	10-130		≤1.5
Carbamazepine	25-200		≤1.5	70-160		≤1.5	70-150		≤1.5
Cefotaxime	10-300		≤6	65-300		≤6	70-300		≤6
Ciprofloxacin	25-180		≤6	70-180		≤6	70-130		≤6
Clarithromycin	50-160		≤1.5	50-200		≤1.5	70-250		≤1.5
Clinafloxacin	25-300		≤6	70-180		≤6	70-200		≤6
Cloxacillin [2]	70-130		≤3	70-220		≤3	70-250		≤3
Dehydronifedipine	35-160		≤0.6	70-180		≤0.6	70-200		≤0.6
Digoxigenin	50-150		≤6	70-160		≤6	50-200		≤6
Digoxin	35-200		≤6	60-180		≤6	70-250		≤6
Diltiazem	20-160		≤0.3	70-135		≤0.3	70-200		≤0.3
1,7-Dimethylxanthine	30-300		≤60	70-180		≤60	70-250		≤60
Diphenhydramine	70-130		≤0.6	70-150		≤0.6	60-130		≤0.6
Enrofloxacin	30-220		≤3	70-150		≤3	70-130		≤3
Erythromycin - H_2O	70-130		≤0.3 [3]	70-145		≤0.3 [3]	70-130		≤0.3 [3]

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
Flumequine	40-160		≤1.5	70-180		≤1.5	60-200		≤1.5
Fluoxetine	60-150		≤1.5	65-135		≤1.5	70-130		≤1.5
Lincomycin	10-300		≤3	40-250		≤3	70-300		≤3
Lomefloxacin	50-250		≤3	70-160		≤3	70-150		≤3
Miconazole	35-130		≤1.5	55-145		≤1.5	5-130		≤1.5
Norfloxacin	10-250		≤15	70-200		≤15	70-150		≤15
Norgestimate	35-130		≤3	40-130		≤3	5-130		≤3
Ofloxacin	60-250		≤1.5	70-180		≤1.5	70-200		≤1.5
Ormetoprim	70-150		≤0.6	70-145		≤0.6	70-130		≤0.6
Oxacillin [2]	20-130		≤3	70-180		≤3	70-200		≤3
Oxolinic Acid	60-150		≤0.6	70-180		≤0.6	70-130		≤0.6
Penicillin G [2]	10-130		≤3	70-200		≤3	20-130		≤3
Penicillin V	40-140		≤3	70-250		≤3	70-250		≤3
Roxithromycin	50-140		≤0.3	45-160		≤0.3	50-200		≤0.3
Sarafloxacin	50-200		≤15	70-180		≤15	50-130		≤15
Sulfachloropyridazine	60-160		≤1.5	70-200		≤1.5	70-200		≤1.5
Sulfadiazine	70-130		≤1.5	70-180		≤1.5	70-300		≤1.5
Sulfadimethoxine	35-160		≤0.3	50-130		≤0.3	70-130		≤0.3
Sulfamerazine	60-140		≤0.6	70-135		≤0.6	70-200		≤0.6
Sulfamethazine	70-130		≤0.6	70-135		≤0.6	70-130		≤0.6

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
Sulfamethizole	30-140		≤0.6	55-135		≤0.6	60-130		≤0.6
Sulfamethoxazole	70-130		≤0.6	70-130		≤0.6	70-130		≤0.6
Sulfanilamide	2-160		≤15	50-150		≤15	50-300		≤15
Sulfathiazole	30-180		≤1.5	35-130		≤1.5	70-130		≤1.5
Thiabendazole	60-150		≤1.5	70-160		≤1.5	70-130		≤1.5
Trimethoprim	50-150		≤1.5	70-135		≤1.5	70-130		≤1.5
Tylosin	70-130		≤6	30-145		≤6	60-200		≤6
Virginiamycin M1	15-300		≤3	70-180		≤3	30-200		≤3
Surrogate Standard									
$^{13}C_2$, ^{15}N-Acetaminophen	30-160	30-160		50-160	30-160		30-150	30-250	
$^{13}C_3$-Caffeine	40-140	40-140		50-150	40-140		30-150	20-250	
d_{10}-Carbamazepine	40-150	40-150		50-150	40-150		30-150	30-150	
$^{13}C_3$, ^{15}N-Ciprofloxacin	7-150	7-150		40-160	7-150		30-150	30-200	
$^{13}C_2$-Erythromycin - H_2O	35-130	35-130		40-180	35-130		30-206	5-200	
d_5-Fluoxetine	10-160	10-160		50-160	10-160		30-150	20-150	
$^{13}C_6$-Sulfamethazine	30-160	30-160		40-150	30-160		30-150	30-150	
$^{13}C_6$-Sulfamethoxazole	30-140	30-140		50-180	30-140		30-150	10-150	
d_6-Thiabendazole	25-180	25-180		30-150	25-180		30-150	30-150	
$^{13}C_3$-Trimethoprim	30-140	30-140		40-150	30-140		30-150	30-200	
Recovery Standard									

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
$^{13}C_3$-Atrazine									
List 2 Compounds (TCYC)									
Anhydrochlortetracycline (ACTC)	15-200		≤15	9-145		≤15			
Anhydrotetracycline (ATC)	20-160		≤15	25-150		≤15			
Chlortetracycline (CTC)	30-250		≤6	40-180		≤6			
Demeclocycline	35-180		≤15	20-130		≤15			
Doxycycline	35-180		≤6	35-220		≤6			
Epianhydrochlortetracycline (EACTC)	6-130		≤60	5-130		≤60			
Epianhydrotetracycline (EATC)	15-200		≤15	20-160		≤15			
Epichlortetracycline (ECTC)	25-180		≤15	30-200		≤15			
Epioxytetracycline (EOTC)	25-180		≤6	9-145		≤6			
Epitetracycline (ETC)	35-200		≤6	20-250		≤6			
Isochlortetracycline (ICTC)	25-180		≤6	35-140		≤6			
Minocycline	1-250		≤60	9-400		≤60			
Oxytetracycline (OTC)	20-200		≤6	15-150		≤6			
Tetracycline (TC)	20-200		≤6	25-180		≤6			
Surrogate Standard									
d_6-Thiabendazole	25-140	25-140		30-150	25-140				
Recovery Standard									
$^{13}C_3$-Atrazine									

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
List 3 Compounds (ANEG)									
Bisphenol A	70-130		≤500	70-130		≤500	70-150		≤500
Furosemide	70-130		≤40	70-130		≤40	70-130		≤40
Gemfibrozil	70-130		≤1.5	70-130		≤1.5	70-140		≤1.5
Glipizide	70-130		≤6	70-130		≤6	70-140		≤6
Glyburide	70-130		≤3	70-130		≤3	70-150		≤3
Hydrochlorothiazide	70-130		≤20	70-130		≤20	70-130		≤20
2-hydroxy-ibuprofen	70-130		≤80	70-130		≤80	70-130		≤80
Ibuprofen	70-130		≤15	70-130		≤15	70-130		≤15
Naproxen	70-130		≤3	70-135		≤3	70-150		≤3
Triclocarban	70-130		≤3	70-145		≤3	70-130		≤3
Triclosan	70-130		≤60	70-140		≤60	70-150		≤60
Warfarin	70-150		≤1.5	70-140		≤1.5	70-150		≤1.5
Surrogate Standards									
d_6-Bisphenol A	50-150	50-150		50-150	50-150		50-150	50-150	
d_5-Furosemide	50-150	50-150		10-150	10-150		10-150	10-150	
d_6-Gemfibrozil	50-150	50-150		50-150	50-150		50-150	50-150	
d_{11}-Glipizide	50-150	50-150		50-150	50-150		50-150	50-150	
d_3-Glyburide	50-150	50-150		50-150	50-150		50-150	50-150	
$^{13}C_1$-d_2-Hydrochlorothiazide	22-160	22-160		45-150	45-150		35-150	35-150	

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
d_6-2-hydroxy-Ibuprofen	39-150	39-150		50-150	50-150		50-150	50-150	
$^{13}C_6$-Ibuprofen	50-150	50-150		28-150	28-150		50-150	50-150	
d_3-Naproxen	50-150	50-150		50-150	50-150		50-150	50-150	
$^{13}C_6$-Triclocarban	35-150	35-150		18-150	18-150		13-150	13-150	
$^{13}C_6$-Triclosan	50-150	50-150		20-150	20-150		40-150	40-150	
d_5-Warfarin	50-150	50-150		50-150	50-150		50-150	50-150	
Recovery Standard									
$^{13}C_3$-Ibuprofen									
$^{13}C_1$-d_3-Naproxen									
List 4 Compounds (BPOS)									
Albuterol	50-160		≤0.3	70-180		≤0.3	60-130		≤0.3
Amphetamine	50-160		≤1.5	70-200		≤1.5	70-130		≤1.5
Atenolol	70-130		≤0.6	70-220		≤0.6	70-130		≤0.6
Atorvastatin	20-130		≤1.5	25-130		≤1.5	70-150		≤1.5
Cimetidine	15-130		≤0.6	70-145		≤0.6	30-130		≤0.6
Clonidine	70-130		≤1.5	70-220		≤1.5	70-130		≤1.5
Codeine	70-130		≤3	70-250		≤3	70-130		≤3
Cotinine	70-130		≤1.5	70-145		≤1.5	70-130		≤1.5
Enalapril	70-130		≤0.3	70-150		≤0.3	70-130		≤0.3
Hydrocodone	70-130		≤1.5	70-220		≤1.5	70-130		≤1.5

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
Metformin	70-160		≤3	70-200		≤3	70-130		≤3
Oxycodone	65-130		≤0.6	70-180		≤0.6	70-150		≤0.6
Ranitidine	25-140		≤0.6	30-130		≤0.6	NQ-150 [6]		≤0.6
Triamterene	70-140		≤0.3	50-145		≤0.3	70-130		≤0.3
Surrogate Standards									
d_3-Albuterol	20-140	20-140		50-150	20-140		20-150	5-150	
d_5-Amphetamine	20-130	20-130		40-150	20-130		30-150	5-150	
d_7-Atenolol	50-130	50-130		50-150	50-130		30-150	30-300	
d_5-Atorvastatin	25-130	20-130		10-130	10-130		40-150	15-150	
d_3-Cimetidine	4-130	15-130		30-150	15-130		20-150	NQ-500 [6]	
d_4-Clonidine	50-130	50-130		50-160	50-130		30-150	30-300	
d_6-Codeine	50-130	50-130		50-150	50-130		10-150	5-150	
d_3-Cotinine	50-140	50-140		50-250	50-140		30-150	30-300	
d_5-Enalapril	50-130	50-130		50-180	50-130		30-150	10-150	
d_3-Hydrocodone	50-130	50-130		40-150	50-130		30-150	20-150	
d_6-Metformin	3-130	3-130		10-150	3-130		10-150	5-200	
d_6-Oxycodone	50-150	50-150		40-150	50-150		30-150	30-150	
d_6-Ranitidine	4-130	10-130		30-130	10-130		10-130	10-130	
d_5-Triamterene	50-130	50-130		20-130	50-150		30-150	40-130	
Recovery Standards									

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
d_9-Albuterol									
d_3-Amitriptyline									
List 5 Compounds (APOSX)									
Alprazolam	70-130		≤0.3	70-130		≤0.15	70-130		≤0.15
Amitriptyline	70-130		≤0.3	70-130		≤0.15	70-130		≤0.15
Amlodipine	60-130		≤1	70-150		≤0.5	70-130		≤0.5
Benzoylecgonine	70-140		≤0.15	70-130		≤0.15	70-130		≤0.15
Benztropine	70-130		≤0.7	70-130		≤0.35	70-150		≤0.35
Betamethasone	70-130		≤1.5	70-130		≤0.75	70-250		≤0.8
Cocaine	70-130		≤0.15	70-130		≤0.15	70-130		≤0.15
DEET	70-160		≤5	70-140		≤3	70-150		≤2
Desmethyldiltiazem	70-130		≤0.15	70-130		≤0.15	60-130		≤0.15
Diazepam	70-130		≤0.5	70-130		≤0.3	70-130		≤0.3
Fluocinonide	70-130		≤2	70-130		≤1	70-140		≤1
Fluticasone propionate	70-130		≤2	70-130		≤1	60-130		≤1
Hydrocortisone	70-130		≤6	70-130		≤3	70-150		≤3
10-hydroxy-amitriptyline	70-130		≤0.15	60-130		≤0.15	40-130		≤0.15
Meprobamate	70-130		≤1.5	70-150		≤1	70-130		≤0.8
Methylprednisolone	70-130		≤4	70-130		≤2	70-130		≤2
Metoprolol	70-130		≤0.5	70-130		≤0.3	70-130		≤0.3

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
Norfluoxetine	70-130		≤0.5	70-130		≤0.3	70-130		≤0.3
Norverapamil	70-150		≤0.15	70-150		≤0.15	70-160		≤0.15
Paroxetine	70-130		≤1	70-130		≤0.5	60-130		≤0.5
Prednisolone	70-130		≤4	70-130		≤2	60-130		≤2
Prednisone	70-130		≤6	70-145		≤3	70-130		≤3
Promethazine	70-130		≤0.3	70-130		≤0.15	70-130		≤0.15
Propoxyphene	70-130		≤0.3	70-130		≤0.15	70-130		≤0.15
Propranolol	70-130		≤0.3	70-130		≤0.15	70-130		≤0.15
Sertraline	70-130		≤0.3	70-150		≤0.15	60-130		≤0.15
Simvastatin	70-130		≤2	70-130		≤1	70-130		≤1
Theophylline	70-130		≤2	70-130		≤1	70-130		≤1
Trenbolone	70-130		≤2	70-130		≤1	70-130		≤1
Trenbolone acetate	70-130		≤0.3	60-130		≤0.15	70-130		≤0.15
Valsartan	70-130		≤4	70-130		≤2	70-130		≤2
Verapamil	70-130		≤0.15	70-130		≤0.15	70-130		≤0.15
Surrogate Standards									
d_5-Alprazolam	50-150	50-150		50-150	40-150		50-150	30-150	
d_6-Amitriptyline	50-150	50-150		50-150	40-150		50-150	10-150	
d_4-Amlodipine (2-aminoethoxy-d_4)	30-150	30-150		40-150	5-130		50-150	30-150	
d_8-Benzoylecgonine	50-150	30-150		50-150	5-150		50-150	20-150	

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
d_3-Benztropine	40-140	50-150		50-150	5-150		50-150	30-150	
d_5-Betamethasone	50-150	50-150		50-150	40-150		50-150	30-150	
d_3-Cocaine	50-150	50-150		50-150	5-140		50-150	20-150	
d_7-DEET	35-150	30-150		30-150	5-160		30-150	30-150	
d_4-Desmethyldiltiazem	50-150	50-150		50-150	50-150		50-150	20-150	
d_5-Diazepam	50-150	50-150		50-150	20-160		50-150	20-160	
d_5-Fluticasone Propionate	50-150	40-150		20-150	30-150		20-150	20-150	
d_4-Hydrocortisone	40-150	50-150		50-150	30-240		40-150	20-150	
d_3-Methylprednisolone	50-150	50-150		50-150	40-150		50-150	30-150	
d_7-Metoprolol	50-150	40-150		50-150	5-140		50-150	30-150	
d_5-Norfluoxetine	20-130	50-150		50-150	5-130		40-150	5-150	
d_6-Paroxetine	35-130	50-150		50-150	50-150		50-150	20-150	
d_8-Prednisone	40-140	30-150		50-150	20-150		50-150	20-150	
d_4-Promethazine	30-130	40-150		40-150	5-140		40-150	20-150	
d_5-Propoxyphene	50-150	50-150		50-150	5-150		50-150	30-200	
d_7-Propranolol	50-150	50-150		40-150	5-150		50-150	5-150	
d_3-Sertraline	50-150	50-150		50-150	10-150		50-150	20-150	
d_6-Simvastatin	40-140	30-150		40-140	20-150		10-150	10-150	
$^{13}C_1$-$^{15}N_2$-Theophylline	50-150	20-150		30-130	20-200		50-150	10-150	
d_5-Trenbolone	50-150	50-150		20-150	30-150		40-150	20-150	

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
d_3-Valsartan	50-150	40-150		40-150	20-150		10-150	20-150	
d_7-Verapamil	40-140	50-150		50-150	5-150		50-150	30-150	
Recovery Standards									
$^{13}C_3$-Atrazine									
d_4-Amlodipine (2-chlorophenyl-d_4)									
d_7-Propranolol (1-methylethyl-d_7)									
List 6 Compounds (APOSY) [7]									
Amsacrine	70-130		≤ 0.8	70-130		≤ 1.6	70-130		≤ 0.3
Azathioprine	70-130		≤ 8	70-130		≤ 8	70-130		≤ 1.3
Busulfan	70-130		≤ 8	70-130		≤ 8	70-130		≤ 1.3
Citalopram	70-130		≤ 2	70-130		≤ 1.6	70-130		≤ 0.3
Clotrimazole	70-130		≤ 2	70-130		≤ 1.6	70-130		≤ 0.3
Colchicine	70-130		≤ 2	70-130		≤ 2	70-150		≤ 0.4
Cyclophosphamide	70-130		≤ 1.6	70-130		≤ 8	70-130		≤ 0.3
Daunorubicin	70-130		≤ 8	70-130		≤ 8	70-130		≤ 3
Diatrizoic acid	60-130		≤ 320	70-140		≤ 160	70-130		≤ 26
Doxorubicin	30-130		≤ 80	70-130		≤ 80	50-130		≤ 13
Drospirenone	70-130		≤ 24	70-130		≤ 24	70-130		≤ 4
Etoposide	70-150		≤ 8	70-130		≤ 8	70-130		≤ 3
Iopamidol	70-130		≤ 4	70-130		≤ 4	70-130		≤ 0.8

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
Medroxyprogesterone acetate	60-130		≤ 4	70-130		≤ 4	70-130		≤ 0.8
Melphalan	70-130		≤ 320	70-130		≤ 320	60-130		≤ 52
Metronidazole	70-130		≤ 8	70-130		≤ 8	70-130		≤ 2
Moxifloxacin	70-130		≤ 8	70-130		≤ 8	70-130		≤ 2
Oxazepam	70-130		≤ 4	70-130		≤ 4	70-130		≤ 0.8
Rosuvastatin	70-130		≤ 4	70-130		≤ 20	70-130		≤ 0.8
Tamoxifen	70-130		≤ 1.6	70-130		≤ 1.6	70-130		≤ 0.3
Teniposide	70-130		≤ 4	40-130		≤ 4	70-130		≤ 0.8
Venlafaxine	70-130		≤ 3.2	70-130		≤ 1.6	70-130		≤ 0.3
Zidovudine	70-130		≤ 80	70-130		≤ 80	70-130		≤ 13
Surrogate Standards									
$^{13}C_4$-Azathioprine	50-150	50-150		50-170	50-150		50-150	20-150	
d_8-Busulfan	50-150	50-150		50-150	50-150		50-150	50-150	
d_6-Citalopram	50-150	50-150		50-150	20-150		50-150	20-150	
d_5-Clotrimazole	50-150	50-150		50-150	50-150		50-150	40-150	
d_6-Colchicine	50-150	50-150		50-150	50-150		30-150	40-150	
d_4-Cyclophosphamide	50-150	50-150		50-150	50-150		50-150	40-150	
$^{13}C_1$-d_3-Daunorubicin	40-150	50-150		50-150	30-200		50-150	30-150	
d_6-Diatrizoic acid	5-130	5-150		5-130	5-150		5-150	10-150	
$^{13}C_3$-Drospirenone	50-150	50-150		50-150	50-150		50-150	50-150	

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
d_3-Etoposide	50-150	50-150		50-150	20-150		50-150	20-150	
d_8-Iopamidol	15-150	15-150		10-130	10-150		10-150	40-150	
d_6-Medroxyprogesterone acetate	50-150	50-150		50-150	50-160		50-150	40-150	
d_8-Melphalan	20-150	30-150		10-130	10-150		15-150	10-150	
d_4-Metronidazole	40-150	50-150		50-160	50-150		50-150	40-150	
$^{13}C_1$-d_3-Moxifloxacin	40-150	50-150		50-150	30-160		50-150	50-180	
d_5-Oxazepam	50-150	50-150		50-150	50-160		50-150	50-180	
d_6-Rosuvastatin	50-150	50-150		50-150	50-150		50-150	50-180	
d_5-Tamoxifen	50-150	30-150		50-150	50-150		50-150	40-150	
d_6-Venlafaxine	50-150	50-150		50-150	50-150		50-150	40-150	
d_3-Zidovudine	50-150	50-150		50-160	50-150		50-150	40-150	
Recovery Standards									
$^{13}C_3$-Atrazine									
d_4-Amlodipine (2-chlorophenyl-d_4)									
d_7-Propranolol (1-methylethyl-d_7)									
HM-APOS									
Allyl trenbolone	70-130		≤ 0.8	70-130		≤ 0.8	n.a.		≤ 0.8
Androstenedione	70-130		≤ 2	70-130		≤ 2	70-130		≤ 2
Androsterone	70-130		≤ 20	70-150		≤ 20	70-150		≤ 20

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
Desogestrel	60-160		≤ 120	70-135		≤ 120	70-130		≤ 120
17beta-Estradiol 3-benzoate	65-150			70-130			n.a.		
Mestranol	70-150		≤ 40	70-135		≤ 40	45-130		≤ 40
Norethindrone	70-130		≤ 4	70-130		≤ 4	70-130		≤ 4
Norgestrel	70-130		≤ 4	65-150		≤ 4	50-150		≤ 4
Progesterone	70-130		≤ 0.8	70-140		≤ 0.8	70-130		≤ 0.8
Testosterone	70-130		≤ 0.8	70-130		≤ 0.8	70-130		≤ 0.8
Surrogate Standards									
$^{13}C_3$-Androstenedione	50-150	50-150		50-150	50-150		50-150	50-150	
d_4-Androsterone	50-150	50-150		50-160	50-160		14-800	14-800	
$^{13}C_2$,d_2-Desogestrel	25-150	25-150		12-150	12-150		3-180	3-180	
d_3-17beta-Estradiol 3-benzoate	35-150	35-150		30-150	30-150		n.a.	n.a.	
d_4-Mestranol	45-150	45-150		50-150	50-150		35-400	35-400	
d_6-Norethindrone	50-150	50-150		50-200	50-200		50-180	50-180	
d_6-Norgestrel	50-150	50-150		50-160	50-160		50-150	50-150	
d_9-Progesterone	50-150	50-150		50-150	50-150		50-150	50-150	
$^{13}C_3$-Testosterone	50-150	50-150		50-170	50-170		50-200	50-200	
Recovery Standards									
$^{13}C_3$-17alpha-Hydroxyprogesterone									

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	Aqueous Samples (and POCIS [5])			Solid/Biosolid[10] Samples			Tissue Samples		
	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)	OPR recovery (% rec.)	Surrogate recovery in samples (% rec.)	Blank limit [4] (ng)
HM-ANEG									
17alpha-Dihydroequilin	70-130		≤ 4	70-130		≤ 4	40-130		≤ 4
Equilenin	70-150		≤ 0.8	70-150		≤ 0.8	70-220		≤ 0.8
Equilin	70-130		≤ 8	70-130		≤ 8	70-130		≤ 8
17alpha-Estradiol	70-140		≤ 4	70-130		≤ 4	70-130		≤ 4
17beta-Estradiol	70-130		≤ 4	70-130		≤ 4	70-130		≤ 4
Estriol	70-140		≤ 48	70-130		≤ 48	70-130		≤ 48
Estrone	70-130		≤ 4	70-130		≤ 4	70-130		≤ 4
17alpha-Ethinylestradiol	70-150		≤ 20	70-130		≤ 20	65-150		≤ 20
Surrogate Standards									
d_3-Equilenin	50-150	50-150		50-150	50-150		45-150	45-150	
d_3-17alpha-Estradiol	50-150	50-150		50-150	50-150		50-150	50-150	
d_4-17beta-Estradiol	50-150	50-150		50-150	50-150		50-150	50-150	
d_3-Estriol	20-150	20-150		30-150	30-150		14-150	14-150	
$^{13}C_3$-Estrone	50-150	50-150		50-150	50-150		50-150	50-150	
$^{13}C_2$-17alpha-Ethinylestradiol	50-150	50-150		50-150	50-150		50-150	50-150	
Recovery Standards									
d_4-Estrone									

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Footnotes:

[1] OPR recovery, surrogate recovery and blank limits derived from actual method performance data.

[2] Analysis result is classified as “Information Value” of estimated concentration.

[3] Background level of Erythromycin - H_2O in the associated labeled surrogate may elevate the Erythromycin - H_2O blank value. Sample results may be blank corrected where acceptable by contract.

[4] Higher blank levels are acceptable where sample concentrations exceed 10 times the blank limits.

[5] POCIS samples have the same acceptance limits as aqueous samples, but for POCIS samples the limits are interim guidelines only.

[6] NQ = Not Quantifiable. Low recovery rate may preclude quantification.

[7] The acceptance limits for List 6 compounds are guidelines based on initial estimates; recoveries outside of these limits do not invalidate results.

[8] Recoveries outside limits may be accepted based on application and professional judgment

[9] List 2 compounds have not been validated for tissue matrix.

[10] List 2 compounds are not available for soil/sediment samples

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QC Specification Table: Instrumental Acceptance Specifications

QC Parameter	Specification
Instrument Sensitivity	Daily, S:N ≥ 3:1 for all analytes for lowest calibration point.
Initial Calibration (native compounds)	For Lists 1 and 2: Initial, (1/X) weighted linear regression using a minimum of 5 calibration concentrations; 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 – coefficient of determination r2 ≥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. For hydrocortisone, an increased frequency of Initial Calibration variance from method acceptance limits has been observed and is attributed to transient instrumental instability of response correctable by instrumental re-analysis. If the results are deemed to be fit for the intended purpose the hydrocortisone data may be flagged and reported with an explanation of the variance, otherwise instrumental re-analysis to correct the QC variance is required. For initial calibration using only 4 calibration concentrations the following requirements must be met: Calculated concentrations of native compounds 70-130%, Correlation coefficient r2 ≥0.985 (this is a requirement, not a guideline). For List 3, 4, 5, 6, Hormones (POS) and Hormones (NEG): Constant RRF calibration. %RSD of RRF ≤ 25%. Calculated concentrations 70-130%, one point per compound may be 60-140%.
Initial Calibration (labeled compounds)	Calculated concentrations 50-150%.
OPENING Calibration Verification	Native compounds: A mid-level calibration solution is analyzed every 12 hours or every 20 samples, whichever occurs first. Determined concentrations within 70-130% of actual. Allowable exception: A maximum of 1 compound per List or 10% of the compounds on a List, whichever is greater, may fall outside 70-130% provided they are in the range 60-140% of actual. Labeled compounds: Calculated concentrations 50-150%.
CLOSING Calibration Verification	Native compounds: 1) Determined concentrations shall be within 70-130% of actual. Results for the greater of 1 compound or 10% of the compounds on a List may fall outside 70-130% provided they are: a) within 60-140% or, b) within 50-150% and the RPD between the CLOSING result and the OPENING result is ≤40%. 2) Closing calibration verification limits do not apply to Furosemide and Hydrochlorothiazide. Labeled compounds: Calculated concentrations 50-150%.
Instrumental Carryover And Instrument Background	Every Initial Calibration, Cal/Ver, or SPM: ≤ 0.3% carryover. Area response of analytes in instrument blank ≤ ½ of response in A-Cal 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., RPD ≤ 40% for 60% of analytes

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* Specifications for duplicate samples and MS/MSD are guidelines. Wider limits may be applied based on sample characteristics and professional judgment.

APPENDIX I: LIMITATIONS TO PERFORMANCE

1. SOIL/SEDIMENT SAMPLES

Experience has shown that recoveries of certain compounds from some soil/sediment matrices may be low. Sample data must be evaluated with consideration of the recoveries achieved for each analyte in the matrix spike sample prepared with each analytical batch. Poor performance of specific analytes in the matrix spike may result in those analytes being flagged (NQ) in related project samples.

In particular, the surrogates listed in the table below can show recoveries in soil and sediment samples that do not meet method criteria. The exact reason is not known, as recoveries are in the normal range for other matrices including biosolids samples that undergo identical processing, and for aqueous samples as well. The interaction of dissolved inorganic components of the matrix with the analytes and the material in the Oasis HLB cartridge is the most likely cause for compounds in List 1 and List 5 showing low recovery.

Surrogate	List	Issue
$^{13}C_3$,^{15}N-Ciprofloxacin	List 1	Low Recovery
^{13}C-d_3-Naproxen	List 3	Low Recovery
$^{13}C_3$-Ibuprofen	List 3	Low Recovery
$^{13}C_6$-Triclocarban	List 3	Low Recovery
d_5-Warfarin	List 3	Low Recovery
d_6-Bisphenol A	List 3	Low Recovery
d_6-Gemfibrozil	List 3	Low Recovery
d_4-Desmethyldiltiazem	List 5	Low Recovery
d_6-Paroxetine	List 5	Low Recovery
d_3-Sertraline	List 5	Low Recovery
^{13}C,d_3-Daunorubicin[1]	List 6	Low Recovery
^{13}C,d_3-Moxifloxacin	List 6	Low Recovery

[1] ^{13}C,d_3-Daunorubicin must have a minimum recovery of 30% to report both Doxorubicin and Daunorubicin in sediment samples. ^{13}C,d_3-Daunorubicin shows generally poor recovery in soil/sediments and it is unlikely that this specification will be met.

Analytes and surrogates not meeting method criteria are flagged using protocols detailed in SGS AXYS Document QDO-027 *"Rules for the Application of Non-Quantifiable Flags (NQ) to MLA-075 Results"*.

2. 1,7-DIMETHYLXANTHINE, THEOPHYLLINE AND THEOBROMINE

1,7-Dimethylxanthine is an analyte in List 1, Theophylline or 1,3-dimethylxanthine is an analyte in List 5 of the same method. These analytes are isomers, and hence co-elute in both List 1 and List 5 instrumental runs, leading to a systematic over-reporting of each compound in the Spiked Matrix

(SPM) samples. The recovery criteria for these compounds takes into account the effect of the cross interference on data accuracy. Any positive detection of either analyte is presumed to be a sum of the two analytes. Neither the HPLC, nor the mass spectrometer, can differentiate between the two compounds.

3. ROXITHROMYCIN, CLARITHROMYCIN AND TYLOSIN REQUANTIFICATION

Roxithromycin, Clarithromycin and Tylosin are all quantified against ^{13}C-Sulfamethazine. This surrogate is chemically different from the analytes and can sometimes show low recovery in samples even when the three analytes are not affected. If the recovery of ^{13}C-Sulfamethazine is less than 10%, upon request, Roxithromycin, Clarithromycin and Tylosin are requantified against the recovery standard ^{13}C-Atrazine and flagged as estimated minimum concentrations if detected. The data is evaluated and flagged using procedures outlined in SGS AXYS Document QDO-027 *"Rules for the Application of Non-Quantifiable Flags (NQ) to MLA-075 Results"*.

4. METHYL ESTER INTERFERENCE OF BETA-LACTAM ANTIBIOTICS

Cloxacillin, oxacillin and penicillin G are reported as 'Information Values' of estimated concentration. These compounds are determined by LC-MS/MS using ions from the methanol adduct of the compound ($M+CH_3OH$). There is indication that methyl esters of these compounds can also form in standard solutions over time. Ions from these methyl esters cannot be distinguished from methanol adduct ions formed from the parent compound. The consequence of this reaction could be a slow, but continuous increase of instrument response for these compounds in the calibration solutions. The rate of change in response is different for each compound. This behavior has not yet been observed/documented in client samples. The result of this standard transformation is to confer greater uncertainty on measured concentrations of these three compounds.

5. POTENTIAL AMPHETAMINE INTERFERENCE

The presence of an interfering compound with potential to obscure or cause false positive detection of amphetamine has been observed in some water and solids samples. Use of the secondary transition response, itself prone to interference, is not reliable in overcoming the interference problem. Partial or complete chromatographic resolution of the interfering compound has been observed - i.e. a shift of the native compound peak RT (retention time) relative to that of the d5-amphetamine surrogate is indicative of the interference. Where evidence of this interference is observed, amphetamine results are flagged in reports as "estimated maximum possible values". The typical level of this interference is between the range of the A- and B-Calibration standards.

i. Extracts will not be routinely diluted and reinjected for amelioration of the amphetamine interference alone, as there is no evidence of efficacy.

ii. Where multiple injection data for a sample are available (e.g. a neat and a diluted run), instrument analysts will report amphetamine from the chromatogram producing the most definitive result based on an evaluation of peak shape and peak resolution. The result will be quantified as amphetamine but flagged as an "estimated maximum possible concentration" on reports. The flag must be edited by hand in LIMS; EMPC, K or NDR dependent on client flagging requirements.

iii. For amphetamine with a high peak area response above the SPM, the 1st channel should be confirmed by the 2nd channel. If no peak is present in the 2nd channel, the peak in the 1st channel is possibly not amphetamine and should be removed from the 1st channel.

6. POTENTIAL DEGRADATION OF RANITIDINE IN THE STANDARD SOLUTION

Degradation of ranitidine in the standard solution used to prepare OPR tests has been observed intermittently under the specific conditions of the storage. Where OPR test results indicate the possibility of spiking solution degradation, the ranitidine OPR assigned value is adjusted based on the results of a secondary QC test solution (SAR) prepared from the same ampoule that has been analyzed alongside samples. This problem has been demonstrated to have no impact on sample data accuracy

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APPENDIX II: EFFECTS OF ADDING ASCORBIC ACID TO SAMPLES.

Ascorbic acid is added to quench free chlorine in aqueous samples that have been chlorinated. The presence of free chlorine has severe effects on the recovery of analytes and most surrogate compounds. 50 mg/L of ascorbic acid is usually added to samples. The vast majority of analytes and standards are not affected by ascorbic acid addition. It is possible that some analytes may show enhanced recovery. The effects of ascorbic acid on each analyte/standard is shown below.

Analyte	List	Effect	Surrogates	List	Effect
Acetaminophen	List 1	Normal	$^{13}C_2$,^{15}N-Acetaminophen	List 1	Normal
Azithromycin	List 1	Normal	$^{13}C_3$-Caffeine	List 1	Normal
Caffeine	List 1	Normal	$^{13}C_3$,^{15}N-Ciprofloxacin	List 1	Normal
Carbadox	List 1	Normal	$^{13}C_2$-Erythromycin-H_2O	List 1	Normal
Carbamazepine	List 1	Normal	d5-Fluoxetine	List 1	Normal
Cefotaxime	List 1	Normal	$^{13}C_6$-Sulfamethazine	List 1	Normal
Ciprofloxacin	List 1	Normal	$^{13}C_6$-Sulfamethoxazole	List 1	Normal
Clarithromycin	List 1	Normal	d6-Thiabendazole	List 1	Normal
Clinafloxacin	List 1	Normal	$^{13}C_3$-Trimethoprim	List 1	Normal
Cloxacillin	List 1	Normal	d6-Thiabendazole	List 2	Normal
Dehydronifedipine	List 1	Normal	d6-Bisphenol	List 3	Normal
Diphenhydramine	List 1	Marginal low bias	d6-Gemfibrozil	List 3	Normal
Diltiazem	List 1	Marginal low bias	d11-Glipizide	List 3	Normal
Digoxin	List 1	Normal	d3-Glyburide	List 3	Normal
Digoxigenin	List 1	Normal	$^{13}C_3$-Ibuprofen	List 3	High bias
Enrofloxacin	List 1	Normal	^{13}C-d3-Naproxen	List 3	Normal
Erythromycin-H_2O	List 1	Normal	$^{13}C_6$-Triclocarban	List 3	Normal
Flumequine	List 1	Normal	$^{13}C_{12}$-Triclosan	List 3	Normal
Fluoxetine	List 1	Normal	d5-Warfarin	List 4	Normal
Lincomycin	List 1	Normal	d3-Albuterol	List 4	Normal
Lomefloxacin	List 1	Normal	d6-Metformin	List 4	Normal
Miconazole	List 1	Normal	d3-Cotinine	List 4	Normal
Norfloxacin	List 1	Normal	d3-Cimetidine	List 4	Normal
Norgestimate	List 1	Normal	d5-Enalapril	List 4	Normal
Ofloxacin	List 1	Normal	d6-Oxycodone	List 4	Normal
Ormetoprim	List 1	Normal	d4-Clonidine	List 4	Normal
Oxacillin	List 1	Normal	d5-Amphetamine	List 4	Normal
Oxolinic Acid	List 1	Normal	d6-Codeine	List 4	Normal
Penicillin G	List 1	Normal	d3-Hydrocodone	List 4	Normal
Penicillin V	List 1	Normal	d7-Atenolol	List 4	Normal
Roxithromycin	List 1	Normal	d5-Alprazolam	List 5	Normal
Sarafloxacin	List 1	Normal	d6-Amitriptyline	List 5	Normal
Sulfachloropyridazine	List 1	Normal	d8-Benzoylecgonine	List 5	Normal
Sulfadiazine	List 1	Normal	d3-Benztropine	List 5	Normal
Sulfadimethoxine	List 1	Normal	d3-Cocaine	List 5	Normal

Sulfamerazine	List 1	Normal	d7-DEET	List 5	Normal
Sulfamethazine	List 1	Normal	d5-Diazepam	List 5	Normal
Sulfamethizole	List 1	Normal	d3-Methylprednisolone	List 5	Normal
Sulfamethoxazole	List 1	Normal	d7-Metoprolol	List 5	Normal
Sulfanilamide	List 1	Normal	d5-Norfluoxetine	List 5	Normal
Sulfathiazole	List 1	Normal	d6-Paroxetine	List 5	Normal
Thiabendazole	List 1	Normal	d4-Promethazine	List 5	Normal
Trimethoprim	List 1	Normal	d5-propoxyphene	List 5	Normal
Tylosin	List 1	Normal	d7-Propranolol	List 5	Normal
Virginiamycin M1	List 1	Normal	$^{13}C,^{15}N_2$-Theophylline	List 5	Normal
1,7- Dimethylxanthine	List 1	Normal	d4-Hydrocortisone	List 5	Normal
CTC	List 2	Normal	$^{13}C_3$-Androstenedione	HM-APOS	TBD
ECTC	List 2	Normal	d_4-Androsterone	HM-APOS	TBD
ACTC	List 2	Normal	$^{13}C_3$-d_2-Desogestrel	HM-APOS	TBD
EACTC	List 2	Normal	d_3-17alpha-Estradiol	HM-ANEG	TBD
ICTC	List 2	Normal	$^{13}C_2$-17beta-Estradiol	HM-ANEG	TBD
Demeclocycline	List 2	Normal	d_4-17beta-Estradiol	HM-ANEG	Normal
Doxycycline	List 2	Normal	d_3-17beta-Estradiol 3-Benzoate	HM-APOS	TBD
OTC	List 2	Normal	d_3-Estriol	HM-ANEG	TBD
EOTC	List 2	Normal	$^{13}C_3$-Estrone	HM-ANEG	TBD
TC	List 2	Normal	$^{13}C_2$-17alpha-Ethinyl Estradiol	HM-ANEG	TBD
ETC	List 2	Normal	d_3-Equilenin	HM-ANEG	TBD
EATC	List 2	High Bias	d_4-Mestranol	HM-APOS	TBD
ATC	List 2	Normal	d_6-Norethindrone	HM-APOS	Normal
Minocycline (458>441)	List 2	Normal	d_6-Norgestrel	HM-APOS	Normal
Bisphenol A	List 3	Normal	d_9-Progesterone	HM-APOS	Normal
Furosemide	List 3	Normal	$^{13}C_3$-Testosterone	HM-APOS	TBD
Gemfibrozil	List 3	Normal			
Glipizide	List 3	Normal			
Glyburide	List 3	Normal			
Hydrochlorothiazide	List 3	Normal			
2-hydroxy-ibuprofen	List 3	Normal			
Ibuprofen	List 3	Normal			
Naproxen	List 3	Normal			
Triclocarban	List 3	Normal			
Triclosan	List 3	Normal			
Warfarin	List 3	Normal			
Albuterol	List 4	Normal			
Amphetamine	List 4	Normal			
Atenolol	List 4	Normal			
Atorvastatin	List 4	Normal			
Cimetidine	List 4	Normal			
Clonidine	List 4	Normal			
Codeine	List 4	Normal			
Cotinine	List 4	Normal			
Enalapril	List 4	Normal			
Hydrocodone	List 4	Normal			
Metformin	List 4	Normal			
Oxycodone	List 4	Normal			

Ranitidine	List 4	Normal			
Triamterene	List 4	Normal			
Alprazolam	List 5	Normal			
Amitriptyline	List 5	Normal			
Amlodipine	List 5	Normal			
Benzoylecgonine	List 5	Normal			
Benztropine	List 5	Normal			
Betamethasone	List 5	Normal			
Cocaine	List 5	Normal			
DEET	List 5	Normal			
Desmethyldiltiazem	List 5	Normal			
Diazepam	List 5	Normal			
Fluocinonide	List 5	Normal			
Fluticasone Propionate	List 5	Normal			
Hydrocortisone	List 5	Normal			
10-hydroxy-amitriptyline	List 5	Normal			
Meprobamate	List 5	Normal			
Methylprednisolone	List 5	Normal			
Metoprolol	List 5	Normal			
Norfluoxetine	List 5	Normal			
Norverapamil	List 5	Normal			
Paroxetine	List 5	High Bias			
Prednisolone	List 5	Normal			
Prednisone	List 5	Normal			
Promethazine	List 5	Normal			
Propoxyphene	List 5	Normal			
Propranolol	List 5	Normal			
Sertraline	List 5	Normal			
Simvastatin	List 5	Normal			
Theophylline	List 5	Normal			
Trenbolone	List 5	Normal			
Trenbolone acetate	List 5	Normal			
Valsartan	List 5	Normal			
Verapamil	List 5	Normal			
Allyl trenbolone	HM-APOS	Normal			
Androstenedione	HM-APOS	Normal			
Androsterone	HM-APOS	Normal			
Desogestrel	HM-APOS	Normal			
17beta-Estradiol 3-benzoate	HM-APOS	TBD			
Mestranol	HM-APOS	Normal			
Norethindrone	HM-APOS	Normal			
Norgestrel	HM-APOS	Normal			
Progestrone	HM-APOS	Normal			
Testosterone	HM-APOS	Normal			
17alpha-Dihydroequilin	HM-ANEG	Normal			
Equilenin	HM-ANEG	Normal			
Equilin	HM-ANEG	Normal			
17alpha-Estradiol	HM-ANEG	Normal			

17beta-Estradiol	HM-ANEG	Normal			
Estriol	HM-ANEG	Normal			
Estrone	HM-ANEG	Normal			
17alpha-Ethinyl-estradiol	HM-ANEG	Normal			

APPENDIX III: SUMMARY COMPARISON OF USEPA METHOD 1694 AND SGS AXYS METHOD MLA-075.

Area	EPA 1694	MLA-075
Applicable Matrices	Aqueous, Solids	Aqueous, Solids, Tissue, POCIS samplers
Analytes Offered	73 compounds, 2 fractions, 4 instrumental runs	158 compounds, 2 fractions, 8 instrumental runs
Sample Containers	Amber glass	Amber glass or HDPE
Chlorine Quenching (water samples)	80 mg sodium thiosulfate per liter, ascorbic acid allowable alternative	50 mg ascorbic acid per liter
Sample Preservation	pH 5-9 if hold time >48hr or freeze	None
Sample Storage Temperature	< 6ºC or frozen (aqueous, solids)	Aqueous: < 4 ºC; Solids: <-20 ºC
Sample Hold Time (guideline only)	Aqueous, 7 days at < 6ºC, undefined for frozen storage Solids, 7 days at <-10 ºC	Aqueous: 7 days for < 4 ºC storage Solids: 7 days for -20 ºC storage
Extract Hold Time	40 days	40 days
Extraction (separate acid, base fractions)	Aqueous: adjust to pH 2 or pH 10, stabilize with EDTA. Solids: adjust to pH 2 or pH 10, stabilize with EDTA, ultrasonic extract into buffered acetonitrile, exchange to water solution.	Aqueous: adjust to pH 2 or pH 10, stabilize with EDTA. Solids: adjust to pH 2 or pH 10, stabilize with EDTA, ultrasonic extract into buffered acetonitrile, exchange to water solution. Tissue: Ultrasonic extract into acetonitrile and pH 2 or pH 10 buffer, exchange to water solution, stabilize with EDTA. POCIS: Dialysis with solvent.
Clean-up (separate acid, base fractions)	SPE (HLB), elute in methanol	SPE (HLB), elute in methanol
Instrumental Acquisition	LC-MS/MS, 3 +ESI runs, 1 -ESI run	UPLC/MS/MS, 4 +ESI runs, 2 -ESI runs LC/MS/MS 2 +ESI runs

Calibration Range for LC-MS/MS, ng/mL in standard	Minimum 5 points, range 0.25- 25000 mg/mL	
Calibration Range for UPLC/MS/MS, ng/mL in standard		Minimum 5 points, range 0.02- 4000 ng/mL
Calibration Model	Multi-level, constant RRF; alternative models allowable	Multi-level, 1/x weighted linear regression for list 1 and 2 compounds, average RRF for list 3, 4 5, 6 and hormones
Initial Calibration Limits	RSD of RRF >20% (isotope dilution) or <35% (internal standard)	Calculated points 70-130% of actual (allowable exception per compound 60-140%)
Calibration Verification Limits	70-130%	Calculated points 70-130% of actual (allowable exception one compound per list or 10% of compounds per list may be 60-140%)
Quantification Type	Isotope dilution or internal standard	Isotope dilution or internal standard
Quantification References	18 isotopically labeled compounds	100 isotopically labeled compounds UPLC : List 3: 14; List 4: 14; List 5: 26; List 6: 20; Hormones: 15; HPLC: List 1: 10; List 2: 1
Initial Precision and Recovery (IPR) Limits, %	range 6-180%	performance based, generally 3-250%
On-Going Precision and Recovery (OPR) Limits, %	range 5-200%	performance based, generally 2-300%
Blank Limits, ng per sample	range 1-500 ng	performance based, generally 0.15–320 ng
Surrogate Recovery Limits, %	range 5-200%	performance based, generally 3-250%
Lower Reporting Limit, ng per sample based on low calibration standard	range 1–500 ng	performance based, generally 0.3–500 ng

AXYS Analytical Services Ltd.

Summary of AXYS Method MLA-080 Rev 02 Ver 04:

Analytical Procedure for the Analysis of Alkylphenols and Alkylphenol Ethoxylates in Tissue by LC-MS/MS

AXYS Method MLA-080 describes the analysis of 4-nonylphenol (NP), 4-n-octylphenol (OP), 4-nonylphenol monoethoxylate (NP1EO) and 4-nonylphenyl diethoxylate (NP2EO) in tissue samples. Detection limits vary by compound and may depend on the level of compounds detected in the laboratory blanks. Estimated detection limits, based on analysis of a 2 gram wet weight sample, are in the approximate range of 5 - 50 ng/g.

Target Analytes

4-nonylphenol (NP)	4-nonylphenol monoethoxylate (NP1EO)
4-n-octylphenol (OP)	4-nonylphenyl diethoxylate (NP2EO)

EXTRACTION AND CLEANUP

Sample size may be up to 2 g (wet weight). After dispersion in water and addition of isotopically labelled surrogate standards the sample is extracted into isooctane by steam distillation. The extract is cleaned up by solid phase extraction (SPE) using disposable cartridges containing aminopropyl sorbent. The SPE eluate is prepared in methanol, spiked with recovery standards and analyzed by LC-MS/MS. The final extract volume is 1 mL.

INSTRUMENTATION

Analysis of the sample extract is performed on a high performance liquid chromatography reversed phase C18 column using a solvent gradient. The column is coupled to a triple quadrupole mass spectrometer run at unit mass resolution in the Multiple Reaction Monitoring (MRM) mode. Each sample extract is analyzed in two separate LC-MS/MS runs, one run in the ESI negative mode (for nonyl-phenol and n-octyl-phenol), and the other run in the ESI positive mode (for NP1EO and NP2EO).

ESI Negative Analytes: Ions and Quantification References

Target Analyte	Typical Retention Time (minutes)	Parent Ion Mass	Daughter Ion Mass	Quantified Against	Cone Voltage (V)	Coll E (eV)
4-NP	10.29	219	133	$^{13}C_6$-4-NP	27	28
4n-OP	10.63	205.2	106	$^{13}C_6$-4-NP	30	20
Surrogate Standard						
$^{13}C_6$-4-NP	11.43	225	112	d6-BPA	35	20
Recovery Standard						
d6-BPA	5.01	233	215	-	18	18

ESI Positive Analytes: Ions and Quantification References

Target Analyte	Typical Retention Time (minutes)	Parent Ion Mass	Daughter Ion Mass	Quantified Against	Cone Voltage (V)	Coll E (eV)
NP1EO	11.48	282.2	127	$^{13}C_6$-NP1EO	21	9
NP2EO	11.55	326.3	183	$^{13}C_6$-NP1EO	21	14
Surrogate Standard						
$^{13}C_6$-NP1EO	12.43	288.2	127	$^{13}C_3$-Atrazine	25	18
Recovery Standard						
$^{13}C_3$-Atrazine	5.31	219.5	176.9	-	27	19

CALIBRATION

Average relative response factors, determined from a bracketing calibration standard with known amounts of native, surrogate and recovery compounds analyzed at the beginning and end of each sample batch (and at least once every twelve hours) are used to convert sample responses to concentrations.

AXYS Analytical Services Ltd.

Nominal Concentrations of Authentic, Surrogate and Recovery Standard Solutions

Compound Name Authentic Standards	Conc. in Std Solution (ng/mL)	Typical Amount added to Spiked Reference Sample (ng)	Typical volume Std Solution added (µL)
4-Nonylphenol (4-NP)	10000	1000	100
4-n-Octylphenol (4n-OP)	10000	1000	100
4-Nonylphenol monoethoxylate (NP1EO)	20000	2000	100
4-Nonylphenol diethoxylate (NP2EO)	1000	100	100
Labelled Surrogate Standards		**Typical Amount added to each sample (ng)**	
$^{13}C_6$-4-nonylphenol	25000	2000	80
$^{13}C_6$-4-Nonylphenol monoethoxylate	62500	5000	80
$^{13}C_6$-4-Nonylphenol diethoxylate [1]	3125	250	80
Recovery Standards			
d_6-Bisphenol A	4000	200	50
$^{13}C_3$-Atrazine	1200	60	50

[1] Not used for quantification of analytes but included for diagnostic purposes

Nominal Concentrations of the Single Bracketing Calibration Solution

Compound Name Authentic Standards	Conc. in Cal. Solution (ng/mL)
4-Nonylphenol (4-NP)	1000
4-n-Octylphenol (4n-OP)	1000
4-Nonylphenol monoethoxylate (NP1EO)	2000
4-Nonylphenol diethoxylate (NP2EO)	100
Labelled Surrogate Standards	
$^{13}C_6$-4-Nonylphenol	2000
$^{13}C_6$-4-Nonylphenol monoethoxylate	5000
$^{13}C_6$-4-Nonylphenol diethoxylate	250
Recovery Standards	
d_6-Bisphenol A	200
$^{13}C_3$-Atrazine	60

ANALYTE IDENTIFICATION

Positive identification of target compounds, surrogate standards 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 time from the daily bracketing calibration standard. Natives with labelled surrogate standards must elute within 1 minute of the associated labelled surrogate.

QUANTIFICATION AND DATA REPORTING PROCEDURES

Target concentrations are determined with respect to labelled surrogates. Average relative response factors (RRF), determined from a mid-level bracketing calibration standard run at the beginning and at the end of the samples, are used to convert raw peak areas in sample chromatograms to final concentrations as follows:

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

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

The results are recovery corrected by the method of quantification. Surrogate recoveries are determined against the recovery standard and are used as general indicators of overall analytical quality.

Sample specific detection limits (SDL) are determined from the analysis data by converting the minimum detectable area to a concentration following the same procedures used to convert target peak responses to concentrations. The estimated minimum detectable area is determined as three times the height of the noise in the m/z channel of interest, converted to an area using the area height ratio of the corresponding labelled surrogate peak.

NP1EO results are reported as maximum values with potential high bias if $^{13}C_6$-NP1EO is recovered at less than 50%.

QUALITY ASSURANCE / QUALITY CONTROL

All samples are analyzed in batches of the following composition:

- Batch Size - Each batch consists of up to twenty test samples and additional QC samples.
- Blanks - One procedural blank is analyzed for each batch. The procedural blank is prepared by spiking an aliquot of the surrogate standard solution into 300 mL of ultrapure water.
- Duplicates - Sample duplicates are analyzed (provided sufficient sample is available) for batches with 7-20 test samples, or when specified by the contract.
- One SAR (Surrogate/Authentic/Recovery) sample is analyzed with every batch. The SAR sample is prepared by spiking surrogate and authentic standard solutions into a solvent rinsed centrifuge tube. The composition of the SAR sample is the same as the single point bracketing calibration solution.
- Spiked Samples - Spiked reference samples are analyzed with each batch to demonstrate the accuracy of the data. Spiked reference samples are prepared by adding an aliquot of authentic spiking solution to a reference matrix (known to contain low background levels of target analytes).

QC Acceptance Limits for the Determination of Alkylphenols in Tissue Samples

Compound	Blank Limit, ng	OPR Recovery limit, %	Sample Surrogate Recovery Limit, % *
NP	40	60 – 130	
n-OP	1	70 – 130	
NP1EO	10	70 – 150	
NP2EO	2	40 – 130	
Surrogate			
$^{13}C_6$-NP		40 – 130	40 – 130
$^{13}C_6$-NP1EO		50 – 130	30 – 130

* Sample surrogate recovery specifications should be adhered to whenever possible as the accuracy of target analytes can be compromised by lower surrogate recoveries. Where it is necessary to report results with lower surrogate recoveries, the results must be flagged as potentially compromised.

Single point bracketing calibration limit:	≤ 40% RPD (relative percent difference)

Elements of the QA/QC program at AXYS Analytical Services are documented in the Quality Manual QDO-001 "QA/QC Policies and Procedures".

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SUMMARY OF AXYS METHOD MLA-115 REV. 01 VER. 02:

ANALYTICAL METHOD FOR THE DETERMINATION OF TRICLOSAN AND METHYL TRICLOSAN IN TISSUES

This method describes the determination of the concentrations of triclosan and methyl triclosan in tissues. Samples are spiked with isotopically labeled surrogate standards and extracted by Soxhlet extraction. The extracts are derivatized with acetic anhydride to acetylate triclosan and labeled triclosan, cleaned up by column chromatography and analyzed by high resolution gas chromatography with high resolution magnetic sector mass spectrometric detection (HRGC/HRMS).

Compound	CAS Registry Number
Triclosan	3380-34-5
Methyl triclosan	4640-01-1

Typical reporting limits are 1 ng triclosan per sample and 0.1 ng methyl triclosan per sample.

1.0 EXTRACTION AND CLEANUP PROCEDURES

Samples (up to 5 g wet weight) are spiked with isotopically labelled surrogate standards and Soxhlet extracted. After cleanup by gel permeation chromatography the extract is derivatized with acetic anhydride and finally cleaned up on a Florisil column. The final extract is spiked with isotopically labelled recovery (internal) standards prior to instrumental analysis.

2.0 INSTRUMENTATION

Analysis of triclosan and methyl triclosan is performed using a capillary gas chromatograph coupled to a high-resolution mass spectrometer. A DB-5 capillary column (60 m, 0.25 mm i.d. x 0.1 µm film thickness) is coupled to the MS source. The mass spectrometer is operated at a static (8000) mass resolution (10% valley) in the electron ionization (EI) mode using multiple ion detection (MID) acquiring two characteristic ions for each target analyte and surrogate standard. A splitless/split column injection sequence is used.

3.0 CALIBRATION

A series of derivatized calibration solutions covering the working response range of the instrument and containing the native target analytes and labelled surrogate and recovery standards is used to establish initial, multi-level calibration of the GC/MS. Calibration is verified at least once every 12 hours by analysis of a mid-level calibration solution. Bracketing calibration procedures may optionally be employed. use mean RRFs from the analysis of the mid-level calibration solution (or an SAR) prior to and after samples to calculate analyte concentrations.

The sample equivalent lower method calibration limit (LMCL) is defined as the concentration of the lowest calibration solution pro-rated for sample size analyzed and final extract volume.

Nominal Concentration of Calibration Standard Solutions (ng/mL)

Compound Name	Typical Concentration of Calibration Standards (ng/mL)						
	Level A	Level B	Level C	Level D (Mid-Level)	Level E	Level F	Level G
Triclosan	5	7.5	10	20	50	100	500
Methyl triclosan	0.5	1.0	5	50	500	1,000	5,000
Labeled Surrogates							
d_3-triclosan	100	100	100	100	100	100	100
$^{13}C_{12}$-methyl triclosan	60	60	60	60	60	60	60
Recovery Standard							
$^{13}C_{12}$-PCB-52	50	50	50	50	50	50	50

4.0 QUANTIFICATION PROCEDURES

Calculations

Target concentrations are determined with respect to a labeled surrogate. Mean relative response factors (RRF), determined from either a multi-level initial calibration series or a mid-level calibration standard run at the beginning and end of the samples, are used to convert raw peak areas in sample chromatograms to final concentrations as follows:

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

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

and the Qt std is either the surrogate or the internal standard

Concentration results for target compounds are recovery corrected by the method of quantification. Surrogate recoveries are determined similarly against the recovery (internal) standard and are used as general indicators of overall analytical quality.

4.1 Reporting Limits

Sample specific detection limits (SDL's) are determined from the analysis data by converting the minimum detectable area to a concentration following the same procedures used to convert target peak responses to concentrations. The estimated minimum detectable area is determined as three times the height of the noise in the m/z channel of interest, converted to an area using the area height ratio of the corresponding labeled surrogate peak.

Analyte Ions Monitored, and Surrogates Used

Compound Name	Quantified against	Typical Retention time (min)	Mass 1	Mass 2	Mass 1 / Mass 2 ratio	Ion Ratio Tolerance (+/- %)
Authentics						
Triclosan	D_3-triclosan	26.53	287.9512	289.9483	1.03	20
Methyl Triclosan	$^{13}C_{12}$-methyl triclosan	25.8	301.9668	303.964	1.03	20
Surrogates						
d_3-triclosan	$^{13}C_{12}$-PCB-52	26.5	290.9700	292.9672	1.03	20
$^{13}C_{12}$-methyl triclosan	$^{13}C_{12}$-PCB-52	25.78	314.0071	316.0042	1.04	20
Recovery						
$^{13}C_{12}$-PCB-52		23.18	301.9626	303.9597	0.78	20
Lock Mass			318.9792			

5.0 QUALITY ACCEPTANCE CRITERIA

Samples are analyzed in batches consisting of a maximum of twenty samples, one procedural blank and one spiked matrix (OPR) sample. A duplicate is analyzed, provided there is sufficient sample, with batches containing 7-20 samples. 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.

QC Acceptance Criteria [1]

	Typical Detection Limits (ng/g)	Procedural Blank Level (ng/g)	Tissue (% Recovery)
Native Analytes			**OPRs**
Triclosan	0.2 ng/g	0.2 ng/g	70 - 130
Methyl Triclosan	0.02 ng/g	0.02 ng/g	70 - 130
Surrogate Standards			**SARs, OPRs and Samples**
d_3-triclosan			30 - 130
$^{13}C_{12}$-methyl triclosan			50 - 130
Typical Sample Size:	5 g		
Typical final vol., µL	200		

[1] The QC acceptance criteria are based on limited data. Limit exceedance may be accepted based on application and professional judgment.

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QC Parameter	Specification
Analysis Duplicate	The relative difference must be ≤40%, i.e., the duplicates must agree to within ±20% of the mean (applicable to concentrations >10 times the DL).
Procedural Blank	See above or <10% of analyte value.
Matrix Spike Recovery	See above.
GC Resolution	No criteria other than good peak shape.
Instrument Sensitivity	S/N ratio ≥5:1 for 5 pg injection of triclosan and 0.5 pg injection of methyl triclosan.
Instrument Linearity	Linearity is determined by at least a 5-point calibration with a relative standard deviation of the RRFs ≤20% for targets and ≤35% for all labelled compounds.
RRF: Bracketing Cal	RRFs for the opening and closing calibration standards over a 12 hour period must agree to within ±25% of the mean, i.e., ≤ 50 RPD. Note that 50 RPD is equivalent to 35.4% RSD.
RRF Continuing CAL VER	RRFs from opening/closing Cal Vers must be within ±20% of the mean RRFs from the initial calibration for all targets. RRFs from opening Cal Vers must be within ±35% of the mean RRFs from the initial calibration for all labelled compounds. RRFs from closing Cal Vers must be within ±50% of the mean RRFs from the initial calibration for all labelled compounds.
Analyte/Surrogate Ratios	Response must be within the calibrated range of the instrument. IA Chemists may use data from more than one chromatogram to get the responses in the calibrated range.
Ion Ratios	Ion ratios for all target and surrogate compounds must fall within ±20% (±30% for Level A CAL, if fail, A2-CAL is used and must be within ±20%) of the theoretical values for all of targets and surrogates.
Retention Times	The relative retention time of each analyte must be within two seconds of that predicted from the daily calibration runs and the surrogate standard retention times.

Appendix B: Summary of King County Field Staff Duties

The outline below is a summary of roles and responsibilities for King County Toxicology and Contaminant Assessment (TCA) Unit staff and KCEL and SGS AXYS laboratory staff and the order of events regarding this project. Unless otherwise noted, TCA staff will follow all protocols laid out in the respective freshwater and marine tissue monitoring programs (King County 2015b; King County 2017, and addenda).

1. Preparing for field activities:
    a. Obtain fish collection permits from NOAA and WDFW.
    b. Schedule sampling vessel and/or coordinate date(s) for sampling with WDFW.
    c. Obtain LIMS fish ID numbers from KCEL and create labels for the fish sample containers – including extra for backup fish.
    d. Print datasheet forms in Appendix D on rite-in-rain (or equivalent) paper
    e. Coordinate with WDFW staff and/or fishing vessel staff to obtain sample fish:
        i. Alert KCEL staff of expected date that fish will arrive there for freezing and immediate shipment, and need for dry ice.
        ii. Provide list of desired fish, datasheets, labeled bags, pre-cut PFAS-free foil, sample packaging instructions, and cooler with ice to WDFW for collection of rockfish at the Pier 62 location.
    f. Coordinate with KCEL staff to ship samples:
        i. Alert KCEL regarding dates of sampling for receiving fish.
        ii. Fill out, or send KCEL instructions for filling out, SGS AXYS COC form for all samples.
        iii. Request KCEL to send one 4oz clear wide-mouth sample jar with polypropylene lid per fish (plus a few extra) to SGS AXYS for return of fish homogenate aliquots for KCEL analysis of mercury, metals and solids analysis.
2. Fish collection and storage:
    a. Freshwater fish collector:
        i. June 2021 - Obtain fish that have had gastric lavage from WDFW staff:
            1. Fill out “Freshwater Fish - Lake Union Tissue Monitoring Event Datasheet” (Appendix D).
            2. Select up to 18 largemouth bass (preferred) or smallmouth bass of appropriate size (>200 mm fork length) for this study.
                a. 9 will be for shipping and 9 for backup
            3. Fill out top of “Freshwater Fish - Lake Union Sample Processing Datasheet” (Appendix D) and do the following:

a. Assign each fish a fish ID.
b. Measure and record fork and total length and weight of each fish.

4. Wrap each fish in PFAS-free foil and place into individual polyethylene (e.g., Ziploc®) bags pre-labeled with fish IDs
5. Record date and location of capture and species on bag label and on field forms or in the field notebook, accompanied by the fish ID
6. Immediately transport bagged fish in coolers on wet ice to KCEL lab for freezing and shipping to SGS AXYS
7. Fill out COC form (Appendix D) when fish are handed over to KCEL

b. Marine fish - two sampling dates anticipated, preference is for fish from Pier 62 then Myrtle Edwards:
   i. May 20, 2021 - Obtain rockfish fish from Pier 62 from WDFW staff
   ii. June 2021 - Obtain fish from Myrtle Edwards and Alki stations during routine King County monitoring efforts, use these to supplement the rockfish from Pier 62 until 18 whole fish samples are obtained.
   iii. Once fish are obtained perform the following:
      1. Fill out "Marine Fish - Trawl Record Datasheet" (Appendix D)
      2. Select up to 18 brown, quillback or copper rockfish with the largest (regardless of species) preferred, or English sole (if rockfish are not available) of appropriate size:
         a. >100 mm total length for rockfish
         b. >230 mm total length for English sole
      3. Fill out top of "Marine Fish - Sample Processing Datasheet" (Appendix E) and do the following:
         a. Assign each fish a Fish ID
         b. Measure and record total length of each fish
      4. Wrap each fish in PFAS-free foil and place into individual bags pre-labeled with matching fish IDs
      5. Record date and location of capture and species on bag label and on field forms, accompanied by the fish ID
      6. Immediately transport collected fish to KCEL lab for freezing and shipping to SGS AXYS
      7. Fill out SGS AXYS COC form (Appendix D) when fish are handed over to KCEL.

Appendix C: Summary of KCEL and SGS AXYS Lab Duties

KCEL staff

1. Send sample jars to SGS AXYS.
    a. Coordinate with TCA staff to ship 4 oz clear wide-mouth sample jar with polypropylene lids to SGS AXYS for return of fish homogenate samples for mercury, metals and solids analysis at KCEL.
2. Send fish to SGS AXYS.
    a. Obtain pre-labeled, bagged fish from TCA staff for shipping to SGS AXYS - do not open bags or unpack fish.
    b. Freeze and maintain all fish samples at -18° C.
    c. Note and separate bags of fish that are to be shipped immediately to SGS AXYS and those to be held as back-up.
        i. Backup fish will only be used if the first shipment of fish fails to arrive at SGS AXYS in proper condition (i.e. still frozen to 4° C).
    d. Shipping fish to SGS AXYS:
        i. Work with Principal Investigator (Jennifer Lanksbury) to fill out SGS AXYS COC form (Appendix D), including which marine and freshwater samples are to be shipped back as homogenate aliquots from SGS AXYS.
        ii. Ship via fish overnight delivery service on the first available Monday or Tuesday after they arrive at KCEL, to ensure arrival at the SGS AXYS lab during a weekday.
            1. Fish should arrive at SGS AXYS within 7 days of collection.
        iii. Pack frozen fish in approximately 25 lbs of dry ice per 40 qt cooler – this is the amount recommended by SGS AXYS to keep fish at proper temperature for an estimated 2-day shipping time.
        iv. Include SGS AXYS COC form (Appendix D) in shipment.
        v. Notify TCA staff when shipment has been sent to SGS AXYS.
            1. If shipment fails for any reason, coordinate with TCA staff to send backup fish.
3. Fish homogenate aliquot samples back from SGS AXYS:
    a. Coordinate with TCA and SGS AXYS staff on return shipment of frozen homogenate aliquots for analysis. Samples should be packed with approximately 25 lbs of dry ice per 40 qt cooler.

SGS AXYS staff

1. Notify Principal Investigator (Jennifer Lanksbury) each time fish arrive and whether they are in acceptable condition for analysis.
2. Process freshwater and marine whole fish as outlined below:
    a. 9 freshwater fish = 9 homogenate samples for analysis
        i. Species will be largemouth bass or smallmouth bass
        ii. Homogenize whole fish for analysis
    b. 9 marine fish = 9 homogenate samples for analysis.
        i. Species will be Brown, Quillback, or Copper rockfish, or English sole
        ii. Weigh (to nearest gram) and record weight of each fish
            1. TCA staff cannot weigh these fish in the field before they are packaged and frozen, so SGS AXYS is asked to provide this data
        iii. Dissect out ONLY the stomachs of each fish (marine fish only), record the weight, and discard
        iv. Homogenize whole fish (minus stomachs) for analysis
3. Analyze homogenized samples for the following classes of compounds:
    a. SGS AXYS Method MLA080 (LC-MS/MS) – Nonylphenols
        i. Analyze all 9 freshwater fish samples
        ii. Analyze all 9 marine fish samples
    b. SGS AXYS Method MLA115 (GC/HRMS) – Triclosan
        i. Analyze all 9 freshwater fish samples
        ii. Analyze all 9 marine fish samples
    c. SGS AXYS Method MLA075 (LC-MS/MS) – PPCPs Lists 1 - 6
        i. Analyze all 9 freshwater fish samples
        ii. Analyze 8 marine fish samples, use the 9th sample for an extra MS/MSD pair for List 1 and List 2 PPCPs
4. Create 2 aliquots each of homogenate from those marine and freshwater fish specified on the COC forms, to be sent back to KCEL:
    a. Aliquots from all but one of the specified fish will be prepared as follows:
        i. 1 aliquot in PFAS-free jar (provided by SGS AXYS) with at least 60 g tissue for organic chemistry analysis.
        ii. 1 aliquot in 4 oz clear glass wide mouth jar (provided by KCEL) with at least 32 g tissue for metals and mercury analysis.
    b. Aliquots from 1 of the specified fish will require more tissue for QA/QC purposes and will be prepared as follows:
        i. 1 aliquot in PFAS-free jar (provided by SGS AXYS) with at least 300 g tissue for OC analysis

ii. 1 aliquot in 4 oz glass jar (provided by KCEL) with at least 32 g tissue for metals and mercury analysis

c. Ship frozen aliquots back to KCEL via overnight delivery service.

Appendix D: Datasheets and Chain of Custody (COC) Forms

SGS AXYS
2045 Mills Road West
Sidney, British Columbia, Canada, V8L 5X2
TEL: (250) 655-5800
FAX: (250) 655-5811

CHAIN OF CUSTODY

SGS AXYS CLIENT #:

Please note Canadian soils and freshwater sediments MAY require a CFIA Movement Certificate prior to shipping. Please call your local CFIA agent to know whether one is required. CFIA contact information is available at: http://www.inspection.gc.ca/english/directory/offbure.shtml
Shipments including soils and freshwater sediments from outside of Canada are required to include SGS AXYS's current soil importation permit.
Your SGS AXYS Project Manager will be pleased to assist you.

REPORT TO:	*INVOICE TO:*	ANALYSIS REQUESTED
Company	Company	
Address	Address	
Contact	Contact	
Phone	Phone	
FAX	FAX	
E-mail	E-mail	
Project Name/Number:	Sampler's Name: Signature:	

Client Sample Identification	Matrix	Sampling Date	Sampling Time	Container Type/No.	SGS AXYS Lab Sample ID (Lab use only)					

Relinquished by (Signature) Date Time	Received by (Signature) Date Time	Courier	Waybill No.
Relinquished by (Signature) Date Time	Received by (Signature) Date Time	Cooler Sample Receipt	
		Temp °C	
		Custody Seal #	
		Seal Intact Y / N	
		Sample Tags Y / N	

SGS AXYS Analytical Services Ltd

CHAIN OF CUSTODY RECORD (cont'd)

Sample IDs____

Analysis_____________________

Relinquished by: (print) / (sign)	Date/Time/Location	Received by: (print) / (sign)
Relinquished by: (print) (sign)	Date/Time/Location	Received by: (print) (sign)
Relinquished by: (print) (sign)	Date/Time/Location	Received by: (print) (sign)
Relinquished by: (print) (sign)	Date/Time/Location	Received by: (print) (sign)
Relinquished by: (print) (sign)	Date/Time/Location	Received by: (print) (sign)
Relinquished by: (print) (sign)	Date/Time/Location	Received by: (print) (sign)
Relinquished by: (print) (sign)	Date/Time/Location	Received by: (print) (sign)

INSTRUMENT DEPARTMENT CHAIN OF CUSTODY

Analysis_____

WG	SAMPLE ID	DATE	TIME	RELEASED FROM	RELEASED TO

Marine Fish - Trawl Record Datasheet

Date of Collection: _____
Approximate Time: _____
Station Name (Trawl Location): _____
Vessel: _____

Trawl Start Coordinates: _____
Trawl End Coordinates: _____

Species	# Taken	# Returned	Observations[1]

[1] Includes general disposition of species

Notes:
_____
_____
_____
_____
_____
_____
_____
_____

Field Personnel/Agency Affiliation:_____

Marine Fish - Sample Processing Datasheet

Sample Date: _____
Station Name/Locator: _____
Recorder: _____

Fish ID (LIMS ID)	Species	Total Length (mm)	Comment

Fish ID (LIMS ID)	Species	Total Length (mm)	Comment

Notes:

_____
_____
_____
_____
_____
_____
_____

Field Personnel/Agency Affiliation:_____

Freshwater Fish - Lake Union Tissue Monitoring Event Datasheet

Date of Collection: _____
Approximate Time: _____
Zone: _____
Equipment: _____

Coordinates: _____

Species	# Taken	# Returned	Observations[1]

[1] Includes general disposition of species

Notes:
_____
_____
_____
_____
_____
_____
_____
_____

Field Personnel/Agency Affiliation:_____

Freshwater Fish - Lake Union Sample Processing Datasheet

Sample Date: _____
Station Name/Locator: _____
Recorder: _____

Fish ID (LIMS ID)	Species	Total Length (mm)	Fork Length (mm)	Mass (g)	Comment

Fish ID (LIMS ID)	Species	Total Length (mm)	Fork Length (mm)	Mass (g)	Comment

Notes:

_____
_____
_____
_____
_____
_____

Field Personnel/Agency Affiliation:_____

Appendix E. Glossaries, Acronyms, and Abbreviations

GLOSSARY OF GENERAL TERMS

Ambient: Background or away from point sources of contamination. Surrounding environmental condition.

Anthropogenic: Human-caused.

Combined Sewer Overflow (CSO): The discharge from a combined sewer system that is caused by stormwater runoff. Combined Sewers are sewer systems that collect stormwater runoff, domestic sewage, and industrial wastewater in the same pipe and bring it to the wastewater treatment facility.

Composite: Sample made up of tissue from more than one fish.

Contaminant of Emerging Concern: A term used by water quality professionals to describe pollutants detected in water bodies that may cause human health or ecological impacts and typically are not regulated under current environmental laws.

Effluent: An outflowing of water from a natural body of water or from a human-made structure. For example, the treated outflow from a wastewater treatment plant.

Gastric lavage: Also commonly called stomach pumping or gastric irrigation, it is the process of cleaning out the contents of the stomach.

Homogenate: A suspension of cell constituents or fragments obtained when tissue is homogenized (i.e. ground up or blended).

Stormwater: The portion of precipitation that does not naturally percolate into the ground or evaporate but instead runs off roads, pavement, and roofs during rainfall or snow melt. Stormwater can also come from hard or saturated grass surfaces such as lawns, pastures, playfields, and from gravel roads and parking lots.

Waste Water Treatment Plant (WWTP): A location where wastewater or sewage is processed to remove contaminants and convert it into an effluent that can be reused for various purposes or returned to the water cycle with acceptable impact on the environment.

ACRONYMS AND ABBREVIATIONS

CEC	Contaminant of emerging concern
COC	Chain of custody
CSO	Combined sewer overflow
CTR	Critical tissue residue
DDT	Dichlorodiphenyltrichloroethane
e.g.	For example
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
ESA	Endangered Species Act
et al.	And others
FEATS	Financial and Ecosystem Accounting Tracking System of EPA
GIS	Geographic Information System software
GPS	Global Positioning System
HPLC-MS/MS	High-performance liquid chromatography, tandem mass spectrometry
i.e.	In other words
KCEL	King County Environmental Laboratory
LC-MS/MS	Liquid chromatography and tandem mass spectrometry
LIMS	Laboratory Information Management System of KCEL
MDL	Method detection limit
MQO	Measurement quality objective
MS/MSD	Matrix spike/matrix spike duplicate
NP	nonylphenol
NPE	nonylphenol ethoxylate
NTA	Puget Sound Partnership's near term action
OP	Octylphenol
OPR	Ongoing precision and recovery
PBDE	Polybrominated diphenyl ethers
PBT	Persistent, bioaccumulative, and toxic substance
PCB	Polychlorinated biphenyls
PCP	Personal care product
PFAS	Per- and polyfluoroalkyl substances
PPCP	Pharmaceuticals and personal care products
QA	Quality assurance
QC	Quality control
RL	Reporting limit
RPD	Relative percent difference

SAP	Sampling and Analysis Plan
SGS AXYS	SGS AXYS Analytical Services Ltd.
SOP	Standard operating procedures
SOW	Scope of Work
SRM	Standard reference materials
SSRI	Selective serotonin reuptake inhibitor
WDFW	Washington Department of Fish and Wildlife
WQX	Water Quality Exchange of EPA
WRIA	Water Resource Inventory Area
WSTMP	Washington State Toxics Monitoring Program
WWTP	Wastewater treatment plant

UNITS OF MEASUREMENT

°C	degrees centigrade
dw	dry weight
ft	feet
g	gram, a unit of mass
kg	kilograms, a unit of mass equal to 1,000 grams
km	kilometer, a unit of length equal to 1,000 meters
m	meter
mm	millimeter
mg	milligram
mgd	million gallons per day
mg/d	milligrams per day
mg/kg	milligrams per kilogram (parts per million)
mg/L	milligrams per liter (parts per million)
mL	milliliter
mmol	millimole or one-thousandth of a mole
mole	an International System of Units (IS) unit of matter
ng/g	nanograms per gram (parts per billion)
ng/kg	nanograms per kilogram (parts per trillion)
ng/L	nanograms per liter (parts per trillion)
pg/g	picograms per gram (parts per trillion)
pg/L	picograms per liter (parts per quadrillion)
µg/g	micrograms per gram (parts per million)
µg/kg	micrograms per kilogram (parts per billion)
µg/L	micrograms per liter (parts per billion)
ww	wet weight

QUALITY ASSURANCE GLOSSARY

Accreditation: A certification process for laboratories, designed to evaluate and document a lab's ability to perform analytical methods and produce acceptable data (Kammin, 2010). For Ecology, it is defined according to WAC 173-50-040: "Formal recognition by [Ecology] that an environmental laboratory is capable of producing accurate and defensible analytical data."

Accuracy: The degree to which a measured value agrees with the true value of the measured property. USEPA recommends that this term not be used, and that the terms *precision* and *bias* be used to convey the information associated with the term *accuracy* (USEPA, 2014).

Analyte: An element, ion, compound, or chemical moiety (pH, alkalinity) which is to be determined. The definition can be expanded to include organisms, e.g., fecal coliform, Klebsiella (Kammin, 2010).

Bias: Discrepancy between the expected value of an estimator and the population parameter being estimated (Gilbert, 1987; USEPA, 2014).

Blank: A synthetic sample, free of the analyte(s) of interest. For example, in water analysis, pure water is used for the blank. In chemical analysis, a blank is used to estimate the analytical response to all factors other than the analyte in the sample. In general, blanks are used to assess possible contamination or inadvertent introduction of analyte during various stages of the sampling and analytical process (USGS, 1998).

Calibration: The process of establishing the relationship between the response of a measurement system and the concentration of the parameter being measured (Ecology, 2004).

Check standard: A substance or reference material obtained from a source independent from the source of the calibration standard; used to assess bias for an analytical method. This is an obsolete term, and its use is highly discouraged. See Calibration Verification Standards, Lab Control Samples (LCS), Certified Reference Materials (CRM), and/or spiked blanks. These are all check standards but should be referred to by their actual designator, e.g., CRM, LCS (Kammin, 2010; Ecology, 2004).

Comparability: The degree to which different methods, data sets and/or decisions agree or can be represented as similar; a data quality indicator (USEPA, 2014; USEPA, 2020).

Completeness: The amount of valid data obtained from a project compared to the planned amount. Usually expressed as a percentage. A data quality indicator (USEPA, 2014; USEPA 2020).

Continuing Calibration Verification Standard (CCV): A quality control (QC) sample analyzed with samples to check for acceptable bias in the measurement system. The CCV is usually a midpoint calibration standard that is re-run at an established frequency during the course of an analytical run (Kammin, 2010).

Control chart: A graphical representation of quality control results demonstrating the performance of an aspect of a measurement system (Kammin, 2010; Ecology 2004).

Control limits: Statistical warning and action limits calculated based on control charts. Warning limits are generally set at +/- 2 standard deviations from the mean, action limits at +/- 3 standard deviations from the mean (Kammin, 2010).

Data integrity: A qualitative DQI that evaluates the extent to which a data set contains data that is misrepresented, falsified, or deliberately misleading (Kammin, 2010).

Data quality indicators (DQI): Commonly used measures of acceptability for environmental data. The principal DQIs are precision, bias, representativeness, comparability, completeness, sensitivity, and integrity (USEPA, 2006).

Data quality objectives (DQO): Qualitative and quantitative statements derived from systematic planning processes that clarify study objectives, define the appropriate type of data, and specify tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions (USEPA, 2006).

Data set: A grouping of samples organized by date, time, analyte, etc. (Kammin, 2010).

Data validation: The process of determining that the data satisfy the requirements as defined by the data user (USEPA, 2020). There are various levels of data validation (USEPA, 2009).

Data verification: Examination of a data set for errors or omissions, and assessment of the Data Quality Indicators related to that data set for compliance with acceptance criteria (MQOs). Verification is a detailed quality review of a data set (Ecology, 2004).

Detection limit (limit of detection)**:** The concentration or amount of an analyte which can be determined to a specified level of certainty to be greater than zero (Ecology, 2004).

Duplicate samples: Two samples taken from and representative of the same population, and carried through and steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variability of all method activities including sampling and analysis (USEPA, 2014).

Field blank: A blank used to obtain information on contamination introduced during sample collection, storage, and transport (Ecology, 2004).

Initial Calibration Verification Standard (ICV): A QC sample prepared independently of calibration standards and analyzed along with the samples to check for acceptable bias in the measurement system. The ICV is analyzed prior to the analysis of any samples (Kammin, 2010).

Laboratory Control Sample (LCS)/LCS duplicate: A sample of known composition prepared using contaminant-free water or an inert solid that is spiked with analytes of interest at the midpoint of the calibration curve or at the level of concern. It is prepared and analyzed in the same batch of regular samples using the same sample preparation method, reagents, and analytical methods employed for regular samples. Monitors a lab's performance for bias and precision (USEPA, 2014).

Matrix spike/Matrix spike duplicate: A QC sample prepared by adding a known amount of the target analyte(s) to an aliquot of a sample to check for bias and precision errors due to interference or matrix effects (Ecology, 2004).

Measurement Quality Objectives (MQOs)**:** Performance or acceptance criteria for individual data quality indicators, usually including precision, bias, sensitivity, completeness, comparability, and representativeness (USEPA, 2006).

Measurement result: A value obtained by performing the procedure described in a method (Ecology, 2004).

Method: A formalized group of procedures and techniques for performing an activity (e.g., sampling, chemical analysis, data analysis), systematically presented in the order in which they are to be executed (USEPA, 2001).

Method blank: A blank prepared to represent the sample matrix, prepared and analyzed with a batch of samples. A method blank will contain all reagents used in the preparation of a sample, and the same preparation process is used for the method blank and samples (Ecology, 2004; Kammin, 2010).

Method Detection Limit (MDL): The minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results (USEPA, 2016). MDL is a measure of the capability of an analytical method of distinguished samples that do not contain a specific analyte from a sample that contains a low concentration of the analyte (USEPA, 2020).

Minimum level: Either the sample concentration equivalent to the lowest calibration point in a method or a multiple of the method detection limit (MDL), whichever is higher. For the purposes of NPDES compliance monitoring, EPA considers the following terms to be synonymous: “quantitation limit,” “reporting limit,” and “minimum level” (40 CFR 136).

Ongoing Precision and Recovery: A test to ensure that the laboratory controls are in effect throughout the period that the samples are analyzed, separating laboratory performance from method performance with the sample matrix. The test consists of a single aliquot of reference material spiked with the analyte of interest and carried through the entire analytical process with each sample batch. Specifications for the permissible range of recovery are defined (USEPA, 1999).

Parameter: A specified characteristic of a population or sample. Also, an analyte or grouping of analytes. Benzene and nitrate + nitrite are all parameters (Kammin, 2010; Ecology, 2004).

Population: The hypothetical set of all possible observations of the type being investigated (Ecology, 2004).

Precision: The extent of random variability among replicate measurements of the same property; a data quality indicator (USGS, 1998).

Quality assurance (QA): A set of activities designed to establish and document the reliability and usability of measurement data (Kammin, 2010).

Quality Assurance Project Plan (QAPP): A document that describes the objectives of a project, and the processes and activities necessary to develop data that will support those objectives (Kammin, 2010; Ecology, 2004).

Quality control (QC): The routine application of measurement and statistical procedures to assess the accuracy of measurement data (Ecology, 2004).

Relative Percent Difference (RPD): RPD is commonly used to evaluate precision. The following formula is used:

$$\text{RPD} = [\text{Abs}(a\text{-}b)/((a + b)/2)] * 100\%$$

where "Abs()" is absolute value and a and b are results for the two replicate samples. RPD can be used only with 2 values. Percent Relative Standard Deviation is (%RSD) is used if there are results for more than 2 replicate samples (Ecology, 2004).

Relative Standard Deviation (RSD): A statistic used to evaluate precision in environmental analysis. It is determined in the following manner:

$$\text{RSD} = (100\% * s)/x$$

where s is the sample standard deviation and x is the mean of results from more than two replicate samples (Kammin, 2010).

Replicate samples: Two or more samples taken from the environment at the same time and place, using the same protocols. Replicates are used to estimate the random variability of the material sampled (USGS, 1998).

Reporting level: Unless specified otherwise by a regulatory authority or in a discharge permit, results for analytes that meet the identification criteria (i.e., rules for determining qualitative presence/absence of an analyte) are reported down to the concentration of the minimum level established by the laboratory through calibration of the instrument. EPA considers the terms "reporting limit," "quantitation limit," and "minimum level" to be synonymous (40 CFR 136).

Representativeness: The degree to which a sample reflects the population from which it is taken; a data quality indicator (USGS, 1998).

Sample (field): A portion of a population (environmental entity) that is measured and assumed to represent the entire population (USGS, 1998).

Sample (statistical): A finite part or subset of a statistical population (USEPA, 1992).

Sensitivity: In general, denotes the rate at which the analytical response (e.g., absorbance, volume, meter reading) varies with the concentration of the parameter being determined. In a specialized sense, it has the same meaning as the detection limit (Ecology, 2004).

Spiked blank: A specified amount of reagent blank fortified with a known mass of the target analyte(s); usually used to assess the recovery efficiency of the method (USEPA, 2014).

Spiked sample: A sample prepared by adding a known mass of target analyte(s) to a specified amount of matrix sample for which an independent estimate of target analyte(s) concentration is available. Spiked samples can be used to determine the effect of the matrix on a method's recovery efficiency (USEPA, 2014).

Split sample: A discrete sample subdivided into portions, usually duplicates (Kammin, 2010).

Standard Operating Procedure (SOP): A document which describes in detail a reproducible and repeatable organized activity (Kammin, 2010).

Surrogate: For environmental chemistry, a surrogate is a substance with properties similar to those of the target analyte(s). Surrogates are unlikely to be native to environmental samples. They are added to environmental samples for quality control purposes, to track extraction efficiency and/or measure analyte recovery. Deuterated organic compounds are examples of surrogates commonly used in organic compound analysis (Kammin, 2010).

Systematic planning: A step-wise process which develops a clear description of the goals and objectives of a project, and produces decisions on the type, quantity, and quality of data that will be needed to meet those goals and objectives. The DQO process is a specialized type of systematic planning (USEPA, 2006).

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