Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS

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Introduction

EPA Method 1694 determines pharmaceuticals and personal care products (PPCPs) in environmental samples by high performance liquid chromatography combined with tandem mass spectrometry (HPLC/MS/MS) using isotope dilution and internal standard quantitation techniques. This method has been developed for use with aqueous, solid, and biosolids matrices.

Disclaimer

This method has been reviewed by the Engineering and Analytical Support Branch of the Engineering and Analysis Division (EAD) in OST. The method is available for general use, but has not been published in 40 CFR Part 136. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Contacts

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Table of Contents

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1.0 Scope and Application

1.1 Method 1694 is for determination of pharmaceuticals and personal care products (PPCPs) in multi-media environmental samples by high performance liquid chromatography combined with tandem mass spectrometry (HPLC/MS/MS).

1.2 This method was developed for use in Clean Water Act (CWA) programs; other applications are possible. It is based on existing EPA methods (Reference 1) and procedures developed at Axys Analytical Services (Reference 2) as well as previous work on pharmaceuticals and personal care products (Reference 3).

1.3 The target analytes and their corresponding Chemical Abstracts Service Registry Numbers (CASRNs) are listed in Table 1.

1.4 The detection limits and quantitation levels in this method are usually dependent on the level of interferences rather than instrumental limitations. The method detection limits (MDLs; 40 CFR 136, appendix B) and minimum levels of quantitation (MLs; 68 FR 11790) in Tables 3, 5, 7, and 9 are the levels at which the analytes can be determined in the absence of interferences.

1.5 This method is restricted to use by or under the supervision of analysts experienced in LC/MS/MS or under the close supervision of such qualified persons. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 9.2.

1.6 This method is performance-based which means that you may modify the method to improve performance (e.g., to overcome interferences or improve the accuracy or precision of the results) provided that you meet all performance requirements in this method. These requirements for establishing equivalency of a modification are in Section 9.1.2. For Clean Water Act (CWA) uses, additional flexibility is described at 40 CFR 136.6. Modifications that are not within the scope of Part 136.6, or in Section 9 of this method may require prior review and approval.

1.7 Some of the compounds in this method are controlled substances. Laboratories performing this method should have all appropriate licenses and certifications and obtain all needed standards and chemicals from licensed sources. For some of the compounds in this method it may be necessary for laboratories to obtain a DEA license.

2.0 Summary of Method

The target analytes in this method are divided into four groups (1 through 4). Each group represents an LC/MS/MS run, as detailed in Tables 2 to 9 in Section 23. Tables 2 and 3 are specific to Group 1. Tables 4 and 5 are specific to Group 2. Tables 6 and 7 are specific to Group 3. Tables 8 and 9 are specific to Group 4.

Groups 1, 2, and 3 are extracted under acidic (pH 2) conditions. Groups 1 and 2 are run in the positive electrospray ionization (ESI+) mode and Group 3 is run in the negative electrospray ionization (ESI-) mode. Group 4 is extracted under basic (pH 10) conditions and is run in the ESI+ mode. Group 3 is specific to the tetracyclines.

The general steps in this method are summarized in Section 2.1 to 2.7. A flow chart that summarizes procedures for sample preparation, cleanup, and analysis is shown in Figure 1.

2.1 Aqueous samples absent visible particles and filtrate from samples with visible particles – The pH of a 1-L sample aliquot is adjusted to 2 with acid. The pH of a second 1-L aliquot of sample is adjusted with 10 with base. Stable, isotopically labeled analogs of the analytes of interest are spiked into their respective acid or base fraction. The acid fraction is stabilized with tetrasodium ethylenediamine-tetraacetate dihydrate (NA4EDTA.2H2O•2H_2O).

2.2 Solid and semi-solid samples, including biosolids and visible particles from aqueous samples – A phosphate buffer and an ammonium hydroxide solution are used to adjust the pH, respectively, of up to 1 g each of dry solids from a solid sample, or 1 g each of dry solids filtered from an aqueous sample. The labeled compounds are spiked into their respective acid and base fractions. The acid fraction is ultrasonically extracted three times with a phosphate buffer/acetonitrile solution and the base fraction is ultrasonically extracted three times with a ammonium hydroxide/acetonitrile solution. The solutions are concentrated to remove the acetonitrile and diluted with reagent water. The acid fraction is stabilized with NA4EDTA.2H2O•2H_2O.

2.3 Sample cleanup – The acid and base fraction solutions are separately cleaned up using solid-phase extraction (SPE) with hydrophilic-lipophillic balance (HLB) cartridges. After cleanup, the fractions are exchanged to methanol, labeled injection internal standards are added, and the final volume is adjusted to 4 mL with the LC elution solvent.

2.4 Determination by LC/MS/MS – The acid extract is analyzed in two positive electrospray ionization (ESI+) LC/MS/MS runs and one negative electrospray ionization (ESI-) run, each specific to a subset of the analytes of interest. The base extract is analyzed in a single ESI+ run. The analytes are separated by the LC and detected by a tandem (1000 resolution) mass spectrometer. A daughter m/z for each compound is monitored throughout a pre-determined retention time window.

2.5 An individual compound is identified by comparing the LC retention time and presence of the daughter m/z with the corresponding retention time and daughter m/z of an authentic standard.

2.6 Quantitative analysis is performed in one of two ways, using selected ion current profile (SICP) areas:

2.6.1 For a compound for which a labeled analog is available, the concentration is determined using the isotope dilution technique and a multipoint calibration of all the target analytes. Isotope dilution provides automatic correction of the target analyte concentrations.

2.6.2 For a compound for which a labeled analog is not available, the concentration is determined using the internal standard technique and a multipoint calibration of all the target analytes. The labeled compounds are used to recovery correct results of those analytes quantitated by the internal standard technique.

2.6.3 Additional labeled compounds may be incorporated into this method, at the user's discretion to determine the concentration of the native compound using the isotope dilution technique provided that all performance requirements in this method are met. Requirements for establishing equivalency are given in Section 9.1.2, and additionally for CWA uses, at 40 CFR 136.6.

2.7 The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and LC/MS/MS systems.

3.0 Definitions and Units of Measure

Definitions and units of measure are given in the glossary at the end of this method.

4.0 Interferences

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts, elevated baselines, matrix enhancement or matrix suppression causing misinterpretation of chromatograms. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Where possible, reagents are cleaned by extraction or solvent rinse.

4.2 Proper cleaning of glassware is extremely important, because glassware may not only contaminate the samples but may also remove the analytes of interest by adsorption on the glass surface.

4.2.1 Glassware should be rinsed with solvent and washed with a detergent solution as soon after use as is practical. Sonication of glassware containing a detergent solution for approximately 30 seconds may aid in cleaning. Glassware with removable parts, particularly separatory funnels with fluoropolymer stopcocks, must be disassembled prior to detergent washing.

4.2.2 After detergent washing, glassware should be rinsed immediately, first with methanol, then with hot tap water. The tap water rinse is followed by another

methanol rinse, then acetone, and then methylene chloride.

4.2.3 Baking of glassware in a kiln or other high temperature furnace (300 – 500 EC) may be useful after particularly dirty samples are encountered. The kiln or furnace should be vented to prevent laboratory contamination by vapors. Baking should be minimized, as repeated baking of glassware may cause active sites on the glass surface that may irreversibly adsorb the compounds of interest. Volumetric ware should not be baked at high temperature.

4.2.4 After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with solvent rinsed aluminum foil.

4.3 All materials used in the analysis must be demonstrated to be free from interferences by running reference matrix method blanks (Section 9.5) initially and with each sample batch (samples started through the extraction process on a given 12-hour shift, to a maximum of 20 samples).

4.3.1 The reference matrix must simulate, as closely as possible, the sample matrix under test. Ideally, the reference matrix should not contain the analytes of interest in detectable amounts, but should contain potential interferents in the concentrations expected to be found in the samples to be analyzed.

4.3.2 When a reference matrix that simulates the sample matrix under test is not available, reagent water (Section 7.6.1) can be used to simulate water samples; playground sand (Section 7.6.2) can be used to simulate soils; and peat moss (Section 7.6.3) can be used to simulate biosolids.

4.4 Interferences co-extracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds may be present at concentrations several orders of magnitude higher than the analytes of interest. Because low levels of PPCPs are measured by this method, elimination of interferences is essential. The cleanup steps given in Section 13 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the PPCPs at the levels shown in Tables 3, 5, 7, and 9.

4.5 It may be useful to number reusable glassware is to associate that glassware with the processing of a particular sample. This will assist the laboratory in tracking possible sources of contamination for individual samples, identifying glassware associated with highly contaminated samples that may require extra cleaning, and determining when glassware should be discarded.

4.6 Contamination from personal care products used by laboratory staff that are also target analytes is possible. Target analytes also include commonly used medications. Therefore, it is important to take precautions to avoid contamination of the samples, for example wearing of protective gloves and clothing (see Section 5).

5.0 Safety

The target analytes in this method have many beneficial uses as pharmaceuticals or over-the-counter products. While their safety is less of a concern than for many environmental contaminants, laboratory staff should avoid direct contact with samples and pure standards. General guidelines are provided below.

5.1 The toxicity or carcinogenicity of each chemical used in this method has not been precisely determined; however, each compound should be treated as a potential health hazard. Pure standards of the compounds should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks. It is recommended that the laboratory purchase dilute standard solutions of the analytes in this method. However, if primary solutions are prepared, they should be prepared in a hood, and a NIOSH/MESA approved toxic gas respirator may be necessary when high concentrations are handled

5.2 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should also be made available to all personnel involved in these analyses. It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this method and that the results of this monitoring be made available to the analyst. Additional information on laboratory safety can be found in References 4 – 7. The references and bibliography at the end of Reference 6 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 The pure PPCPs and samples suspected to contain high concentrations of these compounds should be handled with care.

5.3.1 Facility – When finely divided samples (dusts, soils, dry chemicals) are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak tight or in a fume hood demonstrated to have adequate air flow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in the case of an accident.

5.3.2 Protective equipment – Disposable plastic gloves (Latex or non-Latex (such as nitrile)), apron or lab coat, safety glasses or mask, and a glove box or fume hood should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection (preferably full face shields) should be worn while working with exposed samples or pure analytical standards. Latex or non-Latex (such as nitrile) gloves are commonly used to reduce exposure of the hands.

5.3.3 Training – Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene – Hands and forearms should be washed thoroughly after each operation involving high concentrations of the analytes of interest, and before breaks (coffee, lunch, and shift).

5.3.5 Confinement – Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Waste handling – Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel should be trained in the safe handling of waste. See Section 20 for additional information on waste handling and disposal.

5.4 Biosolids samples may contain high concentrations of biohazards, and must be handled with gloves and opened in a hood or biological safety cabinet to prevent exposure. Laboratory staff should know and observe the safety procedures required in a microbiology laboratory that handles pathogenic organisms when handling biosolids samples.

6.0 Equipment and Supplies

Note: *Brand names, suppliers, and part numbers are cited for illustration purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here. Demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 Sample bottles and caps

6.1.1 Liquid samples (waters, sludges and similar materials containing 5 percent solids or less) – Sample bottle, amber glass, 1 L minimum, with screw cap.

6.1.2 Solid samples (soil, sediment, sludge, filter cake, compost, and similar materials that contain more than 5 percent solids) – Sample bottle, wide mouth, amber glass, 500-mL minimum.

6.1.3 If amber bottles are not available, samples must be protected from light.

6.1.4 Bottle caps – Threaded to fit sample bottles. Caps must be lined with fluoropolymer.

6.1.5 Cleaning – Bottles are washed with detergent and water, then solvent rinsed before use. Liners are washed with detergent and water and rinsed with reagent water before use.

6.2 Compositing equipment – Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Only glass or fluoropolymer tubing must be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing

must be thoroughly rinsed with methanol, followed by repeated rinsing with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

6.3 Equipment for sample preparation

6.3.1 Laboratory fume hood of sufficient size to contain the sample preparation equipment listed below.

6.3.2 Glove box (optional)

6.3.3 Tissue homogenizer – VirTis Model 45 Macro homogenizer (American Scientific Products H-3515, or equivalent) with stainless steel Macro-shaft and Turbo-shear blade.

6.3.4 Vortex mixer

6.3.5 Ultrasonic mixer

6.3.6 Oven – Capable of maintaining a temperature of 110∀5 °C

6.3.7 Desiccator

6.3.8 Balance, analytical – Capable of weighing 0.1 mg

6.3.9 Balance, top loading – Capable of weighing 10 mg

6.4 Apparatus for measuring pH

6.4.1 pH meter, with combination glass electrode

6.4.2 pH paper, wide range (Hydrion Papers, or equivalent)

6.5 Apparatus for ultrasonic and solid-phase extraction

6.5.1 Sonic disrupter – 375 watt with pulsing capability and ½ or ¾ in. disrupter horn (Ultrasonics, Inc., Model 375, or equivalent)

6.5.2 Sonabox (or equivalent), for use with disrupter.

6.5.3 Vac-Elute Manifold (Analytichem International, or equivalent)

6.5.4 Vacuum trap: Made from 500-mL sidearm flask fitted with single-hole rubber stopper and glass tubing.

6.5.5 Vacuum source – Capable of maintaining 25 in. Hg, equipped with shutoff valve and vacuum gauge.

6.5.6 Rack for holding 50-mL volumetric flasks in the manifold.

6.5.7 SPE cartridge – Hydrophilic-Lipophilic-Balance (HLB) 60 mg, Waters Oasis, 20 cc/1 g LP, 60 µm, or equivalent, calibrated per the procedure in Section 10.6.

6.6 Filtration apparatus

6.6.1 Vacuum filtration apparatus – 1-L , including glass funnel, frit support, clamp, adapter, stopper, filtration flask, and vacuum tubing. For wastewater samples, the apparatus should accept 90- or 144-mm disks.

6.6.2 Glass-fiber filter – Whatman GMF 150 (or equivalent), 1 micron pore size, to fit the vacuum filtration apparatus.

6.6.3 Pressure filtration apparatus – Millipore YT30 142 HW, or equivalent.

6.6.4 Whatman GF/A (1.6 µm), or equivalent, differing diameters, to fit the pressure filtration apparatus.

6.6.5 Millipore, 0.2 µm, or equivalent to fit the pressure filtration apparatus.

6.7 Centrifuge – Capable of rotating 500-mL centrifuge bottles or 50-mL centrifuge tubes at 5,000 rpm minimum, equipped with 500-mL centrifuge bottles (glass or polypropylene bottles) with screw-caps, and 50-mL centrifuge tubes with screw-caps, to fit centrifuge.

6.8 Pipet apparatus and pipets

6.8.1 Pipetter – variable volume

6.8.2 Pipet tips, disposable polypropylene, sizes from 1-10 µL to 5 mL

6.8.3 Disposable, Pasteur, 150-mm long x 5-mm ID (Fisher Scientific 13-678-6A, or equivalent)

6.8.4 Disposable, serological, 50-mL (8- to 10- mm ID)

6.9 Rotary evaporator – Buchi/Brinkman-American Scientific No. E5045-10, or equivalent, equipped with a variable temperature water bath and a vacuum source with shutoff valve at the evaporator and vacuum gauge. A recirculating water pump and chiller are recommended, as use of tap water for cooling the evaporator wastes large volumes of water and can lead to inconsistent performance as water temperatures and pressures vary.

6.9.1 Round-bottom flask – 100-mL and 500-mL or larger, with ground-glass fitting compatible with the rotary evaporator

6.9.2 Boiling chips

6.9.2.1 Glass or silicon carbide – Approximately 10/40 mesh, extracted with methylene chloride and baked at 450 EC for one hour minimum

6.9.2.2 Fluoropolymer (optional) – Extracted with methylene chloride

6.10 Water bath – Heated, with concentric ring cover, capable of maintaining a temperature within ∀ 2 EC, installed in a fume hood.

6.11 Nitrogen evaporation apparatus – Equipped with water bath controlled in the range of 30 – 60 EC (N-Evap, Organomation Associates, Inc., South Berlin, MA, or equivalent), installed in a fume hood.

6.12 Amber glass vials, 2- to 5-mL with fluoropolymer-lined screw-cap

6.13 Clear glass vials, 0.3-mL, conical, with fluoropolymer-lined screw or crimp cap

6.14 HPLC/MS/MS System

6.14.1 HPLC system with high pressure inlet, multi-segment gradient capability, and post-column pump for admission of calibrant. The system must be able to produce the LC separations for the analytical runs detailed in Tables 3, 5, 7, and 9 under the instrument conditions detailed in Tables 2, 4, 6, and 8, and must meet other HPLC requirements in this method (Waters 2690, 2795, or equivalent).

6.14.2 LC columns

6.14.2.1 C_{18} – 10.0 cm, 2.1 mm i.d., 3.5 :m particle size (Waters Xtera C18MS, or equivalent)

6.14.2.2 Hydrophilic – 10 cm. 2.1 mm i.d., 3.0 µm particle size (Waters Atlantis HILIC, or equivalent)

6.14.2.3 Alternative columns other than described above have not been tested and are not allowed for this method. EPA may establish criteria for equivalency in later versions of this method.

6.14.3 MS/MS system

6.14.3.1 Tandem MS with the necessary pumps, collision cell, makeup gases, high vacuum system, and capability for positive and negative ion electrospray ionization (ESI) of the effluent from the HPLC. (Waters Quattro Ultima triple quadrupole MS, or equivalent). The system must be able to produce parent-daughter transitions for the groups of compounds in the acid and base fractions of the PPCPs for the analytical runs detailed in Tables 3, 5, 7, and 9.

6.14.3.2 Instrument control and data system – Interfaced to the HPLC and MS/MS to control the LC gradient and other LC and MS/MS operating conditions, and to acquire, store, and reduce LC/MS/MS data. The data system must be able to identify a compound by retention time and parent-daughter m/zs, and quantify the compound using linear or

quadratic multi-point relative responses and response factors by isotope dilution and internal standard techniques.

6.15 Miscellaneous labware – Beakers, 400- to 500-mL; Erlenmeyer flasks; volumetric flasks; pipets; syringes; stainless steel spatulas; etc.

7.0 Reagents and Standards

Note: All r*eagents are ACS Reagent Grade unless specified otherwise.*

7.1 pH adjustment and solution stabilization

7.1.1 Potassium hydroxide – Dissolve 20 g reagent grade KOH in 100 mL reagent water.

7.1.2 Sulfuric acid – Reagent grade (specific gravity 1.84)

7.1.3 Hydrochloric acid – Reagent grade, 6N

7.1.4 Phosphoric acid (H_3PO_4) – Reagent grade (85%), Fisher, or equivalent

7.1.5 Sodium chloride – Reagent grade, prepare at 5% (w/v) solution in reagent water

7.1.6 Ammonium hydroxide (NH_4OH) – Reagent grade, Anachemia, or equivalent

7.1.7 Sodium dihydrogen phosphate monohydrate – Reagent grade, J.T. Baker, or equivalent

7.1.8 Oxalic acid, anhydrous

7.2 Prepurified nitrogen

7.3 Solvents, reagents, and solutions

7.3.1 Acetic acid, acetone, acetonitrile ammonium acetate, formic acid, methanol, methylene chloride, HPLC water, ammonium formate.

7.3.2 Solvents and purchased solutions should be lot-certified to be free of interferences. If necessary, solvents should be analyzed by this method to demonstrate that they are interference free.

7.4 Buffer and elution solutions

7.4.1 Phosphate buffer (sodium phosphate monohydrate/phosphoric acid) – 0.14 M $NaH_2PO_4.H_2O$ /85% H_3PO_4 (1.93 g $NaH_2PO_4.H_2O$ in 99 mL of reagent water + 1 mL of 85% H_3PO_4)

7.4.2 Tetrasodium ethylenediamine tetraacetate hydrate ($Na_4EDTA \bullet 2H_2O$ ~+99.5%

titration), Sigma, used as received

7.4.3 Formic acid solutions – Alfa Aesar, >99 percent purity

7.4.3.1 2% v/v in methanol

7.4.3.2 0.1% v/v in methanol

7.4.3.3 Formic acid/ammonium formate (0.1%) in water – dissolve 4 mL of formic acid and 4 g of ammonium formate in 4.0 L of HPLC water. Mix thoroughly and sonicate for 5 min.

7.4.3.4 Formic acid (0.1%) in methanol:water (75:25) – add 4 mL of formic acid to 3.0 L methanol premixed with 1.0 L HPLC-grade water. Mix thoroughly and sonicate for 5 min.

7.4.4 Acetonitrile:methanol (1:1) – mix 500 mL methanol and 500 mL of acetonitrile. Sonicate for 5 min.

7.4.5 Oxalic acid solution (5 mM) – dissolve 0.45 g anhydrous oxalic acid in 1.0 L of HPLC water. Mix thoroughly and sonicate for 5 min.

7.4.6 Oxalic acid/acetonitrile/methanol (5 mM) – dissolve 0.45 g anhydrous oxalic acid in 500 mL acetonitrile premixed with 500 mL methanol. Mix thoroughly and sonicate for 5 min.

7.4.7 Acetonitrile/water (90%) – Add 400 mL HPLC-grade water to 3600 mL of acetonitrile. Mix thoroughly and sonicate for 5 min.

7.4.8 Ammonium acetate/acetic acid, 1 mM (0.1%) in water – Add 4 g NH_4OAC and 4 mL acetic acid to 4.0 L of HPLC-grade water. Mix thoroughly and sonicate for 5 min.

7.5 Sodium iodide/cesium iodide mass calibration solution – 2 mg/mL NaI and 50 µg/mL CsI in (1:1) isopropyl alcohol:water (Waters 700000889, or equivalent) or other based on manufacture's specifications.

7.6 Reference matrices – Matrices in which the PPCPs and interfering compounds are not detected by this method

7.6.1 Reagent water – Bottled water purchased locally, or prepared by passage through activated carbon

7.6.2 High-solids reference matrix – Playground sand or similar material.

7.6.2.1 Playground sand is used to simulate the base fraction of solids in this method, including biosolids (see Section 7.6.3.1 for simulation of the biosolids acid fraction) – Place 1 g of sand in a 50-mL centrifuge tube. Add 15 mL of reagent water and adjust the pH to 10 ± 0.5 with NH_4OH.

Add 20 mL of acetonitrile and sonicate for 20 minutes. Discard the aqueous phase.

7.6.2.2 Extract with a second 20-mL portion of acetonitrile. Decant and discard the acetonitrile. The sand is now ready for spiking (Section 11.5.4).

7.6.3 Biosolids (sludge) reference matrix – Dry peat moss, purchase from local garden center. Note: Store peat moss in closed container to prevent further drying. Sand may be used for the acid fraction if QC acceptance criteria (Section 9) are met.

7.6.3.1 Peat moss is used to simulate the acid fraction of biosolids in this method (see Section 7.6.2.1 for information on the biosolids base fraction) – Place 1 g of peat moss in a 50-mL centrifuge tube. Add 15 mL of phosphate buffer (Section 7.4.1) and vortex to mix. Extract with 20 mL of acetonitrile and discard the aqueous phase.

7.6.3.2 Extract with a second 20-mL portion of acetonitrile. Decant and discard the acetonitrile. The peat moss is now ready for spiking (Section 11.4.3).

7.6.4 Other matrices – Other reference matrices of interest may be used if the results from the tests given in Section 9.2 demonstrate acceptable performance. Ideally, the matrix should be free of the analytes of interest, but in no case must the background level of the analytes in the reference matrix exceed the minimum levels in Tables 3, 5, 7, and 9. If low background levels of the analytes of interest are present in the reference matrix, the spike level of the analytes used in Section 9.2 should be increased to provide a spike-to-background ratio of approximately 5 (Reference 8).

7.7 Standard solutions – Prepare from materials of known purity and composition or purchase as solutions or mixtures with certification to their purity, concentration, and authenticity. If the chemical purity is 98 % or greater, the weight may be used without correction to calculate the concentration of the standard. Observe the safety precautions in Section 5.

7.7.1 Preparation and storage of solutions - For preparation of stock solutions from neat materials, dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10 to 20 mg of Ampicillin to three significant figures in a 10-mL ground-glass-stoppered volumetric flask and fill to the mark with methanol. After the compound is completely dissolved, transfer the solution to a clean 15-mL vial with fluoropolymer-lined cap. When not being used, store standard solutions in the dark at less than -10 ºC in screw-capped vials with fluoropolymer-lined caps or under a non-reactive gas (e.g., nitrogen) in a flame-sealed glass ampul. Place a mark on the vial or ampul at the level of the solution so that solvent loss by evaporation can be detected. Replace the solution if solvent loss has occurred.

7.7.2 Native (unlabeled; authentic) compound spiking solution – Separately prepare Group 1 to Group 4 native compounds at the concentrations shown in column 3 of Table 10 in methanol, or purchase prepared solutions. If additional native compounds are to be determined, include these compounds in this stock solution.

Stock solutions should be prepared at a frequency necessary to preclude degradation from affecting the analysis. For example, it may be necessary to prepare the tetracycline compounds weekly if concentrations drop more than 30 % of their original concentration. Stock solutions should also be checked for signs of degradation prior to preparation of calibration or performance test standards.

7.7.3 Labeled compound spiking solution – Prepare Group 1 to Group 4 labeled compounds at the concentrations shown in column 3 of Table 10 in methanol, or purchase prepared solutions. If additional labeled compounds are to be used, include these compounds in this solution. Note: The Group 2, acid extracted positive ESI (tetracyclines) contains the same labeled compounds as for Group 1 and 3, acid extracted positive and negative ESI, yet the only labeled compounds used in determination of the Group 2 are Thiabendazole-d6 and 13C3-Atrazine. This minimizes the work required to prepare solutions. Some of those surrogates are used to quantify the Group 1 and 2 and some Group 3 in separate runs of the same extract. This is not a requirement.

7.7.4 Labeled injection internal standard spiking solutions – For the labeled injection internal standards for Groups 1 and 2, prepare ^{13}C-Atrazine in methanol at the concentration shown column 3 of Table 10. For the labeled injection internal standard for Group 3, prepare $^{13}C_6$-2,4,5-Trichlorophenoxyacetic acid (TCPAA) in methanol at the concentration shown in column 3 of Table 10. For the labeled injection internal standards for Group 4, prepare $^{13}C_3$-Atrazine and Continine-d3 in methanol at the concentrations shown in column 3 of Table 10. If additional labeled injection internal standards are to be used, include these compounds in these solutions.

7.7.5 Calibration standards – Combine and dilute the solutions in Sections 7.7.1 and 7.7.2 to produce the calibration solutions in Table 11 or purchase prepared standards for the CS-1 to CS-5 set of calibration solutions. These solutions permit the relative response (labeled to native) and response factor to be determined as a function of concentration. The CS-3 standard is used for calibration verification (VER).

7.8 QC Check Sample – A QC Check Sample should be obtained from a source independent of the calibration standards. Ideally, this check sample would be a Standard Reference Material (SRM) from the National Institute of Standards and Technology (NIST) containing the compounds of interest in known concentrations in a sample matrix similar to the matrix of interest. If no SRM is available, a certified reference material (CRM) may be used or a QC check sample may be prepared from materials from a source or lot of standards separate from those used for calibration and spiked into a clean reference matrix.

7.9 Stability of solutions – standard solutions used for quantitative purposes (Sections 7.7.2 - 7.7.5) should be assayed periodically (e.g., every 6 months) against SRMs from NIST (if available), or against certified reference materials from a source that will attest to the authenticity and concentration, to assure that the composition and concentrations have not changed.

8.0 Sample Collection, Preservation, Storage, and Holding Times

8.1 Collect samples in amber glass containers following conventional sampling practices (Reference 9).

8.2 Aqueous samples

8.2.1 Samples that flow freely are collected as grab samples or in refrigerated bottles using automatic sampling equipment. Collect 1-L each for the acid and base fractions (2 L total). If high concentrations of the analytes of interest are expected, collect two smaller volumes (e.g., 100 mL each) in addition to the 1-L samples. Do not rinse the bottle with sample before collection.

8.2.2 If residual chlorine is present, add 80 mg sodium thiosulfate per liter of water. Any method suitable for field use may be employed to test for residual chlorine. Ascorbic acid has also been used by a number of other groups as a preservative for a number of pharmaceuticals however it has not been tested for all of the pharmaceuticals covered under this method (Reference 10).

8.2.3 Maintain aqueous samples in the dark at <6 EC from the time of collection until receipt at the laboratory (see 40 CFR 136.6(e), Table II). If the sample will be frozen, allow room for expansion.

8.3 Solid, mixed-phase, and semi-solid samples, including biosolids

8.3.1 Collect samples as grab samples using wide-mouth jars. Collect a sufficient amount of wet material to produce a minimum of 10 g of solids.

8.3.2 Maintain solid, semi-solid, and mixed-phase samples in the dark at <6 EC from the time of collection until receipt at the laboratory. Store solid, semi-solid, and mixed-phase samples in the dark at less than -10 EC.

8.4 Store sample extracts in the dark at less than -10 EC until analyzed. Analyze extracts within 40 days of extraction.

8.5 Holding times

EPA has not conducted formal holding time studies for these analytes to date. Use the information below as guidance. Exceeding these default holding times does not invalidate the sample results.

8.5.1 Aqueous samples – Anecdotal evidence suggests that some may degrade rapidly in aqueous samples. Therefore, begin sample extraction within 7 days of collection (within 48 hours is strongly encouraged). Extracts should be analyzed within 40 days of extraction. Freezing of aqueous samples is encouraged to minimize degradation, in which case, samples should be extracted within 48 hours of removal from the freezer.

8.5.2 Biosolid, solid, mixed-phase, and semi-solid samples – Anecdotal evidence suggests that some may degrade rapidly in these samples. Therefore, begin sample extraction within 7 days of collection (within 48 hours is strongly encouraged). Extracts should be analyzed within 40 days of extraction. Freezing of biosolids, mixed phase and semisolid samples is encouraged to minimize degradation, in which case, samples should be extracted within 48 hours of removal from the freezer..

8.5.3 If extraction within 48 hours is not practical, samples should be frozen to increase the holding time to seven days.

8.5.4 If the sample will not be extracted within 48 hours of collection, the laboratory should adjust the pH of aqueous samples to 5.0 to 9.0 with a sodium hydroxide or sulfuric acid solution. Record the volume of acid or base used. If aqueous samples are stored frozen, extraction should begin within 48 hours of removal from the freezer.

9.0 Quality Assurance/Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 11). The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with labeled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

If the method is to be applied to sample matrix other than water (e.g., soil, sediment, filter cake, compost) the most appropriate alternate reference matrix (Sections 7.6.1 – 7.6.4) is substituted for the reagent water matrix (Section 7.6.1) in all performance tests.

9.1.1 The laboratory must make an initial demonstration of the ability to generate acceptable precision and recovery with this method. This demonstration is given in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, and to overcome matrix interferences, the laboratory is permitted certain options to improve separations or lower the costs of measurements. These options include alternate extraction, concentration, and cleanup procedures, and changes in columns and detectors (see also 40 CFR 136.6). Alternate determinative techniques, such as the substitution of spectroscopic or immunoassay techniques, and changes that degrade method performance, are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or greater than the specificity of the techniques in this method for the analytes of interest.

9.1.2.1 Each time a modification is made to this method, the laboratory is

required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the MDLs (40 CFR Part 136, Appendix B) are lower than one-third the regulatory compliance level or the MDLs in this method, whichever are greater. If calibration will be affected by the change, the instrument must be recalibrated per Section 10. Once the modification is demonstrated to produce results equivalent or superior to results produced by this method as written, that modification may be used routinely thereafter, so long as the other requirements in this method are met (e.g., labeled compound recovery).

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) that performed the analyses and modification, and of the quality control officer that witnessed and will verify the analyses and modifications.

9.1.2.2.2 A list of compounds (s) measured, by name and CAS Registry number.

9.1.2.2.3 A narrative stating reason(s) for the modifications.

9.1.2.2.4 Results from all quality control (QC) tests comparing the modified method to this method, including:

a) Calibration (Section 10).
b) Calibration verification (Section 15.2).
c) Initial precision and recovery (Section 9.2).
d) Labeled compound recovery (Section 9.3).
e) Analysis of blanks (Section 9.5).
f) Accuracy assessment (Section 9.4).

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

a) Sample numbers and other identifiers.
b) Extraction dates.
c) Analysis dates and times.
d) Analysis sequence/run chronology.
e) Sample weight or volume (Section 11).
f) Sample or extract volume prior to each cleanup step (Section 12).
g) Extract volume after each cleanup step (Section 12).
h) Final extract volume prior to injection (Section 12).
i) Injection volume (Sections 10.2.1 and 14.2).

j) Dilution data, differentiating between dilution of a sample or extract (Section 17.5).
k) Instrument and operating conditions.
l) Column (dimensions, material, particle size, etc).
m) Operating conditions (flow rates, elution solvents, gradient, flow rates).
n) Detector (type, operating conditions, etc).
o) Chromatograms, printer tapes, and other recordings of raw data.
p) Quantitation reports, data system outputs, and other data to link the raw data to the results reported.

9.1.3 Analyses of method blanks are required to demonstrate freedom from contamination (Section 4.3). The procedures and criteria for analysis of a method blank are given in Sections 9.5 and 15.4.

9.1.4 The laboratory must spike all samples with labeled compounds to monitor method performance. This test is described in Section 9.3. When results of these spikes indicate atypical method performance for samples, the samples are diluted to bring method performance within acceptable limits. Procedures for dilution are given in Section 17.5.

9.1.5 The laboratory must, on an ongoing basis, demonstrate through calibration verification and the analysis of the ongoing precision and recovery standard (OPR) and blanks that the analytical system is in control. These procedures are given in Sections 15.1 through 15.4.

9.1.6 The laboratory should maintain records to define the quality of data generated. Development of accuracy statements is described in Section 9.4.

9.2 Initial precision and recovery (IPR) – To establish the ability to generate acceptable precision and recovery, the laboratory must perform the following operations.

9.2.1 For aqueous samples containing less than 1% solids, analyze four 1-L aliquots of reagent water (7.6.1) each for the acid and base fractions according to the procedures in Sections 11 through 18. For an alternate sample matrix, four aliquots each for the acid and base fractions of the alternate reference matrix (Sections 7.6.2-7.6.4) are used. All sample processing steps that are to be used for processing samples, including preparation (Section 11), extraction (Section 12), and cleanup (Section 13), must be included in this test.

9.2.2 Using results of the set of four analyses, compute the average percent recovery (X) of the concentration of each compound in each extract and the relative standard deviation (RSD) of the concentration for each compound, by isotope dilution for compounds with a labeled analog, and by internal standard for compounds without a labeled analog and for the labeled compounds.

9.2.3 For each native and labeled compound, compare RSD and X with the corresponding limits for initial precision and recovery in Table 12. If RSD and X

for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual RSD exceeds the precision limit or any individual X falls outside the range for recovery, system performance is unacceptable for that compound. Correct the problem and repeat the test (Section 9.2).

9.3 To assess method performance on the sample matrix, the laboratory must spike all samples with the Labeled spiking solution (Section 7.7.3).

9.3.1 Analyze each sample according to the procedures in Sections 11 through 18.

9.3.2 Compute the percent recovery of the labeled compounds using the internal standard method (Sections 10.4 and 7.2).

9.3.3 The recovery of each labeled compound must be within the limits in Table 12. If the recovery of any compound falls outside of these limits, method performance is unacceptable for that compound in that sample. Additional cleanup procedures must then be employed to attempt to bring the recovery within the normal range. If the recovery cannot be brought within the normal range after all cleanup procedures have been employed, water samples are diluted and smaller amounts of soils, sludges, sediments, and other matrices are analyzed per Section 18.

9.4 Recovery of labeled compounds from samples should be assessed and recorded.

9.4.1 After the analysis of 30 samples of a given matrix type (water, soil, sludge, pulp, etc.) for which the labeled compounds pass the tests in Section 9.3, compute the average percent recovery (R) and the standard deviation of the percent recovery (S_R) for the labeled compounds only. Express the assessment as a percent recovery interval from R ! $2S_R$ to R + $2S_R$ for each matrix. For example, if R = 90% and S_R = 10% for 30 analyses of biosolids, the recovery interval is expressed as 70 to 110%.

9.4.2 Update the accuracy assessment for each labeled compound in each matrix on a regular basis (e.g., after each 5-10 new measurements).

9.5 Method blanks – A reference matrix method blank is analyzed with each sample batch (Section 4.3) to demonstrate freedom from contamination. The matrix for the method blank must be similar to the sample matrix for the batch, e.g., a 1-L reagent water blank (Section 7.6.1), high-solids reference matrix blank (Section 7.6.2), biosolids reference matrix blank (Section 7.6.3) or alternate reference matrix blank (Section 7.6.4).

9.5.1 Process the method blank(s) along with the IPR or batch of samples according to the procedures in Sections 11 through 18. Analyze the blank immediately after analysis of the OPR (Section 15.4) to demonstrate freedom from contamination.

9.5.2 If any compound of interest (Table 1) is found in the blank at greater than the minimum level (Tables 3, 5, 7, or 9) or one-third the regulatory compliance limit, whichever is greater; or if any potentially interfering compound is found in the blank above the minimum level for each native compound in Tables 3, 5, 7, or 9

(assuming a response factor of 1 relative to the quantitation reference in Tables 3, 5, 7, or 9 for a potentially interfering compound; i.e., a compound not listed in this method), analysis of samples must be halted until the sample batch is re-extracted and the extracts re-analyzed, and the blank associated with the sample batch shows no evidence of contamination at these levels. All samples must be associated with an uncontaminated method blank before the results for those samples may be reported or used for permitting or regulatory compliance purposes.

9.6 QC Check Sample – If available, analyze the QC Check Sample (Section 7.8) periodically to assure the accuracy of calibration standards and the overall reliability of the analytical process. It is suggested that the QC Check Sample be analyzed at least quarterly.

9.7 The specifications contained in this method can be met if the apparatus used is calibrated properly and then maintained in a calibrated state. The standards used for calibration (Section 10), calibration verification (Section 15.2), and for initial (Section 9.2) and ongoing (Section 15.4) precision and recovery should be identical, so that the most precise results will be obtained. A LCMSMS instrument will provide the most reproducible results if dedicated to the settings and conditions required for determination of PPCPs by this method.

9.8 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and spiked samples may be required to determine the accuracy of the analysis when the internal standard method is used.

10.0 Calibration and Standardization

10.1 Establish the LC/MS/MS operating conditions for the Group 1 through Group 4 compounds, as suggested in Tables 2, 4, 6, and 8, to meet the retention times in Tables 3, 5, 7, and 9, respectively. The LC conditions may be optimized for compound separation and sensitivity. Once optimized, the same conditions must be used for the analysis of all standards, blanks, IPR and OPR standards, and samples.

10.2 Retention time calibration for the native and labeled compounds

10.2.1 Inject the volume of CS-3 calibration standard (Section 7.7.5 and Table 11) listed in Table 2, 4, 6, or 8, or other volume appropriate to system optimization. Establish the beginning and ending retention times for the parent-daughter descriptors in Tables 3, 5, 7, and 9. Descriptors other than those listed may be used provided the MLs in those tables are met. Store the retention time (RT) for each compound in the data system.

10.2.2 The absolute retention time of last-eluted compound in each of the four Groups must be equal to or greater than its retention time in Tables 3, 5, 7, or 9; otherwise, the LC operating conditions must be adjusted and this test repeated until this minimum retention time criterion is met.

10.3 Mass spectrometer calibration and optimization

10.3.1 Mass calibration – The mass spectrometer must undergo mass calibration according to manufacture's specifications to ensure accurate assignments of m/z's by the instrument. This mass calibration must be performed at least annually to maintain instrument sensitivity and stability. It must be repeated after performing major maintenance on the mass spectrometer.

In the absence of vendor-specific instructions and acceptance criteria, the following procedure may be used.

10.3.1.1 Introduce the NaCsI calibration solution (Section 7.5) to the MS at the flow rate necessary to produce a stable aerosol spray (e.g., 10 µL/min).

10.3.1.2 Scan the MS/MS over the mass range from 20 to 3000 Daltons. Adjust the source parameters to optimize peak intensity and shape across the mass range. The exact m/z's for NaCsI calibration are:

Calibration Masses (Daltons)	
22.9898	1521.9321
132.9054	1671.8264
172.8840	1821.7206
322.7782	1971.6149
472.6725	2121.5091
622.5667	2271.4033
772.4610	2421.2976
922.3552	2571.1918
1072.2494	2721.0861
1222.1437	2870.9803
1372.0379	

10.3.1.3 Mass calibration is judged on the basis of the presence or absence of the exact calibration masses, e.g., a limit of the number of masses that are "missed." Absent vendor-specific instructions, all of the masses from 22.9898 to 1971.6149 must be present. If peaks above 1971 are missing or not correctly identified, adjust the MS/MS and repeat the test. Only after the MS/MS is properly calibrated may standards, blanks, and samples be analyzed.

10.3.2 Mass spectrometer optimization – Prior to measurements of a given analyte Group (Table 2, 4, 6, or 8), the mass spectrometer must be separately optimized for that Group.

10.3.2.1 Using the post-column pump (Section 6.14.1), infuse the CS-3 calibration solution (Table 11 a, b, or c) for the Group of interest.

10.3.2.2 Optimize sensitivity to the daughter m/z's for the high mass compounds in each Group (Table 3, 5, 7, or 9).

10.3.2.3 After MS calibration and optimization and LC/MS/MS calibration (Sections 10.4 and 10.5), MS and LC/MS/MS conditions may not be altered without verifying calibration (Section 15.2).

10.4 Calibration by isotope dilution – Isotope dilution is used for calibration of each native compound for which a labeled analog is available. The reference compound for each native compound is its labeled analog, as listed in Tables 3, 5, 7, and 9. A 5-point calibration encompassing the concentration range is prepared for each native compound. The calibration solutions are listed in Table 11.

10.4.1 To calibrate the analytical system by isotope dilution, inject calibration standards CS-1 through CS-5 (Section 7.7.5 and Table 11). Use the volume shown in identical to the volume chosen in Section 10.2.1, the procedure in Section 14, and the optimized operating conditions from Sections 10.1 - 10.3.

10.4.2 For the compounds determined by isotope dilution, the relative response (RR) (labeled to native) vs. concentration in the calibration solutions (Table 11) is computed over the calibration range according to the procedures below. Determine the response of each compound relative to its labeled analog using the area responses of the daughter m/zs specified in Tables 3, 5, 7, and 9. Use the labeled compounds listed in the tables as the quantitation reference and the daughter m/zs of these labeled compounds for quantitation. The area of the daughter m/z for the native compound is divided by the area of the daughter m/z of the labeled quantitation reference compound.

Note: *Other quantitation references and procedures may be used provided that the results produced are as accurate as results produced by the quantitation references and procedures described in this method.*

10.4.3 Calibrate the native compounds with a labeled analog using the following equation:

$$RR = \frac{A_n\ C_l}{A_l\ C_n}$$

Where:

A_n = The area of the daughter m/z for the native compound

A_l = The area of the daughter m/z for the labeled compound.

C_l = The concentration of the labeled compound in the calibration standard (Table 11) (ng/mL).

C_n = The concentration of the native compound in the calibration standard (Table 11) (ng/mL).

10.4.4 Compute the average (mean) RR, and the standard deviation and relative standard deviation (RSD) of the 5 RRs.

10.4.5 Linearity – If the RR for any compound is constant (less than 20% RSD), the average RR may be used for that compound; otherwise, the complete calibration curve for that compound must be used over the calibration range.

10.5 Calibration by internal standard – Internal standard calibration is applied to determination of the native compounds for which a labeled compound is not available, and to determination of the labeled compounds for performance tests and intra-laboratory statistics (Sections 9.4 and 15.4.4). The reference compound for each native compound is listed in Table 3, 5, 7, or 9. For the labeled compounds, calibration is performed at a single concentration using data from the 5 points in the calibration (Section 10.4).

10.5.1 Response factors – Internal standard calibration requires the determination of response factors (RF) defined by the following equation:

$$RF = \frac{A_n\ C_{is}}{A_{is}\ C_n}$$

Where:

A_n = The area of the daughter m/z for the native compound
A_{is} = The area of the daughter m/z for the internal standard.
C_{is} = The concentration of the internal standard (Table 11) (ng/mL).
C_n = The concentration of the native compound in the calibration standard (Table 11) (ng/mL).

10.5.2 To calibrate the analytical system for compounds that do not have a labeled analog, and for the labeled compounds, use the data from the 5-point calibration (Section 10.4 and Table 11).

10.5.3 Compute and store the response factor (RF) for all native compounds that do not have a labeled analog. Use the labeled compounds and daughter m/zs listed in Tables 3, 5, 7, and 9 as the quantitation references.

10.5.4 Compute and store the response factor (RF) for the labeled compounds using the labeled injection internal standard as the quantitation reference, as given in Tables 3, 5, 7, and 9.

10.5.5 Linearity – If the RF for any native compound without a labeled analog or for any labeled compound is constant (less than 35% RSD), the average RF may be used for that compound; otherwise, the complete calibration curve for that compound must be used over the calibration range.

10.6 SPE cartridge performance check

In order to be used for extraction of aqueous samples or cleanup of solid-sample extracts, the performance of the HLB SPE cartridges must be checked at least once for each manufacturer's lot of cartridges. This performance check is accomplished by processing a spiked reagent water sample through the extraction procedure is Section 12 and analyzing the extract. Separate checks are performed for the acid and base fractions. Labeled compounds are not added to these check samples before extraction because the

recovery correction inherent in isotope dilution will mask problems with the cartridges. Cartridge performance is acceptable if the recoveries of the native analytes are within the QC acceptance criteria for the OPR in Table 12. Perform this cartridge check as outlined below. Note – This performance check is performed when a new lot number of cartridges is purchased.

10.6.1 Acid fraction – Acidify a 1.0-L aliquot of reagent water to pH 2.0 ± 0.5. Add 500 mg Na_4EDTA (Section 7.4.2) and spike with the Group 1, 2, and 3 native compounds (Section 7.7.2 and Table 10). Do not spike the labeled compounds. Process the solution through the SPE HLB procedure for the acid fraction in Section 12. After processing, spike the solution with the Group 1, 2, and 3 labeled compounds (Section 7.7.3 and Table 10) and complete the analysis per Sections 12 - 15. Recovery of the native compounds must be within the QC acceptance crieria for the OPR in Table 12. If the compounds are not recovered in this range, adjust the elution volumes or reject the cartridge batch.

10.6.2 Base fraction – Adjust the pH a 1.0-L aliquot of reagent water to pH 10.0 ± 0.5 and spike with the Group 4 native compounds (Section 7.7.2 and Table 10). Do not spike the labeled compounds. Process the solution through the SPE HLB procedure for the base fraction in Section 12. After processing, spike the extract with the Group 4 labeled compounds (Section 7.7.3 and Table 10) and complete the analysis per Sections 12 - 15. Recovery of the native compounds must be within the QC acceptance criteria for the OPR in Table 12. If the compounds are not recovered in this range, adjust the elution volumes or reject the cartridge batch.

11.0 Sample Preparation

Sample preparation involves modifying the physical form of the sample so that the analytes can be extracted efficiently. In general, the samples must be in a liquid form or in the form of finely divided solids in order for efficient extraction to take place. Table 13 lists the phases and suggested quantities for extraction of various sample matrices. For samples known or expected to contain high levels of the analytes, the smallest sample size representative of the entire sample should be used (see Section 18).

Biosolids and solid samples are prepared per Section 11.4, extracted per Sections 12.3 and 12.4, and cleaned up using SPE HLB cleanup in Sections 12.1 and 12.2.

Aqueous samples - Because the analytes may be bound to suspended particles, the preparation of aqueous samples is depends on the presence of visible particles. Aqueous samples absent visible particles are prepared per Section 11.3 and processed using SPE HLB cleanup in Sections 12.1 and 12.2.

Aqueous samples with visible particles - If visible particles can be seen in aqueous samples they should be filtered and the solids and aqueous portions of these samples should be extracted and combined prior to clean up as follows. Filtration of particles - assemble a clean filtration apparatus (Section 6.6). Apply vacuum to the apparatus, and pour the entire contents of the

sample bottle through the filter, swirling the sample remaining in the bottle to suspend any particles. Rinse the sample bottle twice with approximately 5 mL portions of reagent water to transfer any remaining particles onto the filter. Rinse any particles off the sides of the filtration apparatus with small quantities of reagent water. Weigh the empty sample bottle to ±1 g. Determine the weight of the sample by difference. Save the bottle for further use. Prepare and extract the filtrate using the procedure in Section 11.3. Prepare and extract the filter containing the particles using the same procedure for biosolids and solid samples in Section 11.4, Sections 12.3 and 12.4, and Sections 12.1 and 12.2. These extracts should be combined prior to analysis (Section 14) or results of separate analysis combined. It should be noted that the judgment of the analyst must be used to determine the need to analyze samples with visible particles that compose less than 1 % of the sample weight per Section 11.1.

Procedures for grinding, homogenization, and blending of various sample phases are given in Section 11.5.

Note: *Each sample batch (Section 4.3) is accompanied by a blank and an OPR. If the acid fraction (Groups 1, 2, and 3) only is to be analyzed then 1 acid blank and OPR must be used. If both the acid (Groups 1, 2, and 3) and base (Group 4) fractions are to be analyzed, 1 acid blank and OPR as well as 1 base blank and OPR must accompany the batch. If the base fraction (Group 4) only is to be analyzed, a base blank and OPR must accompany the base batch.*

11.1 Determination of solids content

The solids content of the bulk sample is determined from a subsample that is used only for the solids determination. Separate procedures are used for the solids determination, based on the sample matrix, as described below.

11.1.1 Aqueous liquids and multi-phase samples consisting of mainly an aqueous phase.

11.1.1.1 Dry a GF/A filter (Section 6.6.4) and weigh to three significant figures. Mix the bulk sample in the original container (e.g., cap the bottle and shake) and take a 10.0 ∀ 0.2 mL aliquot. Filter that aliquot through the filter. Dry the filter in an oven for a minimum of 12 hours at 110 ∀ 5 EC and cool in a dessicator.

11.1.1.2 Weigh the filter and calculate percent solids as follows:

$$\% \text{ Solids} = \frac{\text{Weight of sample aliquot after drying (g) - weight of filter (g)}}{10 \text{ g}} x\, 100$$

11.1.2 Non-aqueous liquids, solids, semi-solid samples, and multi-phase samples in which the main phase is not aqueous

11.1.2.1 Weigh 5 to 10 g of the bulk sample to three significant figures in a tared beaker, weighing pan, or other suitable container. Dry for a minimum of 12 hours at 110 ∀ 5 EC, and cool in a dessicator.

11.1.2.2 Weigh the dried aliquot and calculate percent solids as follows:

$$\% \text{ Solids} = \frac{\text{Weight of sample aliquot after drying (g)}}{\text{Weight of sample aliquot before drying (g)}} \; x \; 100$$

11.2 Estimation of particle size

Extraction of a sample matrix is affected by the size of particles in the sample. Ideally, the particles should be 1 mm or less. The particle size can be estimated using the sample aliquot filtered or dried in Sections 11.1.1 or 11.1.2. Spread the aliquot on a piece of filter paper or aluminum foil in a fume hood or glove box. Visually estimate the size of the particles in the sample. If the size of the largest particles is greater than 1 mm, use one of the procedures in Section 11.5 to reduce the particle size to 1 mm or less prior to extraction. If the largest particles are 1 mm or less, proceed with sample preparation, using the procedures in Section 11.4

11.3 Preparation of aqueous samples absent visible particles and corresponding QC samples.

Two separate sample aliquots are required to analyze all of the target analytes in this procedure: one aliquot is adjusted to pH 2 ± 0.5 (Section 11.3.3.1) and the other aliquot is adjusted to pH 10 ± 0.5 (Section 11.3.4.1). Following this pH adjustment, both aliquots are filtered separately, and the two filtrates are extracted using the SPE HLB cartridge per Section 12.

11.3.1 Mark the original level of the sample on each of the two sample bottles. Designate one bottle for the acid fraction and the other for the base fraction. Weigh each sample plus bottle to the nearest 1 g. If only one sample bottle was provided, and both the acid and base fractions are to be analyzed, split the sample in half and place each new aliquot in a separate clean container.

11.3.2 For each sample batch (Section 4.3) to be extracted during the same 12-hour shift, transfer four 1-L aliquots of reagent water to clean sample bottles or flasks. Two of these aliquots will serve as method blanks (one for the acid fraction and one for the base fraction) and the other two aliquots will be used to prepare the OPR samples (one acid and one base). (If both acid and base fractions are not required, prepare only the reference matrix aliquots appropriate for the fraction of interest.)

11.3.3 Acid fraction - typically 500 mL to 1 L

11.3.3.1 Acidify the filtrate for the acid fraction to pH 2.0 ± 0.5 with HCl while swirling or stirring the water. Re-adjust the pH as necessary to achieve pH 2.0 ± 0.5. Maintain the pH above 1.95 to preclude deuterium-hydrogen exchange on the deuterium-labeled compounds.

11.3.3.2 Spike the acid fraction (Group 1, 2, and 3) native compounds (Section 7.7.2 and Table 10) into the reagent water aliquot that will serve as the acid fraction OPR. Acidify the OPR aliquot and the

blank aliquot in the same manner as the acid fraction of the field sample (11.3.3.1).

11.3.3.3 Spike the acid fraction (Group 1, 2, and 3) labeled compounds (Section 7.7.3 and Table 10) into the acid fractions of the samples and QC aliquots.

11.3.3.4 Add 500 mg NA4EDTA.2H2O (Section 7.4.2) to each of the acid fraction samples and QC aliquots. Cap the bottles and mix by shaking. Allow the sample and QC aliquots to equilibrate for 1 to 2 hours, with occasional shaking. Proceed to Section 12 for sample extraction.

11.3.4 Base fraction – typically 500 mL to 1 L

11.3.4.1 Adjust the pH of the second of the two sample bottles to pH 10.0 ± 0.5 with NH_4OH while swirling or stirring the water. Re-adjust the pH as necessary to achieve pH 10.0 ± 0.5.

11.3.4.2 Spike the base fraction (Group 4) native compounds (Section 7.7.2 and Table 10) into the reagent water aliquot that will serve as the base fraction OPR. Adjust the pH of the OPR aliquot and the blank aliquot in the same manner as the base fraction of the field sample (11.3.4.1).

11.3.4.3 Spike the base fraction (Group 4) labeled compounds (Section 7.7.3 and Table 10) into the base fractions of the samples and QC aliquots.

11.3.4.4 Cap the bottles and mix by shaking. Allow the sample and aliquots to equilibrate for 1 to 2 hours, with occasional shaking. Proceed to Section 12 for sample extraction.

11.4 Preparation of solid samples and samples from filtered particles and corresponding QC samples.

Filtered solids from aqueous samples are treated as solid matrices, regardless of whether they are pourable liquids or solid materials. Two separate aliquots are required to analyze all of the target analytes in this procedure. If the particle size estimated in Section 11.2 exceeds 1 mm, use one of the six size-reduction procedures in Section 11.5 first. Following addition of buffer solutions, one aliquot is adjusted to pH 2 ± 0.5 and the other aliquot is adjusted to pH 11 ± 0.5. Following pH adjustment, each aliquot is extracted separately per Section 12.

11.4.1 Homogenize the sample in its original container, by shaking samples that are pourable liquids, or by stirring solids in their original container with a clean spatula, glass stirring rod, or other suitable implement.

11.4.2 Using the percent solids data collected in Section 11.1, collect two aliquots of the well-mixed sample sufficient to provide 1.0 g of dry solids, but do not exceed a maximum of 5 g wet weight. For biosolids, do not exceed 0.25 g of wet solids. Place the two sample aliquots in separate clean 50-mL disposable centrifuge tubes. Designate one of the samples as the acid fraction, the other the base fraction.

11.4.3 For each sample batch (Section 4.3) to be extracted during the same 12-hour shift, transfer two 1-g aliquots of peat moss (Section 7.6.3) to clean sample bottles or flasks. These two peat moss aliquots will be used for the method blank and the OPR sample for the acid fraction. Transfer two 1-g aliquots of clean sand (Section 7.6.2) to clean sample bottles or flasks. These two clean sand aliquots will be used for the method blank and the OPR sample for the base fraction. (If both acid and base fractions are not required, prepare only the reference matrix aliquots appropriate for the fraction of interest.)

11.4.4 Acid fraction

11.4.4.1 Add 15 mL of pH 2 phosphate buffer (Section 7.4.1) to the sample, blank, and OPR. Vortex each for 5 min. Check and adjust the pH to 2.0 ± 0.5 with buffer, vortexing the mixture after each addition. Maintain the pH above 1.95 to preclude deuterium-hydrogen exchange on the deuterium-labeled compounds.

11.4.4.2 Spike the acid fraction (Group 1, 2, and 3) native compounds (Section 7.7.2 and Table 10) into the peat moss aliquot that will serve as the acid fraction OPR. Acidify the OPR aliquot and the blank aliquot in the same manner as the acid fraction of the field sample (11.4.4.1).

11.4.4.3 Spike the acid fraction (Group 1, 2, and 3) labeled compounds (Section 7.7.3 and Table 10) into the acid fractions of the samples and QC aliquots.

11.4.4.4 Vortex the samples and QC aliquots. Proceed to Section 12.3 for extraction of the solids acid fraction.

11.4.5 Base fraction

11.4.5.1 Add 15 mL of reagent water to the sample, blank, and OPR. Vortex each for 5 min. Adjust the pH of the sample, blank, and OPR aliquots to 10.0 ± 0.5 by adding NH_4OH solution dropwise. Vortex for 5 min. Check and adjust the pH to 10.0 ± 0.5 with NH_4OH solution, vortexing the mixture after each addition.

11.4.5.2 Spike the base fraction (Group 4) native compounds (Section 7.7.2 and Table 10) into one of the QC aliquots. This aliquot will serve as the OPR. The other will serve as the blank.

11.4.5.3 Spike the base fraction (Group 4) labeled compounds (Section 7.7.3 and Table 10) into the samples and QC aliquots.

11.4.5.4 Vortex the samples and QC aliquots. Proceed to Section 12.4 for extraction of the solids base fraction.

11.5 Sample grinding, homogenization, or blending

Samples with particle sizes greater than 1 mm (as determined in Section 11.2) are subjected to grinding, homogenization, or blending. The method of reducing particle size to less than 1 mm is matrix-dependent. In general, hard particles can be reduced by grinding with a mortar and pestle. Softer particles can be reduced by grinding in a Wiley mill or meat grinder, by homogenization, or in a blender.

11.5.1 Each size-reducing preparation procedure on each matrix must be verified by running the tests in Section 9.2 before the procedure is employed routinely.

11.5.2 The grinding, homogenization, or blending procedures must be carried out in a glove box or fume hood to prevent particles from contaminating the work environment.

11.5.3 Grinding – Amorphous and other solids can be ground in a Wiley mill or heavy duty meat grinder. In some cases, reducing the temperature of the sample to freezing or to dry ice or liquid nitrogen temperatures can aid in the grinding process. Grind the sample aliquots in a clean grinder. Do not allow the sample temperature to exceed 50 EC. Also grind the blank and OPR reference matrix aliquots using a clean grinder.

11.5.4 Homogenization or blending – Particles that are not ground effectively, or particles greater than 1 mm in size after grinding, can often be reduced in size by high speed homogenization or blending. Homogenize and/or blend the particles or filter for the sample, blank, and OPR aliquots.

11.5.5 After size reduction, return to Section 11.4 for preparation of the sample and QC aliquots.

12.0 Extraction and Concentration

This method employs solid-phase extraction (SPE) procedures to extract the target analytes from aqueous samples. Solid samples are extracted using ultrasonic extraction with acetonitrile. The extracts from solid samples contain significant amount of coextracted interferences which can be removed through the use of the same SPE procedure employed for the aqueous samples.

12.1 Extraction of aqueous samples absent visible particles, and cleanup of extracts from filtered solids, solids and biosolids samples.

Extraction of both the acid and base fractions of aqueous samples involve many of the same

steps, beginning with the conditioning of the SPE cartridges.

12.1.1 Assemble the SPE extraction apparatus and attach the SPE HLB cartridges (Section 6.5.7).

12.1.2 Condition an SPE HLB cartridge by eluting it with 20 mL of methanol, and 6 mL of reagent water. Discard these eluants. When extracting the base fraction of a sample, the conditioning steps stop here. Do not let the cartridge go dry at any point during the conditioning process.

12.1.3 When extracting the acid fraction of a sample, complete the cartridge conditioning step by eluting the cartridge with 6 mL reagent water at pH 2.0 ± 0.5. Discard this eluant.

12.1.4 Using the SPE cartridge appropriate for the sample fraction (acid or base), load the sample prepared as described in Sections 11.3.3.4 or 11.3.4.4 onto the cartridge at a flow rate of 5-10 mL/min. Extraction of a 1-L aqueous sample will take 100-200 minutes, thus use of a multi-position extraction manifold is desirable.

12.1.5 Once the entire sample has passed through the cartridge, wash the acid fraction cartridge with 10 mL of reagent water to remove the EDTA. Do not wash the cartridge for the base fraction.

12.1.6 Dry the cartridges for either fraction under vacuum for approximately 5 min.

12.2 Cartridge elution

12.2.1 Acid fraction

12.2.1.1 Elute the analytes with 12 mL methanol. Initiate the elution by vacuum and complete the elution by gravity. Collect the eluant in a clean centrifuge tube.

12.2.1.2 If triclocarban and triclosan are analytes of interest, elute these two analytes with 6 mL of acetone:methanol (1:1). Combine with the methanol eluant.

12.2.1.3 Proceed with concentration of the extract (Section 12.5).

12.2.2 Base fraction

12.2.2.1 Elute the analytes with 6 mL methanol followed by 9 mL of 2% formic acid solution (Section 7.4.3.1). Initiate the elution by vacuum and complete the elution by gravity. Collect the eluant in a clean centrifuge tube.

12.2.2.2 Proceed with concentration of the extract (Section 12.5).

12.3 Acid extraction of solid samples

12.3.1 Add 20 mL acetonitrile to the solid sample and the QC aliquots, sonicate for 30 min, and centrifuge for approximately 5 min at approximately 3000 rpm.

12.3.2 Decant the extracts (supernatants) of the sample and the QC aliquots into separate, clean 250-mL round-bottom flasks.

12.3.3 Add 15 mL of phosphate buffer (Section 7.4.1) to the sample and the QC aliquots. Adjust to pH 2.0 ± 0.5 with HCl. Vortex to resuspend the solids. Check and adjust the pH to 2.0 ± 0.5 with buffer, vortexing the mixture after the addition.

12.3.4 Perform a second extraction by repeating Sections 12.3.1 and 12.3.2, adding the extracts to their respective flasks.

12.3.5 For the third extraction, add 15 mL of acetonitrile only to each of the tubes. Sonicate and centrifuge the tubes, and decant the supernatants into their respective round-bottom flasks.

12.3.6 If particles are visible in the extract, filter through a 110-mm or larger GF/A filter. Using squeeze bottles, rinse the filter three times with reagent water, followed by three rinses with acetonitrile.

12.3.7 Proceed with concentration of the acid extract (Section 12.6) followed by SPE in 12.1 and 12.2.

12.4 Base extraction of solid samples

12.4.1 Add 20 mL acetonitrile to the solid sample and QC aliquots, sonicate for 30 min, and centrifuge for approximately 5 min at approximately 3000 rpm.

12.4.2 Decant the extracts (supernatants) of the sample and QC aliquots into separate, clean 250-mL round-bottom flasks.

12.4.3 Add 15 mL of reagent water to the sample and QC aliquots. Add NH_4OH dropwise to the sample and QC aliquots to pH 10.0 ± 0.5. Vortex to resuspend the solids. Check and adjust the pH to 10.0 ± 0.5 with NH_4OH, vortexing the mixture after the addition.

12.4.4 Perform a second extraction by repeating Sections 12.4.1 and 12.4.2, adding the extracts to their respective flasks.

12.4.5 For the third extraction, add 15 mL of acetonitrile only to the centrifuge tubes. Sonicate and centrifuge the tubes, and decant the supernatants into the round-bottom flasks.

12.4.6 If particles are visible in the extract, filter through a 110-mm or larger GF/A filter. Using squeeze bottles, rinse the filter three times with reagent water, followed by three rinses with acetonitrile.

12.4.7 Proceed with concentration of the base extract (Section 12.6) followed by SPE in Section 12.1 and 12.2.

12.5 Concentration of aqueous sample extracts

Extracts from the acid and base fractions of aqueous samples are concentrated separately to near dryness and the solvent exchanged to methanol, as described below. This same procedure is used to concentration the extracts of solid samples after they have been subjected to the SPE HLB cleanup procedure in Sections 12.1 - 12.2.

12.5.1 Concentrate the extract to near dryness under a gentle stream of nitrogen in a water bath held at 50 ± 5 °C.

12.5.2 Add 3 mL of methanol to the concentrated acid and base extracts, including the blank and OPR aliquots.

12.5.3 Spike the acid extracts with the labeled injection acid internal standards and the base extracts with the labeled injection base internal standard (Table 10).

12.5.4 Bring the acid and base extracts to a final volume of 4.0 ± 0.1 mL with 0.1% formic acid solution (Section 7.4.3.2). Vortex to mix.

12.5.5 If visible particles are present in the extract, or if the extract is cloudy, filter through a 0.2-µm filter (Section 6.6.5.

12.5.6 Transfer 1 mL of each extract to an LC/MS/MS autosampler vial for analysis. Store the remaining 3 mL of extract as backup in a refrigerator. (Other proportions of the extract may be used as long as sensitivity is not compromised).

12.5.7 Proceed to Section 14 for analysis.

12.6 Concentration of the solid sample extracts

Extracts from the acid and base fractions of solid samples are concentrated separately prior to cleanup and the extracts are reconstituted into aqueous solutions that are processed through the aqueous sample SPE HLB extraction procedures (Sections 12.1 - 12.2) as a cleanup step.

12.6.1 Concentrate the extracts from the acid and base fractions of the solid samples and QC aliquots separately, to a final volume of 20 - 30 mL by rotary evaporation at 50 °C. Do not allow the extracts to go dry.

12.6.2 Immediately after concentration, add 200 mL of reagent water and 500 mg of NA4EDTA.2H2O to the acid fraction extract. Swirl to mix.

12.6.3 Immediately after concentration, add 200 mL of reagent water to the base fraction extract. Check that the pH is 10.0 ± 0.5. If necessary, adjust dropwise with

NH_4OH solution. Swirl to mix.

12.6.4 Proceed to Section 13 for cleanup of the extracts of all solid samples and associated QC aliquots.

13.0 Extract Cleanup

As noted in Section 12.6, the extracts from all solid samples are subjected to cleanup using the same SPE procedure used to extract aqueous samples. In essence, the solvent extract is reconstituted with reagent water and the pH adjusted to that appropriate for the analytes of interest. The reconstituted sample is processed through the same SPE procedure and the final extract is concentrated and prepared for instrumental analysis. Because the volume of the reconstituted solid sample extract is about 200 mL, the SPE cleanup will take significantly less time than the extraction of a 1-L water sample. Therefore, it is not recommended that aqueous sample extractions and cleanup of solid sample extracts be performed simultaneously on the same extraction manifold.

13.1 The acid fraction extract of each solid sample in Section 12.6.2 is processed through the SPE procedure, beginning at Section 12.1.1 and proceeding through Section 12.2.1.3. Process the associated QC aliquots (blank and OPR) through the cleanup procedure as well.

13.2 The base fraction extract of each solid sample in Section 12.6.3 is processed through the SPE procedure, beginning at Section 12.1.1 and proceeding through Section 12.1.6, and 12.2.2.1 through 12.2.2.2, but omitting Sections 12.1.3 and 12.1.5. Process the associated QC aliquots (blank and OPR) through the cleanup procedure as well.

13.3 After completing the SPE cleanup, concentrate the acid and base extracts of solid samples and QC aliquots separately per Section 12.5 and proceed to Section 14 for analysis.

14.0 LC/MS/MS Analysis

14.1 Establish the same operating conditions established and optimized in Section 10.1 - 10.3 for the calibration appropriate to the fraction and Group to be analyzed. Analysis is performed using positive electrospray ionization (ESI+) for the acid fraction Group 1 and 2 analytes and the base fraction Group 4 analytes. Analysis is performed by ESI– for the acid fraction Group 3 analytes. Retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation for Groups 1, 2, 3, and 4 are given in Tables 3, 5, 7, and 9, respectively.

14.2 Inject the volume of the concentrated extract specific to the Group into the LC/MS/MS instrument. The volume injected must be identical to the volume chosen in Section 10.2.1 and used for calibration in Section 10.3.1.

14.2.1 Start the gradient according to the program appropriate for the Group (see Table 2, 4, 6, or 8 for recommended conditions). Start data collection prior to elution of the first analyte.

14.2.2 Monitor the daughter m/z's for each analyte throughout its retention time window. Where known, monitor m/z's associated with interferents expected to be present.

14.2.3 Stop data collection after elution of the last analyte in each Group. Return the gradient to the initial mixture for analysis of the next sample extract or standard.

15.0 System and Laboratory Performance

15.1 At the beginning of each 12-hour shift during which analyses are performed, LC/MS/MS system performance and calibration are verified for all native and labeled compounds. For these tests, analysis of the CS-3 calibration verification (VER) standard (Section 7.7.5 and Table 11) must be used to verify all performance criteria. Adjustment and/or recalibration (Section 10) must be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, IPRs, and OPRs be analyzed.

15.2 Calibration verification

15.2.1 Inject the VER (CS-3) calibration standard (Table 10) for the Group being analyzed using the procedure in Section 14.

15.2.2 The LC peak representing each native and labeled compound in the VER standard must be present with a S/N of at least 10; otherwise, the LC/MS/MS system must be adjusted and the verification test repeated.

15.2.3 Compute the concentration of the native compounds that have labeled analogs by isotope dilution and the concentration of the native compounds that do not have labeled analogs and of the labeled compounds by the internal standard technique. These concentrations are computed based on the calibration data in Section 10.

15.2.4 For each compound, compare the concentration with the calibration verification limit in Table 12. If all compounds meet the acceptance criteria, calibration has been verified and analysis of standards and sample extracts may proceed. If, however, any compound fails its respective limit, the measurement system is not performing properly. In this event, prepare a fresh calibration standard or correct the problem and repeat the verification (Section 15.2) tests, or recalibrate (Section 10).

15.3 Retention time

15.3.1 The retention times of the native and labeled compounds in the verification test (Section 15.2) must be within ± 15 seconds of the respective retention times in the most recent calibration verification standard.

15.3.2 If the retention time of any compound is not within the limits specified, the LC is not performing properly. In this event, adjust the LC operating conditions and

repeat the verification test (Section 15.3) or recalibrate (Section 10), or replace the LC column and either verify calibration or recalibrate.

15.4 Ongoing precision and recovery

15.4.1 Analyze the extracts of both the acid and base fractions of the ongoing precision and recovery (OPR) aliquots prior to analysis of samples from the same batch.

15.4.2 Compute the percent recovery of each native compound with a labeled analog by isotope dilution (Section 10.4). Compute the percent recovery of each native compound without a labeled analog and of each labeled compound by the internal standard method (Section 10.5).

15.4.3 For the native and labeled compounds, compare the recovery to the OPR limits given in Table 12. If all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may proceed. If, however, any individual concentration falls outside of the range given, the extraction/concentration processes are not being performed properly for that compound. In this event, correct the problem, re-prepare, extract, and clean up the sample batch and repeat the ongoing precision and recovery test (Section 15.4).

15.4.4 If desired, add results that pass the specifications in Section 15.4.3 to initial and previous ongoing data for each compound in each matrix. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory accuracy for each compound in each matrix type by calculating the average percent recovery (R) and the standard deviation of percent recovery (S_R). Express the accuracy as a recovery interval from R ! $2S_R$ to R + $2S_R$. For example, if R = 95% and S_R = 5%, the accuracy is 85 to 105%.

15.5 Blank – Analyze the method blank extracted with each sample batch immediately following analysis of the OPR aliquot to demonstrate that there is no contamination or carryover from the OPR analysis. If native compounds will be carried from the OPR into the method blank, analyze one or more aliquots of solvent between the OPR and the method blank. Results of analysis of the method blank must meet the specifications in Section 9.5.2 before sample analysis may begin.

16.0 Qualitative Determination

A native or labeled compound is identified in a standard, blank, or sample when the criteria in Sections 16.1 through 16.2 are met.

16.1 The signal-to-noise ratio (S/N) at the LC peak maximum for each native compound at its daughter m/z must be greater than or equal to 2.5 for each compound detected in a sample extract, and greater than or equal to 10 in CALs and VER samples for parent to daughter transition except S/N of 3 in CS-1.

16.2 The retention time of the peak for a native compound must be within ± 15 seconds of its RT in the most recent CS-3 standard (Table 11).

16.3 Because of compound RT overlap and the potential for interfering substances, it is possible that all of the identification criteria (Sections 16.1 - 16.2) may not be met. If identification is ambiguous, an experienced spectrometrist (Section 1.5) must determine the presence or absence of the compound.

16.4 If the criteria for identification in Sections 16.1 - 16.2 are not met, the compound has not been identified and the result for that compound may not be reported or used for permitting or regulatory compliance purposes. If interferences preclude identification, a new aliquot of sample must be analyzed. Refer to Section 18 for guidance.

17.0 Quantitative Determination

17.1 Isotope dilution quantitation

17.1.1 By adding a known amount of a labeled compound to every sample prior to extraction, correction for recovery of the native analog of that compound can be made because the native compound and its labeled analog exhibit similar effects upon extraction, concentration, and chromatography. Relative responses (RRs) are used in conjunction with the calibration data in Section 10.4 to determine the concentration in the final extract, as long as labeled compound spiking levels are constant.

17.1.2 Compute the concentration of each compound in the extract using the RR from the calibration data (Section 10.4) and following equation:

$$C_{ex}\ (ng/mL) = \frac{A_n\ C_l}{A_l\ RR}$$

Where:

C_{ex} = Concentration of the compound in the extract , and the other terms are as defined in Section 10.4.3

17.2 Internal standard quantitation and labeled compound recovery

17.2.1 Compute the concentration of each native compound that does not have labeled analog and each labeled compound using the RF from the calibration data (Section 10.5) and the following equation:

$$C_{ex}\ (ng/mL) = \frac{A_s\ C_{is}}{A_{is}\ RF}$$

Where:

C_{ex} = Concentration of the compound in the extract, and the other terms are as defined in Section 10.5.1

17.2.2 Using the concentration in the extract determined above, compute the percent recovery of the labeled compounds using the following equation:

$$\text{Recovery}\ (\%) = \frac{\textit{Concentration found}\ (ng/mL)}{\textit{Concentration spiked}\ (ng/mL)}\ x\ 100$$

17.3 The concentration of a native compound in the solid phase of the sample is computed using the concentration of the compound in the extract and the weight of the solids, as follows:

$$\textit{Concentration in solid sample}\ (ng/kg) = \frac{C_{ex}\,V_{ex}}{W_s}$$

Where:
C_{ex} = Concentration of the compound in the extract.
V_{ex} = Extract volume in mL.
W_s = Sample weight (dry weight) in kg.

If desired, divide the concentration by 1000 to convert ng/kg to µg/kg.

17.4 The concentration of a native compound in the aqueous phase of the sample is computed using the concentration of the compound in the extract and the volume of water extracted, as follows:

$$\textit{Concentration in aqueous phase (ng/L)} = 1000 \times \frac{(C_{ex} \times V_{ex})}{V_s}$$

Where:
C_{ex} = Concentration of the compound in the extract.
V_{ex} = Extract volume in mL.
V_s = Sample volume in liters.

17.5 If the SICP area at the daughter quantitation m/z for any compound exceeds the calibration range of the system, dilute the sample extract by the factor necessary to bring the concentration within the calibration range, adjust the concentration of the labeled injection internal standard to the original concentration in the extract, and analyze an aliquot of this diluted extract. If the compound cannot be measured reliably by isotope dilution, dilute and analyze an aqueous sample or analyze a smaller portion of a solid, or other sample. Adjust the compound concentration, detection limit, and minimum level of quantitation to account for the dilution.

17.6 Reporting of results

17.6.1 Reporting units and levels

17.6.1.1 Aqueous samples – Report results in ng/L (parts-per-trillion).

17.6.1.2 Samples containing solids (aqueous samples containing visible particles, solids, soils, sediments, filter cake, compost) – Report results in µg/kg (parts-per-billion) based on the dry weight of the sample.

Report the percent solids so that the result may be converted to aqueous units.

17.6.2 Reporting level

17.6.2.1 Report the result for each compound in each sample, blank, or standard (VER, IPR, OPR) at or above the minimum level of quantitation (ML; Table 3, 5, 7, or 9) to 3 significant figures. Report the result below the ML in each sample as <ML (where ML is the concentration at the ML) or as required by the regulatory authority or permit.

17.6.2.2 Blanks – Report the result for each compound below the ML but above the MDL to 2 significant figures. Report results below the MDL as <MDL (where MDL is the concentration at the MDL) or as required by the regulatory authority or permit. In addition to reporting results for the samples and blank(s) separately, the concentration of each compound in a method blank or field blank associated with the sample may be subtracted from the results for that sample, or must be subtracted if requested or required by a regulatory authority or in a permit.

17.6.2.3 Results for a compound in a sample that has been diluted are reported at the least dilute level at which the area at the quantitation m/z is within the calibration range (Section 17.5).

17.6.2.4 For a compound having a labeled analog, report results at the least dilute level at which the area at the quantitation m/z is within the calibration range (Section 17.5) and the labeled compound recovery is within the normal range for the method (Section 9.3 and Table 12).

17.6.2.5 Results from tests performed with an analytical system that is not in control must not be reported or otherwise used for permitting or regulatory compliance purposes, but do not relieve a discharger or permittee of reporting timely results.

18.0 Analysis of Complex Samples

18.1 Some samples may contain high levels (>1 μg/L; >1 mg/kg) of the compounds of interest, interfering compounds, and/or polymeric materials. The concentration of analytes and/or interferences in some extracts may overload the LC column and/or mass spectrometer.

18.2 Analyze a smaller aliquot of the sample (Section 17.5) when the interferences preclude analysis of the full sample volume or amount. If a smaller aliquot of a solid, biosolid, or mixed-phase sample is analyzed, attempt to assure that the smaller aliquot is representative.

18.3 Perform integration of peak areas and calculate concentrations manually when interferences preclude computerized calculations.

18.4 Signal suppression – Coextracted interferences in the sample may suppress signals for the compounds of interest. To detect signal suppression, the labeled injection internal standard(s) must be monitored in the analysis. If the signal for the labeled injection internal standard is suppressed by more than 30%, as compared to the average signal for the labeled injection internal standard in the 5-point calibration, the sample must be further cleaned up and reanalyzed. If the sample cannot be cleaned up further, the sample or extract must be diluted, and a diluted sample or extract must be analyzed (Section 17.5).

18.5 Recovery of labeled compounds – For most samples, recoveries of the labeled compounds will be similar to those from reagent water or from the alternate matrix (Section 7.6 and Table 12).

18.5.1 If the recovery of any of the labeled compounds is outside of the normal range (Table 12), a diluted sample must be analyzed (Section 17.5).

18.5.2 If the recovery of any of the labeled compounds in the diluted sample is outside of normal range, the calibration verification standard (Section 7.7.5 and Table 11) must be analyzed and calibration verified (Section 15.2).

18.5.3 If the calibration cannot be verified, a new calibration must be performed and the original sample extract reanalyzed.

18.5.4 If calibration is verified and the diluted sample does not meet the limits for labeled compound recovery, this method does not apply to the sample being analyzed and the result may not be reported or used for permitting or regulatory compliance purposes. In this case, alternate extraction and cleanup procedures in this method or an alternate LC column must be employed to resolve the interference. If all cleanup procedures in this method and an alternate LC column have been employed and labeled compound recovery remains outside of the normal range, extraction and/or cleanup procedures that are beyond this scope of this method will be required to analyze the sample.

19.0 Pollution Prevention

19.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When wastes cannot be reduced at the source, the Agency recommends recycling as the next best option.

19.2 The compounds in this method are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes

consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

19.3 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872-4477.

20.0 Waste Management

20.1 The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is also required with any sewage discharge permits and regulations. An overview of requirements can be found in *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).

20.2 Samples at pH <2, or pH >12 are hazardous and must be neutralized before being poured down a drain, or must be handled as hazardous waste.

20.3 The compounds in this method decompose above 500 EC. Low-level waste such as absorbent paper, tissues, animal remains, and plastic gloves may be burned in an appropriate incinerator. Gross quantities (milligrams) should be packaged securely and disposed of through commercial or governmental channels that are capable of handling toxic wastes.

20.4 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better-Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

21.0 Method Performance

Method 1694 was validated and preliminary data were collected in a single laboratory (Reference 2). Performance data are given in Table 14.

22.0 References

1 EPA Methods 610, 1668A, and 8321A

2 "Analytical Procedure for the Analysis of Pharmaceutical Compounds in Solid and Aqueous Samples by LC-MS/MS," Axys Analytical Services proprietary.

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8 Provost, L.P., and Elder, R.S., "Interpretation of Percent Recovery Data," *American Laboratory*, 15: 56-83, 1983.

9 "Standard Practice for Sampling Water," ASTM Annual Book of Standards, ASTM, 1916 Race Street, Philadelphia, PA 19103-1187, 1980.

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USEPA EMSL, Cincinnati, OH 45268, EPA-600/4-79-019, March 1979.

23.0 Tables and Flowchart

Table 1. Names and CAS Registry numbers for pharmaceuticals and personal-care products (PPCPs) determined by isotope dilution and internal standard HPLC/MS/MS

Compound	CAS Registry	Labeled analog	CAS Registry
Acetaminophen	103-90-2	$^{13}C_2$-^{15}N-Acetaminophen	
Albuterol	18559-94-9	Albuterol-d_3	
Ampicillin	69-53-4		
Anhydrochlortetracycline (ACTC)	4497-08-9		
Anhydrotetracycline (ATC)	4496-85-9		
Azithromycin	83905-01-5		
Caffeine	58-08-2	$^{13}C_3$-Caffeine	
Carbadox	6804-07-5		
Carbamazepine	298-46-4		
Cefotaxime	63527-52-6		
Chlortetracycline (CTC)	57-62-5		
Cimetidine	51481-61-9		
Ciprofloxacin	85721-33-1	$^{13}C_3$-^{15}N-Ciprofloxacin	
Clarithromycin	81103-11-9		
Clinafloxacin	105956-97-6		
Cloxacillin	61-72-3		
Codeine	76-57-3		
Cotinine	486-56-6	Cotinine-d_3	
Dehydronifedipine	67035-22-7		
Demeclocycline	127-33-3		
Digoxigenin	1672-46-4		
Digoxin	20830-75-5		
Diltiazem	42399-41-7		
1,7-Dimethylxanthine	611-59-6		
Diphenhydramine	58-73-1		
Doxycycline	564-25-0		
Enrofloxacin	93106-60-6		
4-Epianhydrochlortetracycline (EACTC)	158018-53-2		
4-Epianhydrotetracycline (EATC)	4465-65-0		
4-Epichlortetracycline (ECTC)	14297-93-9		
4-Epioxytetracycline (EOTC)	14206-58-7		
4-Epitetracycline (ETC)	23313-80-6		
Erythromycin	114-07-8		
Erythromycin anhydrate	59319-72-1	$^{13}C_2$-Erythromycin anhydrate	
Flumequine	42835-25-6		
Fluoxetine	54910-89-3	Fluoxetine-d_5	
Gemfibrozil	25812-30-0	Gemfibrozil-d_6	

Compound	**CAS Registry**	**Labeled analog**	**CAS Registry**
Ibuprofen	15687-27-1	$^{13}C_3$-Ibuprofen	
Isochlortetracycline (ICTC)	514-53-4		
Lincomycin	154-21-2		
Lomefloxacin	98079-51-7		
Metformin	657-24-9	Metformin-d_6	
Miconazole	22916-47-8		
Minocycline	10118-91-8		
Naproxen	22204-53-1	^{13}C-Naproxen-d_3	
Norfloxacin	70458-96-7		
Norgestimate	35189-28-7		
Ofloxacin	82419-36-1		
Ormetoprim	6981-18-6		
Oxacillin	66-79-5		
Oxolinic acid	14698-29-4		
Oxytetracycline (OTC)	79-57-2		
Penicillin V	87-08-1		
Penicillin G	61-33-6		
Ranitidine	66357-35-5		
Roxithromycin	80214-83-1		
Sarafloxacin	98105-99-8		
Sulfachloropyridazine	80-32-0		
Sulfadiazine	68-35-9		
Sulfadimethoxine	122-11-2		
Sulfamerazine	127-79-7		
Sulfamethazine	57-68-1	$^{13}C_6$-Sulfamethazine	
Sulfamethizole	144-82-1		
Sulfamethoxazole	723-46-6	$^{13}C_6$-Sulfamethoxazole	
Sulfanilamide	63-74-1		
Sulfathiazole	72-14-0		
Tetracycline (TC)	60-54-8		
Thiabendazole	148-79-8	Thiabendazole-d_6	
Triclocarban	101-20-2	$^{13}C_6$-Triclocarban	
Triclosan	3380-34-5	$^{13}C_{12}$-Triclosan	
Trimethoprim	738-70-5	$^{13}C_3$-Trimethoprim	
Tylosin	1401-69-0		
Virginiamycin	11006-76-1		
Warfarin	81-81-2	Warfarin-d_5	
Other standards			
Unlabeled compound spiked into sample and used for recovery correction			
Meclocycline			
Labeled injection internal standard spiked into sample extract prior to injection into LC/MS/MS			
		$^{13}C_3$-Atrazine	
		$^{13}C_6$-2,4,5-Trichlorophenoxyacetic acid ($^{13}C_6$-TCPAA)	

Table 2. Group 1 – Acidic extraction, positive electrospray ionization (ESI+) instrument conditions

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

LC Gradient Program		**LC Flow Rate (mL/min)**	**Gradient**	**General LC Conditions**	
Time (min)	**Flow Mixture[1]**			Column Temp	40 °C
0.0	95% Solvent A 5% Solvent B	0.150	1	Flow Rate	0.15 – 0.30 mL/min
4.0	95% Solvent A 5% Solvent B	0.250	6	Max Pressure	345 Bar
22.5	12% Solvent A 88% Solvent B	0.300	6	Autosampler tray temperature	4ºC
23.0	100% Solvent B	0.300	6	**MS Conditions**	
26.0	100% Solvent B	0.300	6	Source Temp	140°C
26.5	95% Solvent A 5% Solvent B	0.150	6	Desolvation Temp	350°C
33.0	95% Solvent A 5% Solvent B	0.150	6	Cone / Desolvation Gas Rate	80 L/hr / 400 L/hr

[1] Solvent A = 0.3% Formic Acid and 0.1% Ammonium Formate in HPLC water
Solvent B = 1:1 Acetonitrile:Methanol

Table 3. Group 1 acidic extraction, positive electrospray ionization (ESI+) compound retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation.

Analyte	RT (min)	Parent-daughter m/zs	Quantitation reference	Detection limits and minimum levels					
				Water (ng/L)		Other (μg/kg)		Extract (ng/ΦL)	
				MDL	ML	MDL	ML	MDL	ML
Group 1	**Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (+) ESI**								
Native compounds									
Sulfanilamide	2.5	190.0 - 155.8	$^{13}C_6$-Sulfamethazine	8.9	50	48	200	2.2	12.5
Cotinine	2.8	177.0 - 98.0	Cotinine-d_3	3.4	5	1.1	5	0.9	1.25
Acetaminophen	4.6	152.2 - 110.0	$^{13}C_2$-^{15}N-Acetaminophen	27	200	35	200	6.7	50
Sulfadiazine	6.0	251.2 - 156.1	$^{13}C_6$-Sulfamethazine	0.4	5	2.7	10	0.1	1.25
1,7-Dimethylxanthine	6.9	181.2 - 124.0	$^{13}C_3$-Caffeine	120	500	270	1000	30	125
Sulfathiazole	7.7	256.3 - 156.0	$^{13}C_6$-Sulfamethoxazole	0.5	5	1.9	50	0.1	1.25
Codeine	8.3	300.0 - 152.0	$^{13}C_3$-Trimethoprim	1.5	10	3.4	10	0.4	2.5
Sulfamerazine	8.7	265.0 - 156.0	$^{13}C_6$-Sulfamethazine	0.3	2	1.4	5	0.1	0.5
Lincomycin	9.3	407.5 - 126.0	$^{13}C_3$-Trimethoprim	0.8	10	4.7	10	0.2	2.5
Caffeine	9.3	195.0 - 138.0	$^{13}C_3$ Caffeine	15	50	5.4	50	3.6	12.5
Sulfamethizole	10.0	271.0 - 156.0	$^{13}C_6$-Sulfamethoxazole	0.4	2	0.88	5	0.1	0.5
Trimethoprim	10.0	291.0 - 230.0	$^{13}C_3$-Trimethoprim	1.1	5	3.3	10	0.3	1.25
Thiabendazole	10.0	202.1 - 175.1	Thiabendazole-d_6	0.7	5	2.1	10	0.2	1.25
Sulfamethazine	10.1	279.0 - 156.0	$^{13}C_6$-Sulfamethazine	0.6	2	0.83	5	0.2	0.5
Cefotaxime	10.2	456.4 - 396.1	$^{13}C_3$-Trimethoprim	10	20	18	50	2.5	5
Carbadox	10.5	263.2 - 231.2	$^{13}C_3$-Trimethoprim	2.3	5	2.1	10	0.6	1.25
Ormetoprim	10.5	275.3 - 259.1	$^{13}C_3$-Trimethoprim	0.3	2	0.50	2	0.1	0.5
Norfloxacin	10.7	320.0 - 302.0	$^{13}C_3$ ^{15}N-Ciprofloxacin	28	50	15	50	7.0	12.5
Sulfachloropyridazine	10.8	285.0 - 156.0	$^{13}C_6$-Sulfamethazine	1.2	5	1.9	5	0.3	1.25
Ofloxacin	10.8	362.2 - 318.0	$^{13}C_3$ ^{15}N-Ciprofloxacin	1.8	5	3.4	10	0.4	1.25
Ciprofloxacin	10.9	332.2 - 314.2	$^{13}C_3$ ^{15}N-Ciprofloxacin	5.1	20	8.1	20	1.3	5
Sulfamethoxazole	11.2	254.0 - 156.0	$^{13}C_6$-Sulfamethoxazole	0.4	2	1.2	5	0.1	0.5
Lomefloxacin	11.2	352.2 - 308.1	$^{13}C_3$ ^{15}N-Ciprofloxacin	4.9	10	4.4	10	1.2	2.5
Enrofloxacin	11.5	360.0 - 316.0	$^{13}C_3$ ^{15}N-Ciprofloxacin	5.2	10	3.1	10	1.3	2.5
Sarafloxacin	11.9	386.0 - 299.0	$^{13}C_3$ ^{15}N-Ciprofloxacin	170	200	--	200	42	12.5
Clinafloxacin	12.1	366.3 - 348.1	$^{13}C_3$ ^{15}N-Ciprofloxacin	6.9	20	14	50	1.7	5

Analyte	RT (min)	Parent-daughter m/zs	Quantitation reference	Detection limits and minimum levels					
				Water (ng/L)		Other (ng/g)		Extract (ng/ΦL)	
				MDL	ML	MDL	ML	MDL	ML
Digoxigenin	12.6	391.2 - 355.2	$^{13}C_3$-Trimethoprim	5.7	20	9.4	20	1.4	5
Oxolinic acid	13.1	261.8 - 243.8	$^{13}C_3$-Trimethoprim	0.6	2	0.62	2	0.2	0.5
Sulfadimethoxine	13.2	311.0 - 156.0	$^{13}C_6$-Sulfamethoxazole	0.1	1	0.55	2	0.03	0.25
Diphenhydramine	14.5	256.8 - 168.1	$^{13}C_3$-Trimethoprim	0.4	2	0.66	2	0.1	0.5
Penicillin G	14.6	367.5 - 160.2	$^{13}C_3$-Trimethoprim	2.4	10	13	50	0.6	2.5
Azithromycin	14.8	749.9 - 591.6	$^{13}C_3$-Trimethoprim	1.3	5	1.6	5	0.3	1.25
Flumeqine	15.2	262.0 - 173.7	$^{13}C_3$-Trimethoprim	2.7	5	1.4	5	0.7	1.25
Ampicillin	15.3	350.3 - 160.2	$^{13}C_3$-Trimethoprim	--	5	--	5	--	1.25
Diltiazem	15.3	415.5 - 178.0	$^{13}C_3$-Trimethoprim	0.6	2	0.30	2	0.2	0.25
Carbamazepine	15.3	237.4 - 194.2	$^{13}C_3$-Trimethoprim	1.4	5	1.6	5	0.4	1.25
Penicillin V	15.4	383.4 - 160.2	$^{13}C_3$-Trimethoprim	4.4	20	19	50	1.1	5
Erythromycin	15.9	734.4 - 158.0	$^{13}C_2$-Erythromycin	--	1	--	2	--	0.25
Tylosin	16.3	916.0 - 772.0	$^{13}C_2$-Erythromycin anhydrate	13	50	8.1	50	3.2	5
Oxacillin	16.4	434.3 - 160.1	$^{13}C_3$-Trimethoprim	3.3	10	9.4	20	0.8	2.5
Dehydronifedipine	16.5	345.5 - 284.1	$^{13}C_3$-Trimethoprim	0.6	2	0.41	2	0.2	0.5
Digoxin	16.6	803.1 - 283.0	$^{13}C_3$-Trimethoprim	--	50	--	100	--	12.5
Fluoxetine	16.9	310.3 - 148.0	Fluoxetine-d_5	3.7	10	2.8	10	0.9	1.25
Cloxcillin	16.9	469.1 - 160.1	$^{13}C_3$-Trimethoprim	4.3	10	9.2	20	0.1	2.5
Virginiamycin	17.3	508.0 - 355.0	$^{13}C_3$-Trimethoprim	3.6	10	3.4	10	0.9	2.5
Clarithromycin	17.5	748.9 - 158.2	$^{13}C_2$-Erythromycin anhydrate	1.0	5	1.2	5	0.3	1.25
Erythromycin anhydrate	17.7	716.4 - 158.0	$^{13}C_2$-Erythromycin anhydrate	0.4	2	0.46	2	0.1	0.25
Roxithromycin	17.8	837.0 - 679.0	$^{13}C_2$-Erythromycin anhydrate	0.2	1	0.22	1	0.05	0.25
Miconazole	20.1	417.0 - 161.0	$^{13}C_3$-Trimethoprim	1.3	5	0.90	5	0.3	1.25
Norgestimate	21.7	370.5 - 124.0	$^{13}C_3$-Trimethoprim	2.5	10	1.4	10	0.6	2.5
Labeled compounds spiked into each sample									
Cotinine-d_3	2.8	180.0 - 79.9	$^{13}C_3$ Atrazine						
$^{13}C_2$-^{15}N-Acetaminophen	4.5	155.2 - 111.0	$^{13}C_3$ Atrazine						
$^{13}C_3$ Caffeine	9.3	198.0 - 140.0	$^{13}C_3$ Atrazine						
Thiabendazole-d_6	9.8	208.1 - 180.1	$^{13}C_3$ Atrazine						
$^{13}C_3$-Trimethoprim	10.0	294.0 - 233.0	$^{13}C_3$ Atrazine						
$^{13}C_6$ Sulfamethazine	10.1	285.1 - 162.0	$^{13}C_3$ Atrazine						
$^{13}C_3$ ^{15}N-Ciprofloxacin	10.9	336.1 - 318.0	$^{13}C_3$ Atrazine						
$^{13}C_6$-Sulfamethoxazole	11.2	260.0 - 162.0	$^{13}C_3$ Atrazine						
$^{13}C_2$-Erythromycin	15.9	736.4 - 160.0	$^{13}C_3$ Atrazine						

Analyte	RT (min)	Parent-daughter m/zs	Quantitation reference	Detection limits and minimum levels					
				Water (ng/L)		Other (Φg/g)		Extract (ng/ΦL)	
				MDL	ML	MDL	ML	MDL	ML
Fluoxetine-d_5	16.8	315.3 - 153.0	$^{13}C_3$ Atrazine						
$^{13}C_2$-Erythromycin anhydrate	17.7	718.4 - 160.0	$^{13}C_3$ Atrazine						
Injection internal standard									
$^{13}C_3$ Atrazine	15.9	219.5 - 176.9 (134.0)	External standard						

Table 4. Group 2 – Acidic extraction positive electrospray ionization (ESI+) instrument conditions

Instrument	Waters 2690 HPLC or Waters 2795 HPLC, Micromass Quattro Ultima MS/MS
LC Column	Waters Xtera C18, 10.0 cm, 2.1 mm i.d., 3.5 µm particle size
Ionization	Positive Ion Electrospray
Acquisition	MRM mode, unit resolution
Injection Volume	5 µL

LC Gradient Program		**LC Flow Rate (mL/min)**	**Gradient**	**General LC Conditions**	
Time (min)	**Flow Mixture[1]**			Column Temp	40 °C
0.0	10% Solvent A 90% Solvent B	0.20	1	Flow Rate	0.20 – 0.23 mL/min
1.0	10% Solvent A 90% Solvent B	0.20	6	Max Pressure	345 Bar
18.0	40% Solvent A 60% Solvent B	0.23	6	Autosampler tray temperature	4ºC
20.0	90% Solvent A 10% Solvent B	0.23	6	**MS Conditions**	
24.0	90% Solvent A 10% Solvent B	0.23	6	Source Temp	120°C
24.3	10% Solvent A 90% Solvent B	0.20	6	Desolvation Temp	400°C
28	10% Solvent A 90% Solvent B	0.20	6	Cone / Desolvation Gas Rate	70 L/hr / 450 L/hr

[1] Solvent A = 1:1 acetonitrile:methanol, with 5 mM Oxalic Acid
Solvent B = HPLC H_2O, with 5 mM Oxalic Acid

Table 5. Group 2 acidic extraction positive electrospray ionization (ESI+) compound retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation.

Analyte	RT (min)	Parent-daughter m/zs	Quantitation reference	Detection limits and minimum levels					
				Water (ng/L)		Other (ng/g)		Extract (ng/μL)	
				MDL	ML	MDL	ML	MDL	ML
Group 2	**Analytes Extracted Under Acidic Conditions and Analyzed Using Positive Electrospray Ionization (+) ESI.**								
Native compounds									
Minocycline	5.1	458.0 - 441.0	Thiabendazole-d_6	51	200	--	200	13	50
Epitetracycline	8.1	445.2 - 410.2	Thiabendazole-d_6	3.6	20	8.6	20	0.9	5
Epioxytetracycline (EOTC)	8.6	461.2 - 426.2	Thiabendazole-d_6	4.1	20	18	50	1.0	5
Oxytetracycline (OTC)	9.4	461.2 - 426.2	Thiabendazole-d_6	2.1	20	2.2	20	0.5	5
Tetracycline (TC)	9.9	445.2 - 410.2	Thiabendazole-d_6	1.9	20	2.8	20	0.5	5
Demeclocycline	11.7	465.0 - 430.0	Thiabendazole-d_6	6.6	50	7.9	50	1.7	12.5
Isochlortetracycline (ICTC) [1]	11.9	479.0 - 462.2	Thiabendazole-d_6	1.7	20	3.5	20	0.4	5
Epichlortetracycline (ECTC) [1]	12.0	479.0 - 444.0	Thiabendazole-d_6	7.7	50	26	100	1.9	12.5
Chlortetracycline (CTC)	14.1	479.0 - 444.0	Thiabendazole-d_6	1.2	20	2.3	20	0.3	5
Doxycycline	16.7	445.2 - 428.2	Thiabendazole-d_6	2.8	20	2.3	20	0.7	5
Epianhydrotetracycline (EATC)	17.0	426.8 - 409.8	Thiabendazole-d_6	7.7	50	14	50	1.9	12.5
Anhydrotetracycline (ATC)	18.8	426.8 - 409.8	Thiabendazole-d_6	4.6	50	7.1	50	1.2	12.5
Epianhydrochlortetracycline (EACTC)	20.7	461.2 - 444.0	Thiabendazole-d_6	28	200	23	200	7.0	50
Anhdrochlortetracycline (ACTC)	22.1	461.2 - 444.0	Thiabendazole-d_6	5.2	50	11	50	1.3	12.5
Labeled compound spiked into each sample									
Thiabendazole-d_6	7.0	208.1 - 180.1	$^{13}C_3$ Atrazine						
Injection internal standard									
$^{13}C_3$ Atrazine	10.5	219.5 - 176.9 (134.0)	External standard						

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

Table 6. Group 3 – Acidic extraction negative electrospray ionization (ESI-) instrument conditions

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

LC Gradient Program		**LC Flow Rate (mL/min)**	**Gradient**	**General LC Conditions**	
Time (min)	**Flow Mixture[1]**			**Column Temp**	**40°C**
0.0	60% Solvent A, 40% Solvent B	0.2	1	Flow Rate	0.200 mL/min
0.5	60% Solvent A, 40% Solvent B	0.2	6	Max Pressure	345 Bar
7.0	100% Solvent B	0.2	6	Autosampler tray temperature	4ºC
12.5	100%Solvent B	0.2	6	**MS Conditions**	
12.7	60% Solvent A, 40% Solvent B	0.2	6	Source Temp	100°C
16.0	60% Solvent A, 40% Solvent B	0.2	1	Desolvation Temp	350°C
				Cone / Desolvation Gas Rate	50L/hr / 300 L/hr

1. Solvent A = 0.1% Ammonium Acetate and 0.1% Acetic Acid in HPLC water
   Solvent B = 1:1 MethanolAcetonitrile

Table 7. Group 3 acidic extraction negative electrospray ionization (ESI-) compound retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation

Analyte	RT (min)	Parent- daughter m/zs	Quantitation reference	Detection limits and minimum levels					
				Water (ng/L)		Other (Φg/g)		Extract (ng/ΦL)	
				MDL	ML	MDL	ML	MDL	ML
Group 3	**Analytes Extracted Under Acidic Conditions and Analyzed Using Negative Electrospray Ionization (-) ESI.**								
Native compounds									
Naproxen	6.7	228.9 - 168.6	^{13}C-Naproxen-d_3	3.9	10	6.1	20	1.0	2.5
Warfarin	7.1	307.0 - 117.0	Warfarin-d_5	0.9	5	1.6	5	0.2	1.25
Ibuprofen	8.4	205.1 - 161.1	$^{13}C_3$-Ibuprofen	6.0	50	11	50	1.5	12.5
Gemfibrozil	9.5	249.0 - 121.0	Gemfibrozil-d_6	0.8	5	1.2	5	0.2	1.25
Triclocarban	9.6	312.9 - 159.7	$^{13}C_6$-Triclocarban	2.1	10	2.7	10	0.5	2.5
Triclosan	9.7	286.8 - 35.0	$^{13}C_{12}$-Triclosan	92	200	56	200	23	50
Labeled compounds spiked into samples									
^{13}C-Naproxen-d_3	6.6	232.9 - 168.6	$^{13}C_6$-TCPAA						
Warfarin-d_5	7.0	312.0 - 161.0	$^{13}C_6$-TCPAA						
$^{13}C_3$-Ibuprofen	8.5	208.2 - 163.1	$^{13}C_6$-TCPAA						
Gemfibrozil-d_6	9.5	255.0 - 121.0	$^{13}C_6$-TCPAA						
$^{13}C_6$-Triclocarban	9.6	318.9 - 159.7	$^{13}C_6$-TCPAA						
$^{13}C_{12}$-Triclosan	9.7	298.8 - 35.0	$^{13}C_6$-TCPAA						
Injection Internal Standard									
$^{13}C_6$-TCPAA	4.9	258.8 - 200.7	External standard						

Table 8. Group 4 – Basic extraction positive electrospray ionization (ESI+) instrument conditions

Instrument	Waters 2690 HPLC or Waters 2795 HPLC, Micromass Quattro Ultima MS/MS
LC Column	Waters Atlantis HILIC, 10 cm, 2.1 mm i.d., 3.0 µm particle size
Ionization	Positive Ion Electrospray
Acquisition	MRM mode, unit resolution
Purge Solvent	100% CH_3CN (changed from H_2O)
Injection Volume	2.0 µL

LC Gradient Program		**LC Flow Rate (mL/min)**	**Gradient**	**General LC Conditions**	
Time (min)	**Flow Mixture[1]**			**Column Temp**	**40 °C**
0.0	2% Solvent A 98% Solvent B	0.25	1	Flow Rate	0.25 mL/min
5.0	30% Solvent A 70% Solvent B	0.25	6	Max Pressure	345 Bar
12.0	30% Solvent A 70% Solvent B	0.25	6	Autosampler tray temperature	4ºC
12.5	2% Solvent A 98% Solvent B	0.25	6	**MS Conditions**	
16.0	2% Solvent A 98% Solvent B	0.25	6	Source Temp	120°C
				Desolvation Temp	350°C
				Cone / Desolvation Gas Rate	70L/hr / 400 L/hr

1. Solvent A = 0.1% Acetic Acid/Ammonium Acetate Buffer
   Solvent B = Acetonitrile

Table 9. Group 4 basic extraction positive electrospray ionization (ESI+) compound retention times (RTs), parent-daughter transitions, quantitation references, method detection limits, and minimum levels of quantitation

Analyte	RT (min)	Parent-daughter m/zs	Quantitation reference	Detection limits and minimum levels					
				Water (ng/L)		Other (ng/g)		Extract (ng/ΦL)	
				MDL	ML	MDL	ML	MDL	ML
Group 4	**Analytes Extracted Under Basic Conditions and Analyzed Using Positive Electrospray Ionization (+) ESI**								
Native compounds									
Cimetidine	6.9	253.1 - 159.0	Albuterol-d_3	0.6	2	0.78	2	0.2	0.5
Albuterol	9.4	240.0 - 148.0	Albuterol-d_3	0.9	2	0.39	2	0.2	0.5
Ranitidine	10.3	315.0 - 175.9	Albuterol-d_3	0.7	2	1.1	2	0.2	0.5
Metformin	11.0	131.1 - 60.1	Metformin-d_6	23	100	38	100	5.8	25
Labeled compounds spiked into samples									
Albuterol-d_3	9.4	243.0 - 151.0	Cotinine-d_3						
Metformin-d_6	11.0	285.1 - 162.0	Cotinine-d_3						
Injection internal standard									
Cotinine-d_3	5.9	180.0 - 79.9	External standard						
13C3-Atrazine	2.0	219.5 - 176.9 (134.0)	External Standard						

Table 10. Nominal concentrations of native compounds, labelled compounds, and instrument internal standard solutions [1]

Compound Name	**Spiking solution concentration (µg/mL)**	**Typical amount spiked into sample (ng)**
Native compound spike solutions for acid extracted analytes (Groups 1 and 3)		**(Typical spiking volume into sample: 30 µL)**
Acetaminophen	100	3000
Azithromycin	2.5	75
Caffeine	25	750
Carbodox	2.5	75
Carbamazapine	2.5	75
Cefotaxime	10	300
Clarithromycin	2.5	75
Cloxacillin	5	150
Codeine	5	150
Cotinine	2.5	75
Dehydronifedipine (Oxidized Nifedipine)	1	30
Diphenhydramine	1	30
Diltiazem	0.5	15
Digoxin	25	750
Digoxigenin	10	300
Erythromycin	0.5	15
Flumequine	2.5	75
Fluoxetine	2.5	75
Lincomycin	5	150
Miconazole	2.5	75
Norgestimate	5	150
Ormetoprim	1	30
Oxacillin	5	150
Oxolinic acid	1	30
Penicillin G	5	150
Penicillin V	5	150
Roxithromycin	0.5	15
Sulfachloropyridazine	2.5	75
Sulfadiazine	2.5	75
Sulfadimethoxine	0.5	15
Sulfamerazine	1	30
Sulfamethazine	1	30
Sulfamethizole	1	30
Sulfamethoxazole	1	30
Sulfanilamide	25	750

Compound Name	**Spiking solution concentration (μg/mL)**	**Typical amount spiked into sample (ng)**
Sulfathiazole	2.5	75
Thiabendazole	2.5	75
Trimethoprim	2.5	75
Tylosin	10	300
Virginiamycin	5	150
1,7-Dimethylxanthine	250	7500
Ampicillin	2.5	75
Ciprofloxacin	8.75	263
Clinafloxacin	10	300
Enrofloxacin	5	150
Lomefloxacin	5	150
Norfloxacin	25	750
Ofloxacin	2.5	75
Sarafloxacin	22.8	684
Gemfibrozil	2.5	75
Ibuprofen	25	750
Naproxen	5	150
Triclocarban	5	150
Triclosan	100	3000
Warfarin	2.5	75
Native compound spike solutions for tetracyclines (Group 2)		**(Typical spiking volume into sample: 200 µL)**
Tetracycline (TC)	0.5	100
Oxytetracycline (OTC)	0.5	100
Doxycycline	0.5	100
Chlortetracycline (CTC)	0.5	100
Anhydrochlortetracycline (ACTC)	1.25	250
Anhydrotetracycline (ATC)	1.25	250
4-Epianhydrochlortetracycline (EACTC)	5	1000
4-Epianhydrotetracycline (EATC)	1.25	250
4-Epichlortetracycline (ECTC)	1.25	250
4-Epioxytetracycline (EOTC)	0.5	100
4-Epitetracycline (ETC)	0.5	100
Isochlortetracycline (ICTC)	0.5	100
Demeclocycline	1.25	250
Minocycline	5	1000
Native compound spike solutions for base extracted analytes (Group 4)		**(Typical spiking volume into sample: 15 µL)**
Albuterol	1	15
Cimetidine	2	30

Compound Name	**Spiking solution concentration (μg/mL)**	**Typical amount spiked into sample (ng)**
Metformin	100	1500
Ranitidine	2	30
Labeled compound solutions for acid extracted analytes (Groups 1, 2 and 3)		**(Typical spiking volume into sample: 100 µL)**
Meclocycline	8	800
d_{10}-Carbamazepine-10,11-epoxide	2	200
d_3-Cotinine	2	200
d_5-Fluoxetine	1	100
d_6-Gemfibrozil	1	100
$^{13}C_2$, ^{15}N-Acetaminophen	4	400
$^{13}C_6$-Sulfamethoxazole	1	100
^{13}C, d_3-Naproxen	3	300
$^{13}C_6$-Triclocarban	0.5	50
$^{13}C_3$-Trimethoprim	1	100
d_6-Thiabendazole	1	100
$^{13}C_3$-Caffeine	3	300
$^{13}C_2$-Erythromycin	1	100
$^{13}C_{12}$-Triclosan	4	400
d_5-Warfarin	1	100
$^{13}C_6$-Sulfamethazine	1	100
$^{13}C_3$, ^{15}N-Ciprofloxacin	4	400
$^{13}C_3$-Ibuprofen	4	400
Labeled compound solutions for base extracted analytes (Group 4)		**(Typical spiking volume into sample: 100 µL)**
d_3-Albuterol	1	100
d_6-Metformin	4	400
Instrument internal standard solutions for acid extracted analytes (Groups 1, 2 and 3)		**(Typical spiking volume into extract: 80 µL)**
$^{13}C_3$-Atrazine	2.5	200
$^{13}C_6$-2,4,5-Trichlorophenoxyacetic acid	2.5	200
Instrument internal standard solutions for base extracted analytes (Group 4)		**(Typical spiking volume into extract: 100 µL)**
$^{13}C_3$-Atrazine	2	200
d_3-Cotinine	2	200

1. See Sections 7.8 – 7.9 for solution details

Tables 11a-c. Concentrations of calibration solutions (ng/mL)

Table 11a Concentrations of calibration standards for Group 1 and Group 3 compounds (ng/mL) (Acid extraction, positive and negative ESI). CS=calibration standard.

Compound	CS-1	CS-2	CS-3 (VER)	CS-4	CS-5
Acetaminophen	50	150	750	2500	10000
Azithromycin	1.25	3.75	18.7	62.5	250
Caffeine	12.5	37.5	187.	625	2500
Carbadox	1.25	3.75	18.7	62.5	250
Carbamazapine	1.25	3.75	18.7	62.5	250
Cefotaxime	5	15	75	250	1000
Clarithromycin	1.25	3.75	18.7	62.5	250
Cloxacillin	2.5	7.5	37.5	125	500
Codeine	2.5	7.5	37.5	125	500
Cotinine	1.25	3.75	18.7	62.5	250
Dehydronifedipine (Oxidized Nifedipine)	0.5	1.5	7.5	25	100
Diphenhydramine	0.5	1.5	7.5	25	100
Diltiazem	0.25	0.75	3.75	12.5	50
Digoxin	12.5	37.5	187	625	2500
Digoxigenin	5	15	75	250	1000
Erythromycin	0.25	0.75	3.75	12.5	50
Erythromycin anhydrate	0.25	0.75	3.75	12.5	50
Flumequine	1.25	3.75	18.7	62.5	250
Fluoxetine	1.25	3.75	18.7	62.5	250
Lincomycin	2.5	7.5	37.5	125	500
Miconazole	1.25	3.75	18.7	62.5	250
Norgestimate	2.5	7.5	37.5	125	500
Ormetoprim	0.5	1.5	7.5	25	100
Oxacillin	2.5	7.5	37.5	125	500
Oxolinic acid	0.5	1.5	7.5	25	100
Penicillin G	2.5	7.5	37.5	125	500
Penicillin V	5	15	75	250	1000
Roxithromycin	0.25	0.75	3.75	12.5	50
Sulfachloropyridazine	1.25	3.75	18.7	62.5	250
Sulfadiazine	1.25	3.75	18.7	62.5	250
Sulfadimethoxine	0.25	0.75	3.75	12.5	50
Sulfamerazine	0.5	1.5	7.5	25	100
Sulfamethazine	0.5	1.5	7.5	25	100
Sulfamethizole	0.5	1.5	7.5	25	100
Sulfamethoxazole	0.5	1.5	7.5	25	100
Sulfanilamide	12.5	37.5	187.5	625	2500

Compound	**CS-1**	**CS-2**	**CS-3 (VER)**	**CS-4**	**CS-5**
Sulfathiazole	1.25	3.75	18.7	62.5	250
Thiabendazole	1.25	3.75	18.7	62.5	250
Trimethoprim	1.25	3.75	18.7	62.5	250
Tylosin	5	15	75	250	1000
Virginiamycin	2.5	7.5	37.5	125	500
1,7-Dimethylxanthine	125	375	1870	6250	25000
Ampicillin	1.25	3.75	18.7	62.5	250
Ciprofloxacin	4.4	13.1	65.6	218.	875
Clinafloxacin	5	15	75	250	1000
Enrofloxacin	2.5	7.5	37.5	125	500
Lomefloxacin	2.5	7.5	37.5	125	500
Norfloxacin	12.5	37.5	187	625	2500
Ofloxacin	1.25	3.75	18.7	62.5	250
Sarafloxacin	11.4	34.2	171	570	2280
Gemfibrozil	1.25	3.75	18.7	62.5	250
Ibuprofen	12.5	37.5	187	625	2500
Naproxen	2.5	7.5	37.5	125	500
Triclocarban	2.5	7.5	37.5	125	500
Triclosan	50	150	750	2500	10000
Warfarin	1.25	3.75	18.7	62.5	250
Labeled compounds					
d_3-Cotinine	50	50	50	50	50
d_5-Fluoxetine	25	25	25	25	25
d_6-Gemfibrozil	25	25	25	25	25
$^{13}C_2$, ^{15}N-Acetaminophen	100	100	100	100	100
$^{13}C_6$-Sulfamethoxazole	25	25	25	25	25
^{13}C-d_3-Naproxen	75	75	75	75	75
$^{13}C_6$-Triclocarban	12.5	12.5	12.5	12.5	12.5
$^{13}C_3$-Trimethoprim	25	25	25	25	25
d_6-Thiabendazole	25	25	25	25	25
$^{13}C_3$-Caffeine	75	75	75	75	75
$^{13}C_2$-Erythromycin	25	25	25	25	25
$^{13}C_{12}$-Triclosan	90	90	90	90	90
d_5-Warfarin	25	25	25	25	25
$^{13}C_6$-Sulfamethazine	25	25	25	25	25
$^{13}C_3$, ^{15}N-Ciprofloxacin	100	100	100	100	100
$^{13}C_3$-Ibuprofen	100	100	100	100	100

Compound	CS-1	CS-2	CS-3 (VER)	CS-4	CS-5
Instrument internal standards					
$^{13}C_3$-Atrazine	50	50	50	50	50
$^{13}C_6$-2,4,5-Trichlorophenoxyacetic acid	50	50	50	50	50

Table 11b Concentrations of calibration standards for Group 2 compounds (ng/mL) (Acid extraction, positive ESI). CS=calibration standard.

Compound name	CS-1	CS-2	CS-3 (VER)	CS-4	CS-5
Tetracycline (TC)	5	12.5	25	50	150
Oxytetracycline (OTC)	5	12.5	25	50	150
Doxycycline	5	12.5	25	50	150
Chlortetracycline (CTC)	5	12.5	25	50	150
Anhydrochlortetracycline (ACTC)	12.5	31.25	62.5	125	375
Anhydrotetracycline (ATC)	12.5	31.25	62.5	125	375
4-Epianhydrochlortetracycline (EACTC)	50	125	250	500	1500
4-Epianhydrotetracycline (EATC)	12.5	31.2	62.5	125	375
4-Epichlortetracycline (ECTC)	12.5	31.2	62.5	125	375
4-Epioxytetracycline (EOTC)	5	12.5	25	50	150
4-Epitetracycline (ETC)	5	12.5	25	50	150
Isochlortetracycline (ICTC)	5	12.5	25	50	150
Demeclocycline	12.5	31.2	62.5	125	375
Minocycline	50	125	250	500	1500
Labeled compounds					
d_3-Cotinine	50	50	50	50	50
d_5-Fluoxetine	25	25	25	25	25
d_6-Gemfibrozil	25	25	25	25	25
$^{13}C_2$, ^{15}N-Acetaminophen	100	100	100	100	100
$^{13}C_6$-Sulfamethoxazole	25	25	25	25	25
^{13}C, d_3-Naproxen	75	75	75	75	75
$^{13}C_6$-Triclocarban	12.5	12.5	12.5	12.5	12.5
$^{13}C_3$-Trimethoprim	25	25	25	25	25
d_6-Thiabendazole[1]	25	25	25	25	25
$^{13}C_3$-Caffeine	75	75	75	75	75
$^{13}C_2$-Erythromycin	25	25	25	25	25
$^{13}C_{12}$-Triclosan	90	90	90	90	90
d_5-Warfarin	25	25	25	25	25
$^{13}C_6$-Sulfamethazine	25	25	25	25	25
$^{13}C_3$, ^{15}N-Ciprofloxacin	100	100	100	100	100
$^{13}C_3$-Ibuprofen	100	100	100	100	100
Instrument internal standards					

Compound name	CS-1	CS-2	CS-3 (VER)	CS-4	CS-5
$^{13}C_3$-Atrazine[1]	50	50	50	50	50
$^{13}C_6$-2,4,5-Trichlorophenoxyacetic acid	50	50	50	50	50

1. Note: The Group 2, acid extracted positive ESI (tetracyclines) contains the same labeled compounds as for Group 1 and 3, acid extracted positive and negative ESI, yet the only labeled compounds used in determination of the Group 2 are Thiabendazole-d6 and 13C3-Atrazine. This minimizes the work required to prepare solutions. Some of those surrogates are used to quantify the Group 1 and 2 and some Group 3 in separate runs of the same extract. This is not a requirement.

Table 11c Concentrations of calibration standards for Group 4 (ng/mL) compounds (Base extraction, positive ESI). CS=calibration standard.

Compound name	CS-1	CS-2	CS-3 (VER)	CS-4	CS-5
Albuterol	0.25	0.75	3.75	12.5	50
Cimetidine	0.5	1.5	7.5	25	100
Metformin	25	75	375	1250	5000
Ranitidine	0.5	1.5	7.5	25	100
Labeled compounds					
d_3-Albuterol	25	25	25	25	25
d_6-Metformin	100	100	100	100	100
Instrument internal standards					
$^{13}C_3$-Atrazine	50	50	50	50	50
d_3-Cotinine	50	50	50	50	50

Table 12. QC acceptance criteria for PPCPs in VER, IPR, OPR, and samples.

Compound	VER (%)	IPR RSD (%)	IPR X (%)	OPR (%)	Labeled compound recovery in samples (%)
Acetaminophen	70 - 130	30	55 - 108	50 - 120	
Albuterol	70 - 130	30	55 - 120	50 - 133	
Ampicillin	70 - 130	70	6 - 180	5 - 200	
Anhydrochlortetracycline (ACTC)	70 - 130	30	55 - 121	50 - 135	
Anhydrotetracycline (ATC)	70 - 130	30	8 - 127	7 - 141	
Azithromycin	70 - 130	30	36 - 108	33 - 120	
Caffeine	70 - 130	30	55 - 111	50 - 124	
Carbadox	70 - 130	30	36 - 130	33 - 144	
Carbamazepine	70 - 130	30	23 - 123	21 - 137	
Cefotaxime	70 - 130	36	9 - 168	8 - 186	
Chlortetracycline (CTC)	70 - 130	31	49 - 155	45 - 172	
Cimetidine	70 - 130	47	6 - 108	5 - 120	
Ciprofloxacin	70 - 130	30	55 - 108	50 - 120	
Clarithromycin	70 - 130	30	8 - 139	8 - 154	
Clinafloxacin	70 - 130	37	6 - 180	5 - 200	
Cloxacillin	70 - 130	30	6 - 180	5 - 200	
Codiene	70 - 130	30	37 - 116	34 - 129	
Cotinine	70 - 130	30	55 - 112	50 - 124	
Dehydronifedipine	70 - 130	30	47 - 108	42 - 120	
Demeclocycline	70 - 130	30	6 - 180	5 - 200	
Digoxigenin	70 - 130	30	8 - 165	8 - 183	
Digoxin	70 - 130	45	6 - 133	5 - 148	
Diltiazem	70 - 130	48	13 - 108	11 - 120	
1,7-Dimethylxanthine	70 - 130	30	55 - 124	50 - 138	
Diphenhydramine	70 - 130	30	53 - 108	48 - 120	
Doxycycline	70 - 130	30	24 - 149	22 - 166	
Enrofloxacin	70 - 130	30	55 - 113	50 - 125	
4-Epianhydrochlortetracycline (EACTC)	70 - 130	30	20 - 108	18 - 120	
4-Epianhydrotetracycline (EATC)	70 - 130	30	6 - 180	5 - 200	
4-Epichlortetracycline (ECTC)	70 - 130	30	55 - 135	50 - 150	
4-Epioxytetracycline (EOTC)	70 - 130	30	55 - 127	50 - 142	
4-Epitetracycline (ETC)	70 - 130	30	55 - 156	50 - 173	
Erythromycin hydrate	70 - 130	30	55 - 142	50 - 158	
Flumequine	70 - 130	30	39 - 180	36 - 200	
Fluoxetine	70 - 130	30	54 - 112	49 - 125	
Gemfibrozil	70 - 130	30	55 - 108	50 - 120	
Ibuprofen	70 - 130	30	55 - 108	50 - 120	
Isochlortetracycline (ICTC)	70 - 130	30	6 - 180	5 - 200	
Lincomycin	70 - 130	60	6 - 108	5 - 120	
Lomefloxacin	70 - 130	33	19 - 180	17 - 200	
Metformin	70 - 130	30	55 - 134	50 - 149	
Miconazole	70 - 130	30	29 - 108	27 - 120	
Minocycline	70 - 130	30	6 - 159	5 - 176	

		IPR			
Naproxen	70 - 130	30	55 - 108	50 - 120	
Norfloxacin	70 - 130	30	55 - 121	50 - 135	
Norgestimate	70 - 130	30	39 - 108	36 - 120	
Ofloxacin	70 - 130	30	55 - 180	50 - 200	
Ormetoprim	70 - 130	30	55 - 108	50 - 120	
Oxacillin	70 - 130	30	6 - 180	5 - 200	
Oxolinic acid	70 - 130	30	46 - 112	42 - 124	
Oxytetracycline (OTC)	70 - 130	30	55 - 165	50 - 183	
Penicillin V	70 - 130	30	6 - 180	5 - 200	
Penicillin G	70 - 130	30	6 - 180	5 - 200	
Ranitidine	70 - 130	41	26 - 144	24 - 160	
Roxithromycin	70 - 130	30	42 - 108	38 - 120	
Sarafloxacin	70 - 130	32	18 - 180	17 - 200	
Sulfachloropyridazine	70 - 130	30	55 - 180	50 - 200	
Sulfadiazine	70 - 130	30	6 - 180	5 - 200	
Sulfadimethoxine	70 - 130	30	55 - 108	50 - 120	
Sulfamerazine	70 - 130	30	55 - 133	50 - 148	
Sulfamethazine	70 - 130	30	55 - 128	50 - 142	
Sulfamethizole	70 - 130	30	55 - 108	50 - 120	
Sulfamethoxazole	70 - 130	30	55 - 108	50 - 120	
Sulfanilamide	70 - 130	71	6 - 170	5 - 189	
Sulfathiazole	70 - 130	30	45 - 108	41 - 120	
Tetracycline (TC)	70 - 130	30	55 - 139	50 - 155	
Thiabendazole	70 - 130	30	55 - 108	50 - 120	
Triclocarban	70 - 130	30	55 - 108	50 - 120	
Triclosan	70 - 130	30	55 - 108	50 - 120	
Trimethoprim	70 - 130	30	55 - 114	50 - 126	
Tylosin	70 - 130	30	17 - 134	16 - 149	
Virginiamycin	70 - 130	33	6 - 170	5 - 189	
Warfarin	70 - 130	30	55 - 108	50 - 120	
$^{13}C_2$-^{15}N-Acetaminophen	70 - 130	30	6 - 180	5 - 200	19 – 200
Albuterol-d3	70 - 130	30	38 - 109	35 - 121	39 – 141
$^{13}C_3$-Caffeine	70 - 130	46	6 - 180	5 - 200	31 – 200
$^{13}C_3$-^{15}N-Ciprofloxacin	70 - 130	34	6 - 180	5 - 200	37 – 181
Cotinine-d3	70 - 130	84	6 - 108	5 - 120	5 – 145
$^{13}C_2$-Erythromycin hydrate	70 - 130	30	55 - 108	50 - 120	23 – 120
Fluoxetine-d5	70 - 130	30	55 - 113	50 - 126	40 – 148
Gemfibrozil-d6	70 - 130	30	42 - 110	38 - 122	21 – 123
$^{13}C_3$-Ibuprofen	70 - 130	30	31 - 109	28 - 122	29 – 127
Metformin-d6	70 - 130	30	6 - 127	5 - 141	5 – 200
^{13}C-Naproxen-d3	70 - 130	30	37 - 118	34 - 131	14 – 132
$^{13}C_6$-Sulfamethazine	70 - 130	30	6 - 141	5 - 157	12 – 120
$^{13}C_6$-Sulfamethoxazole	70 - 130	30	55 - 131	50 - 146	40 – 129
Thiabendazole-d6 (A Pos)	70 - 130	30	55 - 132	50 - 146	32 – 140
Thiabendazole-d6 (TCY)	70 - 130	30	55 - 108	50 - 120	30 – 132
$^{13}C_6$-Triclocarban	70 - 130	30	6 - 155	5 - 172	5 – 147

		IPR			
$^{13}C_{12}$-Triclosan	70 - 130	30	6 - 151	5 - 168	5 – 153
$^{13}C_{3}$-Trimethoprim	70 - 130	30	55 - 162	50 - 180	50 – 172
Warfarin-d5	70 - 130	30	55 - 159	50 - 177	50 – 200

Table 13. Suggested sample quantities to be extracted for various matrices [1]

Sample matrix[2]	**Example**	**Percent solids**	**Phase**	**Quantity extracted**
Single-phase				
Aqueous	Drinking water	No visible particles	Aqueous	1000 mL
	Groundwater			
	Treated wastewater			
Solid	Dry soil	>20	Solid	1 g
	Filter cake			
	Compost			
Multi-phase				
Liquid/Solid				
Aqueous/solid[2]	Wet soil	1 - 30	Aqueous and solid	1 g
	Untreated effluent	1 - 5		
	Municipal sludge	1 - 30		0.25 g

1. The quantity of sample to be extracted is adjusted to provide 1 g of solids (dry weight). One liter of aqueous samples containing 0.1% solids will contain 1 gram of solids. For aqueous samples containing greater than 0.1% solids, a lesser volume is used so that 1 gram of solids (dry weight) will be prepared.
2. 1 g of solids (0.25 g for biosolids), or 5 g wet weight if solids content is <20%.

Table 14. Performance Data from single laboratory validation.

Analyte	Solid-Based on 5 samples			Reagent Water-Based on 5 samples			Biosolids-Based on 6 samples		
	Solids Average Recovery	Solids Standard Deviation	Solids Relative Standard Deviation	Water Average Recovery	Water Standard Deviation	Water Relative Standard Deviation	Biosolids Average Recovery	Biosolids Standard Deviation	Biosolids Relative Standard Deviation
Group 3 acidic extraction ESI-									
Warfarin	90.76	5.96	6.57	86.52	3.59	4.15	119.64	13.67	11.42
Ibuprofen	103.16	3.63	3.52	97.43	3.70	3.80	93.82	7.96	8.48
Gemfibrozil	97.94	2.84	2.90	98.11	2.45	2.50	78.35	21.19	27.05
Naproxen	96.57	6.23	6.45	95.41	5.03	5.27	99.94	10.10	10.10
Triclocarban	100.60	2.36	2.34	106.09	4.81	4.53	265.00	190.08	71.73
Triclosan	97.52	6.78	6.95	93.14	3.35	3.60	359.73	500.34	139.09
d5-Warfarin	113.44	8.21	7.23	143.13	8.22	5.74	145.03	23.97	16.52
13C3-Ibuprofen	59.25	2.34	3.95	90.20	8.04	8.91	74.30	21.35	28.74
d6-Gemfibrozil	65.37	2.85	4.36	94.94	3.16	3.33	65.80	28.35	43.09
13C-d3-Naproxen	65.52	5.45	8.31	98.95	3.99	4.03	55.18	20.45	37.06
13C6-Triclocarban	20.36	1.38	6.78	100.55	3.10	3.09	54.18	36.10	66.62
13C12-Triclosan	42.75	3.75	8.77	108.28	5.80	5.36	71.62	34.48	48.15
Group 1 acidic extraction ESI+									
Acetaminophen	104.50	3.53	3.38	100.85	1.17	1.16	94.25	4.97	5.27
Azithromycin	66.08	11.99	18.15	60.95	6.80	11.15	86.53	25.39	29.34
Caffeine	96.41	10.08	10.45	99.14	6.38	6.44	86.08	5.95	6.91
Carbadox	107.42	3.78	3.52	69.50	7.48	10.76	52.91	13.50	25.52
Carbamazepine	98.84	6.85	6.93	59.14	4.92	8.32	91.50	19.27	21.06
Cefotaxime	122.83	4.32	3.52	71.75	23.92	33.33	173.69	15.86	9.13
Ciprofloxacin	95.76	3.54	3.70	99.66	2.20	2.21	73.93	69.08	93.44
Clarithromycin	54.67	4.07	7.44	106.90	2.64	2.47	69.53	12.17	17.50
Clinafloxacin	172.32	31.99	18.57	76.12	5.13	6.74	171.73	24.34	14.17
Cloxacillin	261.54	15.78	6.03	60.77	3.90	6.42	166.24	11.42	6.87
Codeine	97.65	1.79	1.83	64.66	5.36	8.29	141.78	11.87	8.38
Cotinine	96.04	3.79	3.94	102.27	9.75	9.53	92.34	4.56	4.94
Dehydronifedipine	84.14	6.80	8.09	66.50	7.17	10.78	126.82	13.52	10.66
Diphenhydramine	66.76	2.94	4.40	68.99	8.42	12.20	103.43	20.40	19.73
Diltiazem	66.96	3.47	5.19	55.23	20.89	37.83	160.04	114.83	71.75
Digoxin	92.33	13.28	14.39	52.22	18.64	35.69	22.60	18.11	80.13
Digoxigenin	126.01	1.96	1.55	64.36	8.55	13.28	79.57	11.63	14.61
Enrofloxacin	97.44	11.24	11.54	96.37	7.08	7.34	108.72	6.71	6.17
Erythromycin-H2O	136.67	3.12	2.28	113.95	3.09	2.71	100.45	7.46	7.42
Flumequine	151.31	7.43	4.91	91.15	4.08	4.48	92.35	14.79	16.02
Fluoxetine	88.09	16.86	19.14	85.89	4.27	4.97	100.48	19.64	19.55
Lincomycin	55.95	15.38	27.49	17.70	1.70	9.58	198.99	13.38	6.72
Lomefloxacin	179.94	32.28	17.94	106.07	6.19	5.83	79.59	9.63	12.10
Miconazole	51.31	4.73	9.23	73.81	6.50	8.81	55.79	17.71	31.74
Norfloxacin	101.34	6.89	6.80	114.79	5.07	4.41	63.02	7.17	11.37
Norgestimate	58.06	3.52	6.06	48.26	3.61	7.48	49.20	7.61	15.47

Ofloxacin	166.98	26.78	16.04	127.81	12.16	9.51	78.44	34.96	44.57
Ormetoprim	64.83	2.86	4.41	66.94	3.44	5.14	78.76	5.96	7.56
Oxacillin	168.38	15.50	9.20	60.02	6.22	10.36	163.73	11.69	7.14
Oxolinic Acid	96.72	2.48	2.56	69.17	6.30	9.10	108.07	12.09	11.19
Penicillin G	214.04	14.66	6.85	58.83	6.77	11.51	99.09	14.24	14.37
Penicillin V	195.93	9.43	4.81	61.80	7.81	12.63	157.80	9.85	6.24
Roxithromycin	61.28	3.61	5.89	85.57	2.23	2.60	83.70	19.61	23.42
Sarafloxacin	146.84	25.89	17.63	87.70	4.25	4.84	108.36	8.57	7.91
Sulfachloropyridazine	158.30	8.72	5.51	115.36	3.88	3.36	90.24	9.86	10.92
Sulfadiazine	158.51	17.49	11.03	80.11	2.33	2.91	107.55	12.80	11.90
Sulfadimethoxine	78.65	3.44	4.37	87.00	2.95	3.39	67.87	9.14	13.46
Sulfamerazine	115.08	13.12	11.40	90.48	1.01	1.12	136.01	9.46	6.96
Sulfamethazine	119.60	5.59	4.67	100.67	6.19	6.15	103.35	11.96	11.57
Sulfamethizole	75.61	8.69	11.49	93.86	4.52	4.82	70.14	3.00	4.28
Sulfamethoxazole	103.21	2.97	2.88	88.17	3.63	4.12	102.56	12.66	12.34
Sulfanilamide	99.94	29.94	29.96	20.71	0.95	4.59	130.84	8.89	6.79
Sulfathiazole	59.06	3.39	5.74	76.73	3.22	4.20	92.10	8.07	8.76
Thiabendazole	106.47	1.44	1.35	99.83	1.92	1.93	81.89	6.03	7.36
Trimethoprim	103.24	3.23	3.13	80.82	5.65	6.99	98.81	5.35	5.42
Tylosin	60.99	9.93	16.28	103.48	9.59	9.27	47.80	14.56	30.46
Virginiamycin	116.39	10.65	9.15	43.62	15.26	34.99	172.33	42.36	24.58
1,7 DimethylXanthine	100.64	16.81	16.70	95.73	7.16	7.48	137.15	38.02	27.72
13C2-15N-Acetaminophen	258.79	19.66	7.60	112.20	4.48	3.99	137.17	28.54	20.80
13C3-Caffeine	203.04	50.76	25.00	115.82	7.57	6.54	119.47	9.50	7.95
d3-Cotinine	28.08	9.20	32.76	3.20	0.35	10.92	68.57	19.19	27.99
13C3-N15-Ciprofloxacin	66.74	18.81	28.19	144.50	16.99	11.75	132.12	13.74	10.40
13C2-Erythromycin-H2O	97.49	7.73	7.93	86.25	2.83	3.28	54.62	13.12	24.03
d5-Fluoxetine	92.68	8.40	9.07	103.69	6.05	5.84	94.67	35.78	37.79
13C6-Sulfamethazine	54.80	7.77	14.17	105.70	11.12	10.52	50.78	7.28	14.35
13C6-Sulfamethoxazole	85.21	9.29	10.90	111.77	9.32	8.34	72.67	10.63	14.63
d6-Thiabendazole	92.75	8.63	9.30	117.76	4.64	3.94	66.75	5.19	7.78
13C3-Trimethoprim	121.40	12.12	9.98	144.35	9.96	6.90	94.08	12.15	12.91
Group 4 basic extraction ESI+									
Albuterol	100.43	7.12	7.09	90.04	14.09	15.65	96.58	1.88	1.95
Cimetidine	37.37	10.69	28.60	64.93	12.83	19.75	52.77	14.30	27.09
Metformin	115.61	9.40	8.13	103.72	13.16	12.69	89.06	3.32	3.72
Ranitidine	79.99	13.90	17.37	103.66	22.67	21.87	71.15	7.22	10.15
d3-Albuterol	91.52	8.47	9.25	63.83	2.41	3.78	105.42	19.25	18.26
d6-Metformin	94.38	13.05	13.83	51.66	6.86	13.28	161.13	67.48	41.88
Group 2 acidic extraction ESI+									
Chlortetracycline	121.24	6.98	5.75	95.24	22.32	23.44	114.43	45.67	39.91
4-Epichlortetracycline	112.41	8.71	7.75	96.83	15.65	16.16	95.59	32.60	34.11
Anhydrochlortetracycline	92.62	10.43	11.27	102.22	11.51	11.26	50.40	21.73	43.12
4-Epianhydrochlortetracycline	53.57	1.87	3.49	82.28	13.31	16.18	33.88	8.30	24.49
Isochlortetracycline	65.88	5.01	7.61	149.37	16.06	10.75	91.65	25.51	27.83
Demeclocycline	54.53	1.96	3.59	136.58	3.18	2.33	76.03	31.01	40.79
Doxycycline	67.83	2.96	4.36	119.65	1.01	0.85	87.03	34.42	39.55

Oxytetracycline	112.85	3.12	2.77	148.84	5.76	3.87	74.46	16.46	22.10
4-Epioxytetracycline	119.40	6.94	5.81	122.38	6.25	5.11	83.55	18.09	21.65
Tetracycline	93.41	3.95	4.23	124.79	4.69	3.76	77.98	19.24	24.68
4-Epitetracycline	138.95	3.42	2.46	102.11	4.02	3.94	97.37	37.03	38.03
4-Epianhydrotetracycline	70.11	6.87	9.80	170.82	22.25	13.02	67.87	23.27	34.29
Anhydrotetracycline	50.21	4.13	8.23	98.14	2.50	2.55	86.20	34.27	39.76
d6-Thiabendazole	77.07	4.76	6.18	64.80	3.21	4.95	89.82	15.10	16.81

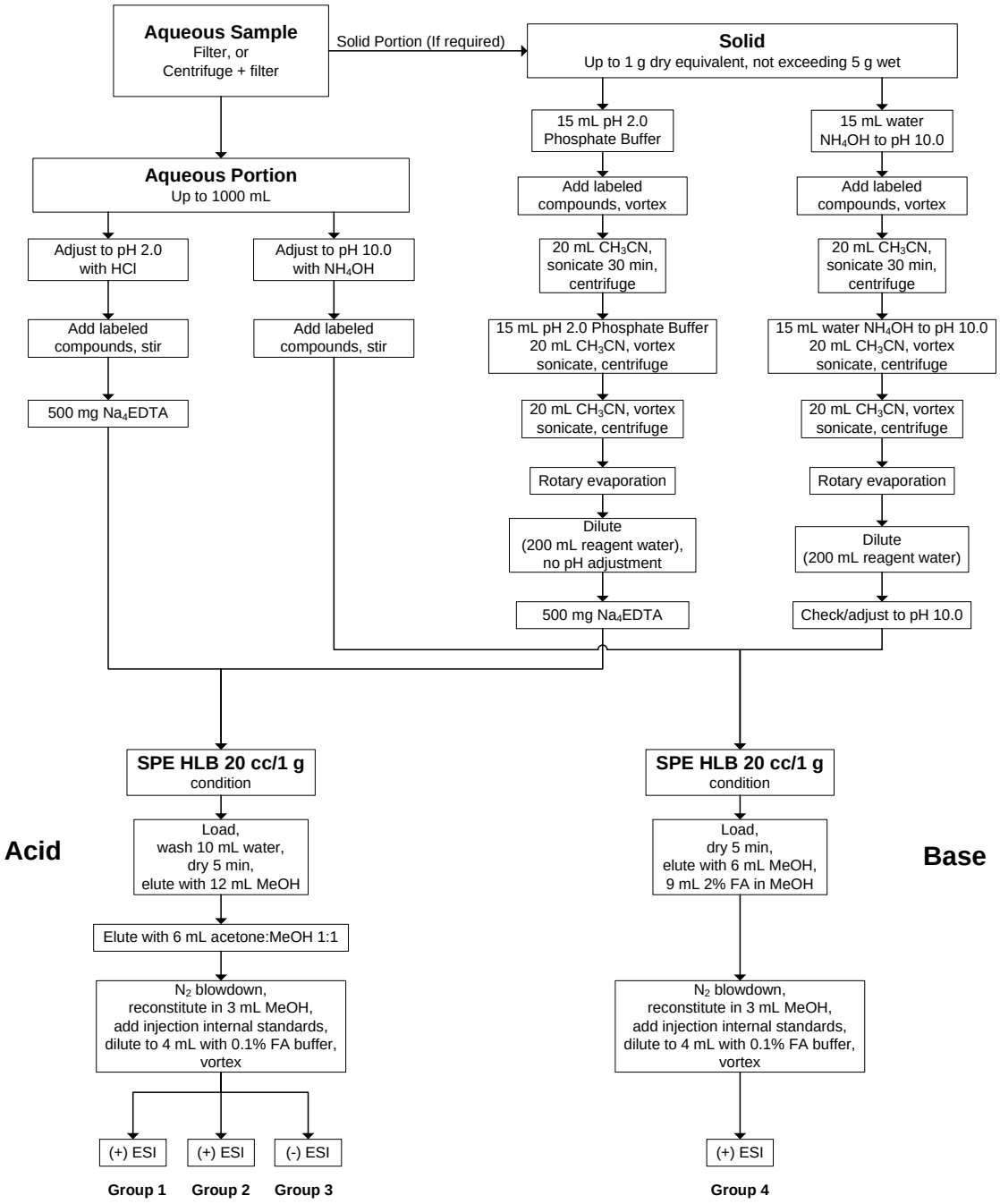

Figure 1 Flow chart for determination of pharmaceuticals and personal-care products by LC/MS/MS

24.0 Glossary

These definitions and purposes are specific to this method but have been conformed to common usage to the extent possible.

24.1 Units of weight and measure and their abbreviations

24.1.1 Symbols

EC	degrees Celsius
ΦL	microliter
Φm	micrometer
<	less than
>	greater than
%	percent

24.1.2 Abbreviations (in alphabetical order)

cm	centimeter
g	gram
h	hour
ID	inside diameter
in.	inch
L	liter
M	Molecular ion
m	mass or meter
mg	milligram
min	minute
mL	milliliter
mm	millimeter
m/z	mass-to-charge ratio
N	normal; gram molecular weight of solute divided by hydrogen equivalent of solute, per liter of solution
OD	outside diameter
pg	picogram
ppb	part-per-billion
ppm	part-per-million
ppq	part-per-quadrillion
ppt	part-per-trillion
psig	pounds-per-square inch gauge
v/v	volume per unit volume
w/v	weight per unit volume

24.2 Definitions and acronyms (in alphabetical order)

Analyte – A pharmaceutical or personal-care product tested for by this method. The analytes are listed in Table 1.

Calibration standard (CAL) – A solution prepared from a secondary standard and/or stock solution and used to calibrate the response of the HPLC/MSMS

instrument.

Calibration verification standard (VER) – The mid-point calibration standard (CS-4) that is used to verify calibration. See Table 4.

CS-1, CS-2, CS-3, CS-4, CS-5, CS-6 – See Calibration standards and Table 4.

Field blank – An aliquot of reagent water or other reference matrix that is placed in a sample container in the field, and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the field blank is to determine if the field or sample transporting procedures and environments have contaminated the sample.

GPC – Gel permeation chromatograph or gel permeation chromatography

HPLC – High performance liquid chromatograph or high performance liquid chromatography

Labeled injection internal standard – A labeled spiked into the concentrated extract immediately prior to injection of an aliquot of the extract into the LC/MS/MS.

Internal standard – a labeled compound used as a reference for quantitation of other labeled compounds and for quantitation of a native compound other than the compound of which it is a labeled analog. See Internal standard quantitation.

Internal standard quantitation – A means of determining the concentration of (1) a naturally occurring (native) compound by reference to a compound other than its labeled analog and (2) a labeled compound by reference to another labeled compound.

IPR – Initial precision and recovery; four aliquots of a reference matrix spiked with the analytes of interest and labeled compounds and analyzed to establish the ability of the laboratory to generate acceptable precision and recovery. An IPR is performed prior to the first time this method is used and any time the method or instrumentation is modified.

Isotope dilution quantitation – A means of determining a naturally occurring (native) compound by reference to the same compound in which one or more atoms has been isotopically enriched. In this method, labeled are enriched with deuterium to produce ^{2}H labeled analogs or carbon-13 to produce ^{13}C-labeled analogs. The labeled analogs are spiked into each sample to allow identification and correction of the concentration of the native compounds in the analytical process.

Labeled compound – A molecule in which one or more of the atoms is isotopically entriched, thereby increasing the mass of the molecule

Laboratory blank – See method blank

Laboratory control sample (LCS) – See Ongoing precision and recovery standard (OPR)

Laboratory reagent blank – See method blank

May – This action, activity, or procedural step is neither required nor prohibited.

May not – This action, activity, or procedural step is prohibited.

Method blank – An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Method detection limit (MDL) – The lowest concentration at which an analyte can be detected under routine operating conditions (see 40 CFR 136, appendix B). MDLs are listed in Table 3, 5, 7, and 9.

Minimum level (ML) – The greater of a multiple of the MDL or the lowest calibration point (see 68 FR 11790, March 12, 2003.) MLs are listed in Tables 3, 5, 7, and 9.

MS – Mass spectrometer or mass spectrometry

Must – This action, activity, or procedural step is required.

Native compound – A molecule in which the atoms all have naturally occuring isotopic abundances

OPR – Ongoing precision and recovery standard (OPR); a method blank spiked with known quantities of analytes. Also known as a “laboratory control sample” (LCS). The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

Preparation blank – See method blank

Quality control check sample (QCS) – A sample containing all or a subset of the analytes at known concentrations. The QCS is obtained from a source external to the laboratory or is prepared from a source of standards different from the source of calibration standards. It is used to check laboratory performance with test materials prepared external to the normal preparation process.

Reagent water – water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte.

Relative standard deviation (RSD) – The standard deviation times 100 divided by the mean. Also termed "coefficient of variation."

RF – Response factor. See Section 10.5

RR – Relative response. See Section 10.4

RSD – See relative standard deviation

Signal-to-noise ratio (S/N) – The height of the signal as measured from the mean (average) of the noise to the peak maximum divided by the width of the noise.

Should – Although this action, activity, or procedural step is suggested and not required, you may be asked to explain why you changed or omitted this action, activity, or procedural step.

SICP – Selected ion current profile; the line described by the signal at an exact m/z.

SPE – Solid-phase extraction; an extraction technique in which an analyte is extracted from an aqueous solution by passage over or through a material capable of reversibly adsorbing the analyte. Also termed liquid-solid extraction.

Stock solution – A solution containing an analyte that is prepared using a reference material traceable to EPA, the National Institute of Science and Technology (NIST), or a source that will attest to the purity and authenticity of the reference material.

VER – See Calibration verification.