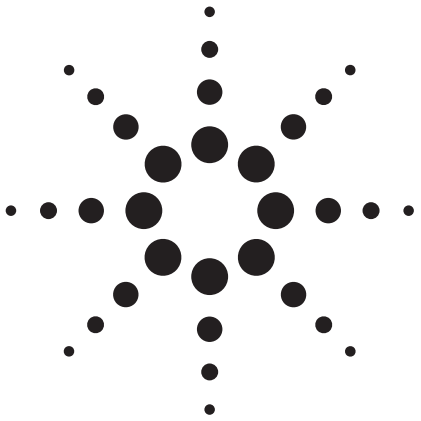

Agilent Standard Operating Procedure

Analysis of Emerging Pharmaceutical Contaminants by UHPLC/MS/MS

With Agilent 1290 Infinity LC and Agilent 6460 Triple Quadrupole Jetstream LC/MS

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E. Michael Thurman, Imma Ferrer
The Center for Environmental Mass Spectrometry
University of Colorado
Boulder, CO 80309

Jerry Zweigenbaum
Agilent Technologies Inc.
Little Falls, Wilmington, DE 19808

Agilent Technologies

Introduction

This Agilent SOP describes a procedure for detecting a select set of pharmaceutical and personal care products (PPCPs) in environmental water samples by ultra-high performance liquid chromatography combined with tandem mass spectrometry (UHPLC/MS/MS) using isotope dilution and internal standard quantitation techniques. This method applies to aqueous matrices using Agilent LCs and triple quadrupole mass spectrometers; specifically the Agilent 1290 Infinity LC with Agilent 6400 series triple quadrupole LC/MS systems, and Agilent LC columns.

An analytical method for the targeted analysis of 33 pharmaceuticals and personal care products in water samples using 14 labeled standards was developed for the Agilent 6460 triple quadrupole mass spectrometer (QQQ) with jet stream technology coupled to an Agilent 1290 Infinity LC system. The method includes commonly occurring pharmaceuticals and other compounds found in water, wastewater, and other sources as determined by a thorough search of recent relevant literature, such as EPA Method 1694 [1-7]. This method is part of an Agilent Solution for the analysis of pharmaceuticals that are emerging as contaminants in water samples by HPLC/MS/MS.

Two distinct chromatographic gradients were used according to the polarity of the analytes: one in positive ion electrospray, and the other in negative ion electrospray. Using UHPLC, with Agilent ZORBAX 1.8 µm columns, the sample was analyzed with two 10-min gradients. The flow rates were controlled at 0.4 mL/min to produce excellent chromatography and peak shape. In addition, the pressure was maintained below 600 bar to allow use of a standard Agilent 1200 Series HPLC or an Agilent 1290 Infinity UHPLC system with this method. Similarly, all transitions in this SOP will perform well with the Agilent 6410 and 6430 triple quadrupole LC systems.

The use of dynamic MRM greatly improved this process of many transitions in a single positive or negative run by choosing only the elution window to monitor the correct transitions. The method was evaluated for both natural river samples and treated wastewater effluents because all of the 33 selected analytes have been found in various water samples by this method. The limits of detection for the pharmaceuticals varied from 1 ng/L to 25 ng/L depending upon the ionization properties of the analyte. The method used a simple semi-automated solid phase extraction procedure with a polymeric adsorbent and a 100-mL water sample.

This method has been adapted from a revision of earlier Agilent application notes 5990-4880EN and 5990-4890EN for EPA Method 1694 and the Agilent application note 5990-4488EN for UHPLC. Other information used in this report include a series of recent journal articles concerning important and widely reported PPCPs in the environment [1-5].

Contacts

Questions concerning this method or its application should be addressed to:

Jerry Zweigenbaum, PhD.
Application Chemist
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1644
302-633-8661

E. Michael Thurman, PhD. and Imma Ferrer, PhD
Center for Environmental Mass Spectrometry
Department of Civil, Environmental and
Architectural Engineering
University of Colorado
303-735-4147 mthurman@ono.com

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Pharmaceuticals and Personal Care Products in Water by UHPLC/MS/MS

1.0 Scope and Application

1.1 This method is for the determination of 33 pharmaceuticals and personal care products (PPCPs) in environmental water samples by ultra-high performance liquid chromatography combined with tandem mass spectrometry (UHPLC/MS/MS).

1.2 This method was developed for use by Agilent customers to analyze an important class of PPCPs in environmental water samples

1.3 The target analytes are listed in Table 1.

1.4 The detection limits and quantitation levels in this method are more dependent on the level of interferences than instrumental limitations.

2.0 Summary of the Method

2.1 The target analytes in this method are divided into two groups: positive electrospray analytes and negative electrospray analytes (Table 1). A total of 32 analytes and 14 isotopically labeled internal standards are used in this method. The method is based on a solid phase extraction of a 100-mL water sample, followed by elution of the analytes and evaporation of the extract. The final extract is transferred to a 1-mL vial for UHPLC/MS/MS analysis.

2.2 The UHPLC analysis consists of a 10-min gradient using acetonitrile/water mobile phases with an acetic acid additive to aid ionization and improve chromatography for both positive and negative ion electrospray.

2.3 The mass spectrometer is run first in electrospray positive mode using two MRM transitions for each analyte and one MRM transition for the isotopic label. Next the mass spectrometer is run in electrospray negative mode using two MRM transitions for each analyte and one MRM transition for the isotopic label.

2.4 Quantitation is performed with the MassHunter Software included with the instrument, using the isotopically labeled internal standard method.

Table 1. Pharmaceuticals and personal care products addressed in this SOP, their use, and instrument and limits of quantitation.

Compound	Drug type	LOD jetstream 6460 (ng/L) instrument LOD	Method LOQ based on 100-mL sample (ng/L)
Positive ion			
Acetaminophen	Analgesic OTC	600	5
Albuterol	Bronchodilator	500	5
Atenolol	Antihypertensive	1000	10
Azithromycin	Antibiotic	6000	30
Bupropion	Antidepressant	600	5
Caffeine	Stimulant	1000	5
Carbamazepine	Anticonvulsant	200	1
Clarithromycin	Antibiotic	500	5
Codeine	Analgesic-narcotic	1000	10
Cotinine	Nicotine-metabolite	500	5
DEET	Insect repellant	500	5
Dehydronifedipine	Antianginal metabolite	300	2
Diazepam	Anxiolytic	1000	10
Diclofenac	Anti-inflammatory	2000	20
Diltiazem	Vasodilator	2000	10
1,7-Dimethylxanthine	Caffeine metabolite	6000	30
Diphenhydramine	Antihistamine	500	5
Erythromycin	Antibiotic	3000	20
Meprobamate	Anxiolytic	5000	25
Metoprolol	Antihypertensive	1000	10
Oxolinic Acid	Antibiotic	300	2
Propanolol	Antihypertensive	1000	10
Sucralose-Na Adduct	Sweetener	10,000	50
Sulfadimethoxine	Antibiotic	500	5
Sulfamethoxazole	Antibiotic	2000	10
Thiabendazole	Fungicide	500	5
Trimethoprim	Antibiotic	5000	25
Negative Ion			
Bisphenol A	Plasticizer	10,000	50
Gemfibrozil	Anti-inflammatory	5000	25
Ibuprofen	Anti-inflammatory	5000	25
Naproxen	Anti-inflammatory	2000	20
Triclocarban	Antibacterial	1000	10
Triclosan	Antibacterial	5000	25

3.0 Interferences and Glassware Cleanup

3.1 Solvents, glassware, pipettes, and reagents may yield interferences. Thus, it is important to use good laboratory practice in the cleansing and use of all glassware and pipettes.

3.2 This means that hot soapy water should be used to clean all glassware, followed by rinses with hot tap water and finally de-ionized water. Ten rinses are recommended. In some cases methanol or acetone may be needed to clean glassware, and these wastes should be disposed of properly. Finally, it may be necessary to muffle glassware that is not used for volumetric measurements, such as beakers and flasks. All sample bottles should also be muffled at a temperature of 550 °C for 3 hours.

3.3 Matrix blanks should be run on all glassware, sample bottles, and other labware to be sure that there is no carryover of PPCP analytes.

3.4 Pipettes should be calibrated according to standard procedure to assure accuracy of analysis during standard preparation.

3.5 Calibration of balances is critical to correct standard preparation.

4.0 Safety

4.1 Care must be taken in the handling of all chemicals, reagents, and PPCP analytes. Read all data safety sheets to know the chemical exposure hazards of all materials.

4.2 Gloves and paper mask should be worn while making standard solutions to prevent direct inhalation or contact of analytes.

4.3 Proper disposal should be applied to any waste solvents or chemicals.

4.4 Personnel should avoid caffeine products and PPCP products during measurement procedures.

5.0 Supplies and Sample Preparation Equipment

5.1 Non-Agilent equipment and supplies specified do not necessarily imply endorsement from Agilent Technologies, Inc. Equivalent materials may be substituted but care must be taken not to change the method performance.

5.2 Sample bottles and caps recommended are 125-mL amber bottles with PTFE-lined caps from VWR catalogue or equivalent.

5.3 Bottles should be rinsed and baked before use as described in section 3.2.

5.4 Recommended equipment for sample preparation.

5.4.1 Automated solid phase extraction apparatus—Gilson GX-271 Aspec or equivalent. Use a vacuum manifold for manual operation.

5.4.2 Automated nitrogen evaporator—Caliper Life Sciences Turbovap system or equivalent

5.4.3 Standard vortex mixer

5.4.4 Muffle furnace capable of 550 °C

5.4.5 Analytical balance capable of 0.1 mg

5.4.6 Balance, top loading

5.4.7 Hood or snorkels to remove fumes during evaporation

5.4.8 SPE cartridge, 200-mg Oasis HLB in 6-mL syringe format

5.4.9 Test tubes, 16 mm × 100 mm for evaporation

5.4.10 Test tubes for elution of SPE

5.4.11 Clear glass vials, 2-mL with white label, compatible with Agilent UHPLC system, p/n 5182-0715

5.4.12 Glass 10-mL vials for standard preparation

6.0 Chromatographic and Mass Spectrometry Equipment

6.1 Agilent 1290 Infinity UHPLC system with autosampler, degasser, and refrigeration unit

6.2 Agilent 6460 Triple Quadrupole LC/MS with Jetstream technology and electrospray source

6.3 Agilent ZORBAX Eclipse Plus C18 Column 2.1 mm × 50 mm, 1.8 µm particle size p/n 959757-902

7.0 Reagents and Standards

7.1 Ultra-high purity acetic acid, doubly distilled in glass

7.2 Reagent grade water, purchased or deionized in a laboratory

7.3 LC/MS grade acetonitrile

7.4 LC/MS grade methanol

7.5 Acetic acid/water solution (0.1%) prepared from items 7.1 and 7.2 by adding 1 mL of the acetic acid to 999 mL of reagent grade water in a 1-L volumetric flask and shaking well.

7.6 Standard Solutions

7.6.1. Prepare from known purity and composition. If the purity is greater than 98% correction is unnecessary. For example, weigh 10 mg of an analyte (acetaminophen, for example) into a 10-mL vial and dissolve with appropriate solvent, such as methanol or acetonitrile. This makes a standard of 1000 ppm as the stock solution. Dilute appropriately from here for all reference standards. Store vials in a dark standard-only freezer at less than –10 °C.

7.7 Dilute stock solutions to 1 ppm for working solutions. These solutions should be brought up in reagent water with 10% methanol.

8.0 Sample Collection, Preservation, Storage, and Holding Times

8.1 Collect samples in 1-L amber baked, glass bottles with PTFE caps and store on ice or in refrigerator until analyzed.

8.2 Rinse the bottle three times with the sample before filling with sample and capping. No preservation chemicals should be added to the bottle.

8.3 Storage time of seven days in the refrigerator is acceptable before sample processing; however, two days or 48 hours is preferred.

8.4 Allow sample solids to settle to the bottom of the bottle before decanting for sample analysis.

9.0 Quality Assurance and Quality Control

9.1 Each laboratory should use a strict quality control program. The minimum for PPCP analysis is to first run a blank control water sample of reagent water through all steps in processing to look for carryover of PPCP analytes. This should be done every 10 samples.

9.2 Do a repeat sample every 10 samples to check for precision and accuracy.

9.3 Do a spike of a real sample matrix with each set of samples to check for matrix suppression and solid phase extraction efficiency. See Section 16.3 for an analysis of this QA/QC sample.

9.4 For QA/QC purposes, all samples should be spiked with at least one isotopic dilution standard for recovery before solid phase extraction, for example, carbamazepine-d10.

9.5 Maintain a laboratory notebook of all sample log-ins, with names of the sample, project, and dates of collection and analysis. Also note any unusual circumstances related to the sample, such as particles, or color.

10.0 Calibration and Standardization

10.1 Mass Calibration-The mass spectrometer must undergo mass calibration according to Agilent's specifications to ensure accurate assignments of *m/z's* by the instrument. This calibration must be performed at least annually to maintain instrument sensitivity and accuracy. It must be repeated after performing major maintenance of the mass spectrometer.

10.2 Mass Spectrometer Optimization-Prior to measurements of a given PPCP, the analyte must be optimized. This has been accomplished. All optimization parameters, such as fragmentation voltage, MRM transitions, and collision energies for positive ion electrospray are given in Table 2 of this SOP. Table 3 provides the method settings for optimized instrument operation for these analytes. Table 4 gives the fragmentation voltage, MRM transitions, and collision energies for negative ion electrospray and Table 5 provides the method settings for optimized instrument operation for these analytes.

10.3 Calibration by isotope dilution—Isotope dilution is used for calibration of each analyte for which a labeled standard is available, (Table 2). For those that do not have an isotope label, the nearest analyte in retention time is used. See Table 2 for all settings. A five-point calibration curve is established using the isotopic label method with relative response and the MassHunter software system. The intercept is forced through zero for this method.

Table 2. MRM transitions and MS operating parameters selected for the analysis of the PPCP compounds in positive ion electrospray. In blue are the labeled standards.

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Acetaminophen	90	152 > 110 152 > 65	15 35
13C2-15N-Acetaminophen	90	155 > 111	15
Albuterol	90	240 > 148 240 > 166	15 5
Atenolol	130	267 > 145 267 > 190	20 15
Azithromycin	130	749.5 > 591.4 749.5 > 158	30 35
Bupropion	80	240 > 184 240 > 166	5 10
Caffeine	110	195 > 138 195 > 110	15 25
13C3-Caffeine	110	198 > 140	15
Carbamazepine	120	237 > 194 237 > 179	15 35
Carbamazepine-d10	120	247 > 204	15
Clarithromycin	110	748.5 > 158 748.5 > 590	25 15
Codeine	130	300 > 165 300 > 215	35 25
Codeine-d3	130	303 > 165	35
Cotinine	90	177 > 98 177 > 80	25 25
Cotinine-d3	90	180 > 80	25
DEET	110	192 > 119 192 > 91	15 30
Dehydronifedipine	130	345 > 284 345 > 268	25 25
Diazepam	160	285 > 154 285 > 193	25 35
Diazepam-d5	160	290 > 154	25
Diclofenac	70	296 > 250 296 > 215	5 15
Diltiazem	130	415 > 178 415 > 150	25 25
1,7-Dimethylxanthine	90	181 > 124 181 > 55	15 35
Diphenhydramine	70	256 >167 256 > 152	15 35
Erythromycin	90	734.5 > 158 734.5 > 576	35 15

(Continued)

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
13C2-Erythromycin	90	736.5 > 160	25
Meprobamate	70	219 > 158 219 > 55	5 20
Metoprolol	135	268 > 116 268 > 56	15 30
Oxolinic Acid	90	262 > 244 262 > 216	15 25
Propanolol	120	260 > 116 260 > 56	15 30
Sucralose (Na Adduct)	110	419 > 239 419 > 221	15 15
Sulfadimethoxine	80	311 > 156 311 > 92	20 35
Sulfamethoxazole	80	254 > 156 254 > 92	10 30
13C6-Sulfamethoxazole	80	260 > 98	30
Thiabendazole	130	202 > 175 202 > 131	25 35
Trimethoprim	110	291 > 230 291 > 261	20 25
13C3-Trimethoprim	110	294 > 233	20

Table 3. PPCP conditions for UHPLC, positive electrospray ionization (ESI+) instrument conditions.

Instrument	Agilent 6460 Triple Quadrupole LC/MS with an Agilent 1290 Infinity UHPLC	
LC Column	Agilent ZORBAX Eclipse Plus C18 narrow bore, 50 mm × 2.1 mm id, 1.8 µm particle size	
Ionization	Positive ion electrospray	
Acquisition	MRM mode, unit resolution	
Injection volume	15 µL	
LC Gradient Program		
Time (min)	**Mobile phase**	**LC flow rate (mL/min)**
0	10% ACN 90% H_2O (0.1% Acetic Acid)	0.4
2	10% ACN 90% H_2O (0.1% Acetic Acid)	0.4
5	27% ACN 73% H_2O (0.1% Acetic Acid)	0.4
10	50% ACN 50% H_2O (0.1% Acetic Acid)	0.4
14	100% ACN 0% H_2O (0.1% Acetic Acid)	0.4
15	100% ACN 0% H_2O (0.1% Acetic Acid)	0.4
General LC Conditions		
Column temperature	25 °C	
Max pressure	600 Bar	
Autosampler tray temperature	8 °C	
MS Conditions		
Sheath gas temperature	375 °C	
Sheath gas flow	11 L/min	
Gas temperature	250 °C	
Desolvation gas flow rate	10 L/min	
Nebulizer pressure	45 psi	
Capillary voltage	4000 V	
Nozzle voltage	0 V	
Delta EMV	400 V	

10.4 The curve should be linear, with a typical r value of 0.9990 or greater. The typical values for r in our studies was 0.9998.

11.0 Sample Preparation

11.1 Decant 123 mL of water into a baked, amber, 125-mL glass bottle. Add 100 µL of the internal standard mixture of acetonitrile. Remember to allow the spiking solution with internal standards to come to room temperature before spiking. Cap and mix the sample.

11.2 Transfer sample to automated sample processing unit GX-271 or equivalent for sample processing.

Table 4. MRM transitions and MS operating parameters selected for the analysis of the pharmaceutical compounds in negative ion electrospray. Isotopically labeled standards are in blue.

Compound	Fragmentor Voltage	MRM transitions (*m/z*)	Collision Energy (eV)
Bisphenol A	120	227 > 212 227 > 133	15 25
Gemfibrozil	70	249 > 121	5
Gemfibrozil-d6	70	255 > 121	5
Ibuprofen	50	205 > 161	0
13C3-Ibuprofen	50	208 > 163	0
Naproxen	50	229 > 169 229 > 170	25 5
13C-Naproxen-d3	50	233 > 170	5
Triclocarban	100	313 > 160 313 > 126	5 15
13C6-Triclocarban	90	319 > 160	5
Triclosan	75	287 > 35 289 > 37	5 5
13C12-Triclosan	75	299 > 35	5

Table 5. PPCP in negative electrospray ionization (ESI–) instrument conditions.

Instrument	Agilent 6460 Triple Quadrupole LC/MS with an Agilent 1290 Infinity UHPLC
LC Column	Agilent ZORBAX Eclipse Plus C18 narrow bore, 50 mm × 2.1 mm id, 1.8 µm particle size
Ionization	Negative ion electrospray
Acquisition	MRM mode, unit resolution
Injection volume	15 µL

LC Gradient Program

Time (min)	Mobile phase	LC flow rate (mL/min)
0	10% ACN 90% H_2O (0.1% Acetic Acid)	0.4
2	10% ACN 90% H_2O (0.1% Acetic Acid)	0.4
5	27% ACN 73% H_2O (0.1% Acetic Acid)	0.4
10	50% ACN 50% H_2O (0.1% Acetic Acid)	0.4
14	100% ACN 0% H_2O (0.1% Acetic Acid)	0.4
15	100% ACN 0% H_2O (0.1% Acetic Acid)	0.4

General LC Conditions	
Column temperature	25 °C
Max pressure	600 Bar
Autosampler tray temperature	8 °C
MS Conditions	
Sheath gas temperature	300 °C
Sheath gas flow	11 L/min
Gas temperature	250 °C
Desolvation gas flow-rate	10 L/min
Nebulizer pressure	45 psi
Capillary voltage	3500 V
Nozzle voltage	1500 V
Delta EMV	400 V

12.0 Sample Extraction and Concentration

12.1 Process 100 mL of the 123-mL sample using an automated solid phase extraction unit, (Gilson GX-271 is recommended). The following procedure can also be performed manually by vacuum manifold, if an automated system is not available.

12.2 Sample Solid-Phase Extraction

12.2.1 Prepare the SPE cartridge for sample addition by first washing with 4 mL of methanol, followed by 6 mL of reagent grade water at 5 mL/min. Be careful to keep the sorbent bed wet.

12.2.2 Apply the water sample at 10 mL/minute.

12.2.3 Leave the residual water in the cartridge

12.2.4 Elute the cartridge with 5 mL of reagent grade methanol.

12.2.5 Force out the last volume of methanol trapped in the cartridge with 5 mL of air.

12.3 Sample Evaporation

12.3.1 Carefully weigh a 16 mm × 100 mm test tube with a top-loading balance to three decimal places. Label the tube first.

12.3. 2 If the eluent is not in a 16 mm × 100 mm test tube, quantitatively transfer the extract to the weighed test tube for evaporation in a Zymark Turbovap or equivalent.

12.3.3 Add 300 µL of reagent grade water before blowing down the extract to approximately 0.500 mL final volume. Then weigh the tube again, compute the volume in the test tube, and record the final volume as a weight in the laboratory notebook. This is used as a check since the labeled standards will be used for exact quantitation.

12.3.4 Vortex the test tube to dissolve any analyte on the walls of the tube and quantitatively transfer to the 2-mL vial for LC/MS/MS analysis.

13.0 UHPLC/MS/MS Analysis

13.1 Establish the method operating conditions outlined in Table 3. These will eventually be downloaded directly from Agilent (Fall 2010). Until this time, they must be entered manually, using the values from Table 3.

13.2 Proceed with Method 1 for electrospray positive analytes first. See the data in Table 2 for the analytes analyzed. The method will inject 15 µL of extract according to the procedure in Table 3.

13.3 Proceed with Method 2 for electrospray negative analytes, using the method in Tables 4 and 5, which will also inject 15 µL of extract for each sample.

13.4 The method will automatically run the appropriate conditions for liquid chromatographic separation and MS/MS analysis with two MRM transitions for each of the PPCP analytes and one transition for each of the isotopically labeled standards.

14.0 System and Laboratory Performance

14.1 At the beginning of each laboratory shift, verify the system performance and calibration of the UHPLC/MS/MS for all native and labeled compounds. This is done visually by looking at the computer derived chromatograms.

14.2 Verify that the correct retention times and analytes are present in a standard containing each set of samples.

14.3 Run the MassHunter software to determine the analytes present in the analyzed samples.

15.0 Qualitative Analysis

15.1 Verify the presence of both the quantitative ion and the qualitative ion. Verify also that the ion ratios are within the 20% window shown on the MassHunter Quantitation software (the qualitative analysis in this case is performed by the quantitation software package).

15.2 Check all 32 analytes and 14 isotopic diluted standards (IS) with the same procedure.

15.3 Check the retention times (RTs) of all analytes versus the IS for accuracy of retention. Typically the IS elutes with or just slightly ahead of the analyte.

15.4 Check for interferences or unusual peaks in the chromatogram that may interfere with the target analytes.

15.5 Check that the blank sample does not contain target analytes. If any are present, note the level. Acceptable values are less than the confirmable LOD and three times less than the lowest standard. Only values larger than the lowest standard are reported. If any carryover is present, clean the source, and add extra wash and solvent blanks to the LC system. If this is not sufficient, check blanks in the solid phase extraction unit and clean. Stop all sample analysis until blanks are clean.

16.0 Quantitative Analysis

16.1 Using the MassHunter Quantitation Software compute the concentrations of all target analytes. Report the results using the Reporting Software package.

16.2 Check the data report for quantitative accuracy by looking at all results from the MassHunter Software screen. The purpose is to be sure that all integrations were properly carried out.

16.3 Check the values for one check of a spiked tap water sample for accuracy and precision before analyzing all the samples.

16.4 Analysis of complex samples may lead to poor recoveries because of solid phase extraction or strong matrix suppression. This is easy to notice due to the area counts of the IS. If the IS has low area counts relative to the blank, (less than 50% or more of its normal response), consider other options. One option may be to dilute the extract and re-analyze. If this is not satisfactory it may be necessary to dilute the water sample and re-analyze through the entire procedure.

17.0 Pollution Prevention and Waste Management

17.1 Treat the PPCP analytes as waste material, since the majority of them are taken as medications.

17.2 Use good laboratory practice for the disposal of all PPCP waste materials, including standards, solvents, and other materials.

17.3 The laboratory is responsible for following all city, county, state, and federal regulations for the proper disposal of waste materials used in this procedure.

18.0 References and Bibliography

1. E. T. Furlong, et al., "USGS Method for US Waters," 2008, Determination of Human Health Pharmaceuticals, USGS Method Paper, Chapter 5.
2. S. Castiglioni, et al., "Pharmaceuticals in Italian Waters," 2005, *J. Chromatogr. A*, v. 1092: 206.
3. B. Kasprzyk-Hordern, "Pharmaceuticals in Surface Water of South Wales, UK," 2008, Water Research, v. 42: 3498.
4. A. L. Batt, et al., "Pharmaceutical in US Waters, EPA Research Methods," 2008,: Analytical Chemistry, v. 80:5021.
5. P. E. Stackelberg, et al.,"USGS Study of Pharmaceuticals in Drinking Water," 2007, Science of the Total Environment. v. 377: 255.
6. "EPA Method 1694 on Agilent Model 6460 Triple Quadrupole with Jetstream,"Agilent Technologies publication 5990-4605EN (2009) .
7. "EPA Method on 6410 Triple Quadrupole," Agilent Technologies publication 5989-9665EN (2008).
8. "Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS," EPA Method 1694: December 2007, EPA-821-R-08-002.

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