Analysis of Pharmaceuticals & Personal Care Products by LC/MS

As performed at the University of Massachusetts, Environmental Engineering Research Laboratory

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Standard Operating Procedures

Analysis of Pharmaceuticals and Personal Care Products by LC/MS

This guidance document was prepared to assist research assistants, post-docs and lab technicians in conducting analysis of pharmaceuticals, personal care products (PPCPs) and related compounds in the UMass Environmental Engineering research laboratories. It aspires to outline our standard operating procedures, as they exist at the present time. It also emphasizes elements of quality control that are necessary to assure high quality data. Please help keep this document current by alerting us to needs for long-term changes in methodology or equipment.

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Faculty QC officer for PPCP analysis

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Scope

This method has been used in the UMass Environmental Engineering Laboratory for several dozen PPCPs and related compounds as listed in Table 1. It has been found to be capable of meeting data quality criteria with raw and treated drinking waters. This method should not be used for other media without further validation.

Extension of this method to additional analytes as listed in Table 1 may be possible, however this has not been validated.

Alternative methods for some PPCPs have employed GC/MS. These are not covered in this SOP.

The standard UMass method presented here is a hybrid of several published methods that are well regarded by experts in the field. No single published method could be used without some modification, due to differences in target analytes and instrumentation. First, the basis for the extraction and isolation protocol is EPA method 1694. Elements of Vanderford and Snyder's (2006) isotope dilution method were incorporated to help strengthen the method. Finally portions of Kasprzyk-Hordern et al., (2008) were used for specifics of the UPLC solvent programs and MS instrument parameters. Final refinement regarding UPLC separation and MS settings (e.g., voltages, gas flows, temperatures) are done through optimization with the UMass analytical equipment and using the actual analytes of interest.

A more recent alternative is to use method 18CD from the WRF-funded round-robin interlaboratory study (Vanderford et al 2012)[1]. Analytes are extracted using HLB Oasis® 200 mg columns. The columns are sequentially preconditioned using 3 mL Dichloromethane (DCM), 5 mL Methanol (MeOH) and 7mL Milli-Q water (H_2O). Samples are spiked with isotopically-labelled internal standards to the extent available (Error! Reference source not found.). Five hundred milliliter samples are then loaded onto the cartridges at 10 mL/min after which the cartridges were rinsed with 3 mL H_2O and 5 mL of 5% MeOH. The cartridges are dried for 40 minutes under vacuum after which the analytes are eluted with 6 mL MeOH followed by 4 mL MeOH/DCM (70:30). The eluate is then dried under a gentle stream of nitrogen at 35 °C to a volume of 0.5 mL and then reconstituted up to 1 mL with MeOH.

Separation and detection of analytes is achieved using Ultra Performance Liquid Chromatography followed by Tandem Mass Spectrometry (UPLC-MS-MS). In the EWRE laboratory we use a Waters Acquity UPLC separation module coupled with a Quattro Premier XE Micromass MS. Analytes are separated using an Acquity BEH C18 (1.7 µm, 2.1 X 50 m) UPLC column. Both ESI (+) and ESI (–) modes are used for detection.

[1] Vanderford,B.,Drewes,J.,Hoppe-Jones,C., Eaton,A., Haghani,A., Guo,Y., Snyder,S., Ternes,T., Schluesener,M.,Wood,C. "Evaluation of Analytical Methods for EDCs and PPCPs via Interlaboratory Comparison." *Water Research Foundation* #4167(2012).

Compound	CAS Registry	Labeled analog	Method			
			Acid			Base
			ESI+		ESI-	ESI+
			1	2	3	4
Acetaminophen	103-90-2	$^{13}C_2{}^{15}N$-Acetaminophen	X			
Albuterol	18559-94-9	Albuterol-d_3				X
Ampicillin	69-53-4		X			
Anhydrochlortetracycline (ACTC)	4497-08-9			X		
Anhydrotetracycline (ATC)	4496-85-9			X		
Azithromycin	83905-01-5		X			
Caffeine	58-08-2	$^{13}C_3$-Caffeine	X			
Carbadox	6804-07-5		X			
Carbamazepine	298-46-4		X			
Cefotaxime	63527-52-6		X			
Chlortetracycline (CTC)	57-62-5			X		
Cimetidine	51481-61-9					X
Ciprofloxacin	85721-33-1	$^{13}C_3{}^{15}N$-Ciprofloxacin	X			
Clarithromycin	81103-11-9		X			
Clinafloxacin	105956-97-6		X			
Cloxacillin	61-72-3		X			
Codeine	76-57-3		X			
Cotinine	486-56-6	Cotinine-d_3	X			
Dehydronifedipine	67035-22-7		X			
Demeclocycline	127-33-3			X		
Digoxigenin	1672-46-4		X			
Digoxin	20830-75-5		X			

Diltiazem	42399-41-7		X			
1,7-Dimethylxanthine	611-59-6		X			
Diphenhydramine	58-73-1		X			
Doxycycline	564-25-0			X		
Enrofloxacin	93106-60-6		X			
4-Epianhydrochlortetracycline (EACTC)	158018-53-2			X		
4-Epianhydrotetracycline (EATC)	4465-65-0			X		
4-Epichlortetracycline (ECTC)	14297-93-9			X		
4-Epioxytetracycline (EOTC)	14206-58-7			X		
4-Epitetracycline (ETC)	23313-80-6			X		
Erythromycin	114-07-8		X			
Erythromycin anhydrate	59319-72-1	$^{13}C_2$-Erythromycin anhydrate	X			
Flumequine	42835-25-6		X			
Fluoxetine	54910-89-3	Fluoxetine-d_5	X			
Gemfibrozil	25812-30-0	Gemfibrozil-d_6			X	

			Method			
			Acid			Base
Compound	**CAS Registry**	**Labeled analog**	ESI+		ESI-	ESI+
			1	2	3	4
Ibuprofen	15687-27-1	$^{13}C_3$-Ibuprofen			X	
Isochlortetracycline (ICTC)	514-53-4			X		
Lincomycin	154-21-2		X			
Lomefloxacin	98079-51-7		X			
Metformin	657-24-9	Metformin-d_6				X
Miconazole	22916-47-8		X			
Minocycline	10118-91-8			X		
Naproxen	22204-53-1	^{13}C-Naproxen-d_3			X	

Norfloxacin	70458-96-7		X			
Norgestimate	35189-28-7		X			
Ofloxacin	82419-36-1		X			
Ormetoprim	6981-18-6		X			
Oxacillin	66-79-5		X			
Oxolinic acid	14698-29-4		X			
Oxytetracycline (OTC)	79-57-2			X		
Penicillin V	87-08-1		X			
Penicillin G	61-33-6		X			
Ranitidine	66357-35-5					X
Roxithromycin	80214-83-1		X			
Sarafloxacin	98105-99-8		X			
Sulfachloropyridazine	80-32-0		X			
Sulfadiazine	68-35-9		X			
Sulfadimethoxine	122-11-2		X			
Sulfamerazine	127-79-7		X			
Sulfamethazine	57-68-1	$^{13}C_6$-Sulfamethazine	X			
Sulfamethizole	144-82-1		X			
Sulfamethoxazole	723-46-6	$^{13}C_6$-Sulfamethoxazole	X			
Sulfanilamide	63-74-1		X			
Sulfathiazole	72-14-0		X			
Tetracycline (TC)	60-54-8			X		
Thiabendazole	148-79-8	Thiabendazole-d_6	X			
Triclocarban	101-20-2	$^{13}C_6$-Triclocarban			X	
Triclosan	3380-34-5	$^{13}C_{12}$-Triclosan			X	
Trimethoprim	738-70-5	$^{13}C_3$-Trimethoprim	X			
Tylosin	1401-69-0		X			
Virginiamycin	11006-76-1		X			
Warfarin	81-81-2	Warfarin-d_5			X	

SPE Protocol for Three well-tested LC/MS Methods

	EPA Method #1694		Batt et al., 2009	Vanderford & Snyder, 2006 (Vanderford et al., 2003)
Step	**Acid Extraction**	**Base Extraction**		
Cartridge	HLB 60 mg, 20cc/lg LP		MCX[2] 150 mg, 6 mL	HLB 200 mg (500 mg)
Conditioning	20 mL MeOH 6 mL water 6 mL 10mM HCl	20 mL MeOH 6 mL water	6 mL ACN 6 mL water	5 mL MTBE 5 mL MeOH 5 mL water
Water Sample	1 L with Na_2EDTA, acidified to pH 2 with HCl	1 L, brought to pH 10 with NH_4OH	0.5L with Na_2EDTA	1 L
Loading rate	5-10 mL/min		3-5 mL/min	15 mL/min
Wash	10 mL water		6 mL 2% HCOOH	5 mL water
Dry	5 min under vacuum		Under vacuum	N_2 for 30 min (60 min)
Elution	12 mL MeOH (6 mL MeOH/Acetone)[3]	6 mL MeOH 9 mL 2% HCOOH	(1) 2x4mL ACN (2) 2x4mL 95/5 ACN/NH_4OH	5 mL MeOH; 5 mL 10/90 MeOH/MTBE
Volume reduction	Near dryness with N_2 @50 C		Dryness with N_2 at 40 C	0.40 mL with N_2 (0.75 mL)
Finish	Add 3 mL MeOH Bring to 4 mL with 0.1% HCOOH		(1) add 0.5mL 20/80 ACN/water (2) add 0.5mL 20/80 MeOH/water	Bring to 0.50 mL with MeOH (1.0 mL)
Analytes by ESI+	Ciprofloxacin Sulfamethoxazole Trimethoprim	Ranitidine	(1) Atorvastatin (2) Atenolol Ranitidine Sulfamethoxazole Trimethoprim	DEET Sulfamethoxazole TCEP Trimethoprim
Analytes by ESI-	Naproxen			Naproxen
Analytes not recovered			Ciprofloxacin	

[2] HLB with a strong cation exchange resin for retention of base analytes; this method recovers 2 separate eluents; neutral (1) and base (2)
[3] 1:1 mix; optional step for triclocarban and triclosan

Method Overview

Reproduced below is a simple, step-by-step outline of our PPCP method for quick reference.

Table 1: Summary of Procedure for PPCP Sample Analysis

1. Prepare **calibration standards** and **QC samples** (**Error! Reference source not found.**)
2. Quench / preserve sample (~1000 mL)
3. Add labelled standards to each sample
4. Filter if needed
5. Adjust pH to either 2 or 10 depending on method
6. Apply sample to pre-conditioned SPE cartridge and elute
7. Solvent exchange to methanol and reduce volume to 4 mL
8. Transfer sample to 2 mL autosampler vial
9. Run LC/MS method

Table 2. Typical Preparation of Calibration Standard

1.
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Table 3. Typical Preparation of QC Samples

1. Prepare Spiked samples for determination of matrix recovery (laboratory fortified sample matrix). Select 10% of analytical samples and set aside an additional aliquot of each.
2. Prepare a continuing calibration check standard at the mid-range level.
3. Prepare any other QC samples as needed.

Detailed Procedures

Basis for Method

As previously mentioned we use a hybrid method based on US EPA method 1694, "Pharmaceuticals and Personal Care Products in Water, Soil, Sediment and Biosolids by HPLC/MS/MS." Please refer to the latest version of this method (currently dated December 2007; attached as Appendix 1) for all details.

For historical reasons and site-specific considerations, we have chosen to depart from method 1694 in several minor ways. The most substantial differences include:

-

Once again, the primary source for our PPCP method is US EPA method 1694. This should be consulted whenever questions arise. However, the analyst should keep in mind that we have made some specific modification. These are itemized below in Table 4.

Table 4. UMass Protocol Departures from US EPA Method 1694

§ from 1694	Step	1694 protocol	UMass protocol

We use our Acquity UPLC with a Waters Micromass Quattro Premier for PPCP analysis. The comparison is shown below in **Error! Reference source not found.**.

Table 5. Instrument Parameters for PPCP Analysis

Step	1694 protocol	UMass protocol
Stationary Phase		
Length		
Internal Diameter		
Particle Size		
Injection Type		
Column Temp	20°C	20°C
Solvent Program		See Table

UMass Detailed Procedures

Sample Preservation

1. **Add one of the following quenches, if there is an oxidant residual.**[4]
   - A. ~ 50 mg/L Ascorbic Acid[5]
   - B. Reduced sulfur oxides S(+II to +IV)
     - ~40 mg/L sodium sulfite dose (Na_2SO_3)
     - ~90 mg/L sodium thiosulfate ($Na_2S_2O_3$)
   - C. Ammonia: convert free chlorine to chloramines
     - ~1 g/L NH_4Cl crystals (Desiccator in 301 Elab II) using dispenser made by glass shop
     - ~1.5 g/L $(NH_4)_2SO_4$ crystals
   - D. ~3 mL/L of 0.1 N sodium arsenite solution[6]

2. **Add a biocide, if samples cannot be extracted within 12 hours**[7]
   - ~1g/L of sodium azide

3. **Place aqueous samples in a dark refrigerator until extraction.**
   - Samples should be extracted and analyzed as soon after collection as possible, but under no circumstances should more than 7 days be allowed to elapse

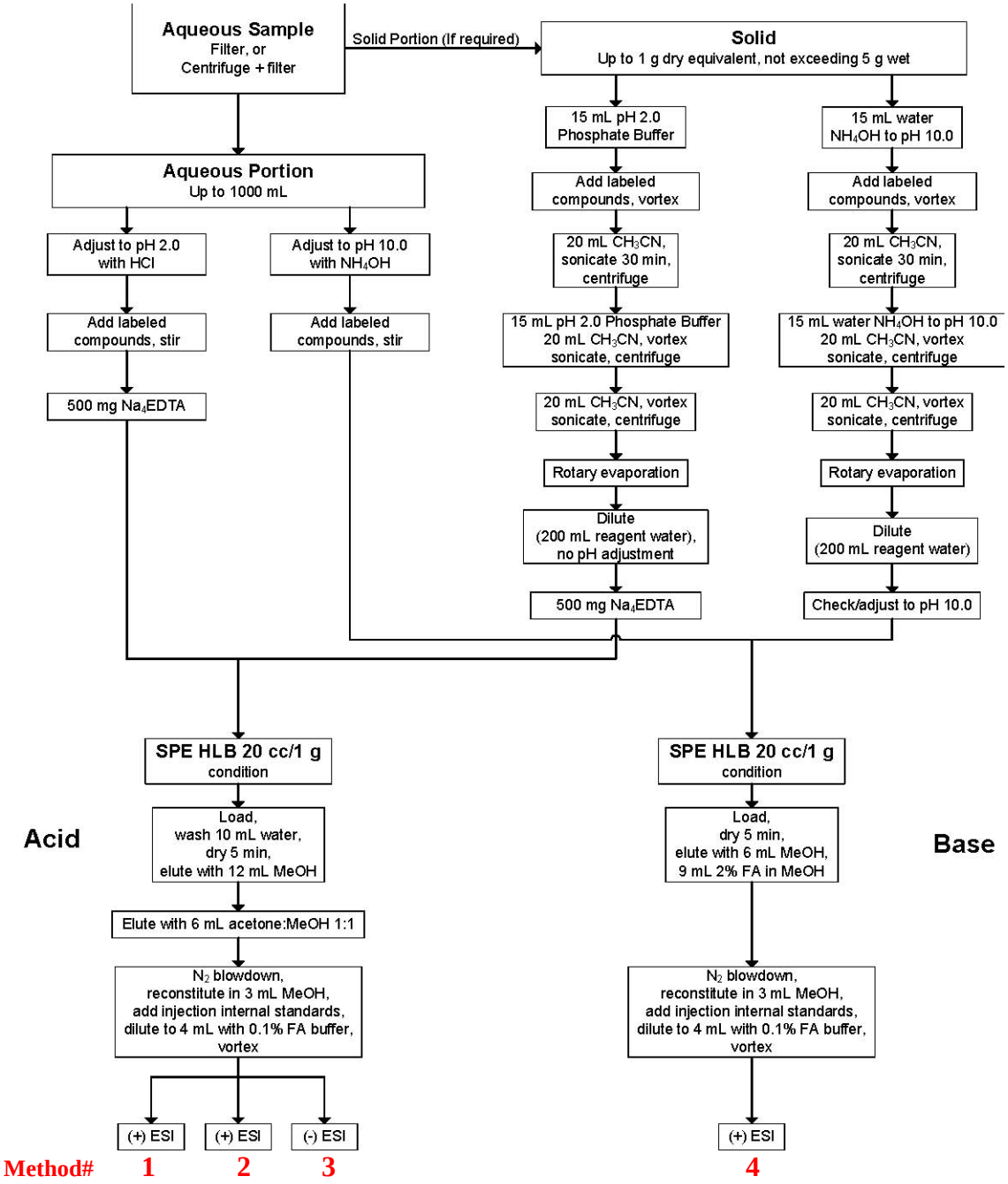
[4] Listed in general order of preference. All except ammonia reagents are added to quench up to about 20 mg/L free chlorine. Amounts can be adjusted up or down as needed. Avoid use of sulfide, as it is a more powerful reactant and reducing agent. Hydrogen peroxide (1M/M, forming O_2 and H_2O) is also effective at reducing chlorine [Worley et al., 2003; JAWWA 95:3:109], but has not been adequately tested for PPCP analysis.
[5] Forms dehydro-ascorbate (2 electron transfer)
[6] kept in refrigerator # 3
[7] distribution system samples having a chlorine residual less than 0.5 mg/L should be treated with the biocide at the time of collection, regardless of when the sample is extracted.

before extraction. The EPA encourages sample extraction within 48 hrs. If the holding time exceeds 48 hours samples must be frozen. Organic extracts can be held for 40 days.

Sample Extraction and Preparation for LC/MS Analysis[8]

1. **Bring analytical samples to room temperature, and aqueous standards and QC samples**
2. **Add isotopic standard mix to each sample**
3. **Divide into two aliquots for acid and base extractions**
4. **Adjust pH to desired level**
   - Acid extract: bring to pH 2 with HCl
   - Base extract: bring to pH 10 with NH_4OH
5. **Add chelator**
   - Acid extract: add 500 mg/L Na_4EDTA
   - Base extract: nothing needed
6. **Pre-condition SPE cartridge**
   - Both: HLB Cartridges (20 cc/1 g) must be pre-conditioned by eluting with 20 mL of methanol and 6 mL of Milli-Q water; do not allow cartridges to go dry
   - Acid extract: continue with elution with 6 mL of acidified (use HCl to reach pH 2) Milli-Q water
7. **Apply sample to SPE Cartridge**
   - At 5-10 mL/min; requires 2-3 hours
8. **Wash and Dry**
   - Acid extract: was with 10 mL of Milli-Q water
   - Both: dry under vacuum for about 5 min
9. **Elute**
   - Acid extract: use 12 mL of methanol[9]
   - Base extract: use 6 mL of methanol, then 9 mL of 2% formic acid
10. **Blowdown and solvent exchange to methanol**
    a) Bring to dryness with N_2 in a 50°C bath
    b) Reconstitute with 3 mL methanol
    c) Dilute to 4 mL with 0.1% formic acid solution
11. **Run samples as quickly as possible**
    - Keep autosampler compartment refrigerated (4C)
    - If they can't be run immediately, store final methanolic extract in an autosampler vial in freezer; do not exceed 40 days holding time

[8] Typical prep time is 4 hours for a run of 30 samples
[9] Follow by 6mL of acetone:methanol (50:50) if triclosan or triclocarban is to be isolated



Analysis by Liquid Chromatography / Mass Spectrometry[10]

[10] typically requires 20 hours of LC/MS time for a run of 30 samples

LC/MS Set up and Initiation of Run

1. **Check there is enough liquid nitrogen and mobile phases**
2. **Load samples into autosampler block**
   - enter information into MassLynx sample list
3. **Prime and stabilize UPLC and MS**
   - prime both channels for 1 minute
   - turn on cone and collision gases in tune window
   - turn MS to operate in tune window
   - start UPLC flow at program flow rate
   - monitor MS signal
   - turn on PDA lamp
4. **start LC/MS run**
5. **Inspect data from first few samples**

LC/MS Shut-down procedure

- turn off UPLC solvent flow
- turn off MS in tune window
- turn off cone and reagent gases

UPLC Solvent Program

Separate solvents and solvent programs are required for positive electrospray (ESI+) and negative electrospray (ESI-). The methods used at UMass are currently:

Table xx. Kasprzyk-Hordern Method 1 Solvent Program for ESI+

Step	Time (min)	Flow (mL/min)	%A	%B	Curve
1	0	0.31	100	0	
2	0.20	0.31	100	0	6
3	1.00	0.31	95	5	6
4	5.00	0.31	90	10	6
5	8.00	0.31	80	20	6
6	10.00	0.31	55	45	6
7	11.50	0.31	55	45	6
8	13.00	0.31	0	100	6
9	15.00	0.31	0	100	6
10	16.00	0.31	100	0	6
11	20.00	0.31	100	0	6

Table xx. Kasprzyk-Hordern Method 1 Mobile Phases for ESI+

Component	Solvent A	Solvent B
Water	94.5%	0%
Methanol	5.0%	99.5%
Acetic Acid	0.5%	0.5%

The solvent programs used with EPA method 1694 are shown in the following tables. While these are not currently used in the UMass lab, they offer possible future alternatives that that could be used for analysis of new analytes.

Table xx. LC Conditions for EPA Method 1694; Group 1 – Acidic extraction, ESI+

LC Gradient Program		LC Flow Rate		General LC Conditions	
Time (min)	Flow Mixture[1]	(mL/min)	Gradient	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

Table xx. LC Conditions for EPA Method 1694; Group 2 – Acidic extraction, ESI+

LC Gradient Program		LC Flow		General LC Conditions	
Time (min)	Flow Mixture[1]	Rate (mL/min)	Gradient	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

Table xx. LC Conditions for EPA Method 1694; Group 3 – Acidic extraction, ESI-

LC Gradient Program		**LC Flow**		**General LC Conditions**	
Time (min)	**Flow Mixture1**	**Rate (mL/min)**	**Gradient**	**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

Table xx. LC Conditions for EPA Method 1694; Group 3 – Basic extraction, ESI+

LC Gradient Program		**LC Flow**		**General LC Conditions**	
Time (min)	**Flow Mixture1**	**Rate (mL/min)**	**Gradient**	**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

Data Analysis & LC/MS Reporting

12. <u>Data Analysis begins with the first injection.</u>

d) The analyst must inspect the first few injections to see that:

- the solvent blank is free from extraneous peaks

- the first standards have all of the transitions expected
- the first standards exhibit good chromatography
- the isotopic internal standard peak areas are within tolerance limits.
- retention times of the analytes are within expected windows (see **Error! Reference source not found.**)
    - Small differences in retention time are expected (from small changes in headloss through the column, temperature, leaking septa, etc.) from one run to the next. Because the retention factors are not as likely to change, retention times may be best re-estimated from them (see equation below). The mobile phase retention time (t_M) may be viewed directly (time for first peak to come off), calculated based on the observed retention time (t_R) from an easily identifiable solute peak (e.g., the internal standard), or from the column void volume (V_M) divided by the gas flow rate through the column.

$$t_R = t_m\left(k' + 1\right)$$

e) The analyst must report on the success or failure of these first few injections by email to the technical QC officer or his/her designee if he/she is not available.
    - The message must also include the address of the Faculty QC officer in the "cc:" line (reckhow@ecs.umass.edu).
    - The subject line of this email message must simply read "PPCP QC report"
    - The report must also include the sample types (e.g., field samples from Stamford), field collection date, laboratory treatment date (if any), and analysis date

Table 6. ESI positive Analyte Parameters

Analyte	Typical Retention Time, t_R (min)	Run#	Transitions			Voltages		
			Parent	Daughters		Cone	Collision	
				D#1	D#2		D#1	D#2
Unretained Solutes	?? (t_M)	All						
Atenolol	4.4	1	266.9	190.1	145	34	19	25
Atenolol-d6		1	273.9	190.1	145			
Ranitidine	4.4	2,3	314.9	176	123.9	26	17	24
Ranitidine-d6		2,3	320.9	176	123.9			
Sulfamethoxazole	9.0	1	253.9	156	107.9	26	16	21
Sulfamethoxazole-d4		1	257.9	160	111.9			
Trimethoprim	8.8	2	290.2	230	123	42	22	22
Trimethoprim-d9		2	299.2	234	123			
Ciprofloxacin	10.1	3	332.5	315.1	289.2	35	20	20
TCEP	12.4	3	285	223	161	30	15	15
DEET	13.0	2	192.1	118.9	90.9	30	19	19
Naproxen	13.3	3	231	185	170	20	15	30
Naproxen-d3		3	234	188	173			
Atorvastatin	13.5	1	559	440	466	40	17	17
Atorvastatin-d5		1	564	445	471			
Propranolol	12.9	2	259.9	183.1	116	34	18	16
Propranolol-d7		2	266.9	183.1	123			

Table 7. ESI negative Analyte Parameters

Analyte	Typical Retention Time, t_R (min)	Run#	Transitions			Voltages		
			Parent	Daughters		Cone	Collision	
				D#1	D#2		D#1	D#2
Unretained Solutes	?? (t_M)	All						
Naproxen	4.2	1	228.9	185.1	170.1	15	8	15
Naproxen-d3		1	231.9	188.1		15	8	
Estrone	6.1	1	269.2	145	143	60	41	53
Estradiol-13C		1	273	185	147	60	42	42

13. Examine samples at end of run

a) The analyst must report on status of the sample extracts and any observed problems by email to the graduate QC officer or his/her designee if he/she is not available.
   - The message must also include the address of the Faculty QC officer in the "cc:" line (reckhow@ecs.umass.edu).
   - The subject line of this email message must simply read "PPCP QC report"
   - The report must include the following information: (1) start date and time for run, (2) approximate average % volume loss for extracts, (3) maximum % volume loss observed; and (4) any other problems noted

14. Preparation of Standard Curves

- We use least squares best linear fit of the standard peak area ratios (PARs) regressed against their known concentrations. Many people use an MS Excel that is re-used as a template. When using these types of files, be careful of the following:
  - All standard data are being used for the standard curves. (this is a problem when standard data have been removed due to outliers, and not replaced in subsequent runs).
  - Reagent blanks are subtracted where appropriate, and not where inappropriate (see: **Error! Reference source not found.**, pg. **Error! Bookmark not defined.**)
- Standard curves must also include the zero standard
- Standard curves must be visually inspected for non-linear behavior and the possible presence of outliers
  - When noted, an outlier may be excluded from the calibration curve, after consultation with the graduate QC officer. Removal of an outlier should:
  - Substantially improve the standard curve linearity or correlation
  - Improve agreement with the calibration check standard
  - Bring the regressed slope closer to the expected values based on recent data from the calibration slope control chart
  - Be careful when removing an outlier in a spreadsheet that you remove it from the range used for graphing as well as from the range used for calculation of regression coefficients. Also be careful that you don't inadvertently replace it with a zero.

15. Evaluation of standard curves and other QC data by the analyst

a) This must be done as soon as possible, but no later than 24 hours from the end of the GC run. Compare with quantitative criteria in Table 9.

b) Send an email report as in #1b above, but this time include the following information:
   i. Calibration curve slopes for all analytes (usually 9)
   ii. Internal standard average area
   iii. Surrogate peak area
   iv. Spike recoveries

16. <u>Validation of QC data</u>

a) The technical QC officer or his/her designee then must compile the analyst's data into the running QC data files, and examine the updated control charts.

b) The technical QC officer or his/her designee then must send an email message to the faculty QC officer stating whether the QC data are within control limits, and if they are not, what actions will be taken.

- Again, the subject line of this email message must simply read "PPCP QC report".
- This must be done as soon as possible, but no later than 24 hours from the time of receipt of the detailed QC report (per #3).

Table xx. US EPA Method 1694 MDLs

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

Table xx. US EPA Method 1694 MDLs (cont)

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						

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						

				Detection limits and minimum levels					
				Water (ng/L)		**Other (Φg/g)**		**Extract (ng/ΦL)**	
Analyte	**RT (min)**	**Parent- daughter m/zs**	**Quantitation reference**	**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						

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						

Glassware Cleaning and Post-run Clean Up

- Clean all non-disposable glassware as follows:
    a. Remove any visible contamination with a brush and detergent
    b. Soak glassware in acid bath for >1 hour
    c. Rinse thoroughly with laboratory reagent water from building tap
    d. Dry and store glassware in a protected environment (e.g., with teflon caps)
- Vials should not be re-used. They should be inactivated.
- SPE cartridges should not be re-used.
- Syringes should be rinsed at least 5 times with methanol or acetone, before and after use.
- Acid baths must be cleaned and refreshed on a weekly basis
- Wear protective gloves to prevent injury and to minimize the possibility of contaminating labware.

Standard Solutions, Solvents and Supplies

Preparation of Aqueous Spike Solutions[11]

The following description is used for tests conducted under Water Research Foundation project #4162.

1. Prepare 11 single-compound aqueous solutions and one two-compound aqueous solution as summarized in the table below. Each should be prepared in a 1-L volume and stored in a refrigerator. Note the dates and prepare fresh every 6 months or more frequent if there are signs of compund loss.

Table 1. Single Compound Standards

#	Compound	Amount of compound added to 1000 mL Water	Volume of solution	Concentration in g/L
1	Estrone	0.0135 g	1000 mL	0.0135
	Ciprofloxacin	0.041g	1000 mL	0.041
2	Naproxen	0.0113 g	1000 mL	0.0113
3	TCEP	0.1 ml	1000 mL	0.142
4	Sulfamethoxazole	0.127 g	1000 mL	0.127
5	Ranitidine	0.1568 g	1000 mL	0.1568
6	Atenolol	0.1341 g	1000 mL	0.1341
7	DEET	0.024 ml	250 mL	0.095808
8	Atorvastatin	0.029 g	1000 mL	0.029

[11] typically requires 20 minutes

9	Trimethoprim	0.146 g	1000 mL	0.146
10	NDMA	1.85 ml	1000 mL	0.03717
11	Perchlorate (Na)	0.0285 g	1000 mL	0.0285
12	Estrone alone			

2. From these solutions, prepared two combined spiking solutions, called the "low level spike solution" and the "high level spike solution". The can be prepared by adding a certain number of mLs from each of the above 12 solutions to make a total volume of 100 mL as listed below.

Table 2. Preparation of Combined Spiking Solutions

#	Compound	Low Level spike soln		High Level spike soln	
		mLs added to 100 mL total	uM/L in LL spike soln	mLs added to 100 mL total	uM/L in HL spike soln
1	Estrone	0.2	1.101	20	10.3
	Ciprofloxacin		0.247		24.7
2	Naproxen	0.2	0.982	20	98.2
3	TCEP	0.02	0.099	4	19.9
4	Sulfamethoxazole	0.02	0.100	4	20.1
5	Ranitidine	0.02	0.100	4	19.9
6	Atenolol	0.02	0.101	4	20.1
7	DEET	0.02	0.100	4	20.0
8	Atorvastatin	0.2	0.100	28	14.0
9	Trimethoprim	0.02	0.101	4	20.1
10	NDMA	0.02	0.100	4	20.1
11	Perchlorate (Na)	5	11.638	4	9.3
12	Estrone alone	27			

3. Then add some of the spike solution to a certain volume of raw water to create the "spiked raw water". This is done for the Low level tests by adding 100 mL of the "LL spike solution" to a 10-L volume of unspiked raw water (resulting in a 100-fold dilution of the LL spike solution). For the high level tests, 3-liters of the unspiked raw water is dosed with 75 mL of HL spike solution (resulting in a 40-fold dilution).

Table 3. Final molar concentrations in the single-compound solutions and the LL and HL spike solutions

	Concentration (uM)		
Compound	**single**	**LL soln**	**HL soln**
naproxen	49.075	0.098	9.815
TCEP	497.390	0.099	19.896
sulfamethoxazole	501.425	0.100	20.057
ranitidine	498.721	0.100	19.949
atenolol	503.499	0.101	20.140
DEET	500.878	0.100	20.035
atorvastatin	49.945	0.100	13.985
trimethoprim	502.897	0.101	20.116
NDMA	501.755	0.100	20.070
perchlorate(Na)	232.767	11.638	9.311
Ciprofloxacin	123.739	0.247	24.748
Estrone	49.932	0.100	9.986

Table 4. Final molar concentrations in the spiked raw waters.

	Conc (nM)	
Compound	LL Raw	**HL Raw**
naproxen	0.98	245
TCEP	0.99	497
sulfamethoxazole	1.00	501
ranitidine	1.00	499
atenolol	1.01	504
DEET	1.00	501
atorvastatin	1.00	350
trimethoprim	1.01	503
NDMA	1.00	502
perchlorate(Na)	116.38	233
Ciprofloxacin	2.47	619
Estrone	1.00	250

	Conc (ng/L)	
Compound	LL Raw	**HL Raw**
naproxen	226	18,833
TCEP	284	47,333
sulfamethoxazole	254	42,333
ranitidine	314	52,267
atenolol	268	44,700
DEET	192	31,936
atorvastatin	580	67,667

trimethoprim	292	48,667
NDMA	74	12,390
perchlorate(Na)	14,250	9,500
Ciprofloxacin	820	68,333
Estrone	270	22,500

4. Aqueous standards were prepared at 25%, 50%, 75% and 100% of the concentrations of the low level spiked raw water

Table xx. Concentration of Aqueous Standards

Concentration Level (% of LL spiked water) →	Concentration (nM)				
	0%	25%	50%	75%	100%
Estrone	0.00	2.75	5.51	8.26	**11.01**
Ciprofloxacin	0.00	0.62	1.24	1.86	**2.47**
Naproxen	0.00	2.45	4.91	7.36	**9.82**
TCEP	0.00	0.25	0.50	0.75	**0.99**
Sulfamethoxazole	0.00	0.25	0.50	0.75	**1.00**
Ranitidine	0.00	0.25	0.50	0.75	**1.00**
Atenolol	0.00	0.25	0.50	0.76	**1.01**
DEET	0.00	0.25	0.50	0.75	**1.00**
Atorvastatin	0.00	0.25	0.50	0.75	**1.00**
Trimethoprim	0.00	0.25	0.50	0.75	**1.01**
NDMA	0.00	0.25	0.50	0.75	**1.00**
Perchlorate (Na)	0.00	29.10	58.19	87.29	**116.38**

5. All aqueous samples intended for SPE concentration (raw waters, treated waters, and aqueous standards) must be spiked with the mix of isotopically-labelled internal standards immediately prior to the start of the extraction procedure. This is done by adding 2 uL of the labeled standard mix for 1 liter of aqueous volume (or 1.3 uL for 650 mL aqueous volume, etc). The labeled standard mix is normally kept in a 5-mL vial (heavy walled, conical bottom) in refrigerator #25. It's blue tape label says "8 labelled PPCP standards". Be careful in adding this. It is challenging to add such a small volume to a large volume and still get good quantitative transfer and good mixing.

Preparation of Instrument Calibration Standards[12]

1. Prepare two PPCP stocks in methanol at 10µM each (100 mL total volume)
   a. 11 compound stock

Compound	Single Compound Stock Conc (mM)	Vol added (mL)
Estrone	0.1	10
Naproxen	0.1	10
TCEP	1	1
Sulfamethoxazole	1.516	0.659631
Ranitidine	1	1
Atenolol	1	1
Propranolol	1.27	0.787402
DEET	1	1
Atorvastatin	0.545	1.834862
Trimethoprim	0.1	10
Ciprofloxacin	0.1	10

   b. 7 compound stock

Compound	Single Compound Stock Conc (mM)	Vol added (mL)
17-B-estradiol	0.231	4.329004
Diclofenac	1	1
Ibuprophen	1	1
Metoprolol	1	1
Cimetidine	1	1
Carbamazepine	1	1
Caffeine	0.520109	1.922673

2. Use these to prepare serial dilutions in Milli-Q water at 8 concentration levels from 6.25 nM to 800 nM in accordance with the table below. Use dedicated labelled volumetric flasks (25 and 10 mL)

Serial Dilution Scheme for preparing Instrument Standards

Standard (nM)	Mother	Vol added (mL)	Total vol (mL)
800	11 & 7 cpd stocks	2 of each	25
400	800	2	10
200	400	2	10
100	200	2	10
50	100	2	10

[12] typically requires 20 minutes

25	50	2	10
12.5	25	2	10
6.25	12.5	2	10
0	None	2	10

3. Add 2 mL of each to a labelled autosampler vial. To each add 3 µL of the combined isotopicall-labelled compound stock.

Preparation of Sodium Azide Preservative[13]

b) Add 800 mg of NaN_3 (99.9+% purity) to a 10-mL volumetric flask
c) Fill flask to mark with Super-Q water
d) Cap and invert 5 times to dissolve
e) Transfer this solution to a septum-capped vial and store in a refrigerator
f) Solution should be prepared fresh every 2 months

Supplies

Table 8. Summary of Supplies for PPCP Analysis

Item	Catalog #	Approx. Price	Approx # used/run[14]
Pasteur Pipettes	Fisher: 13-678-20A	720/ $46.10	10
Autosampler Vials			40
DIUF Water	Fisher: W2-20	$32.29	Not normally used
Methanol			
Sodium Azide	Aldrich: 43,845-6		
H_2SO_4	UMass Stockroom		34 mL

[13] Only necessary for field sampling, or if aqueous samples are to be held for more than 24 hours
[14] Assuming about 30 samples analyzed

Heavy-walled vials	Supelco #3-3293		

Quality Assurance/Quality Control

General Approach

Quality assurance is an essential and integral part of a research study. The purpose of any QA plan is to insure that valid and reliable procedures are used in collecting and processing research data. The procedures outlined are designed to eliminate or reduce errors in experiments, sample preparation and handling, and analytical methods. Emphasis must be given throughout one's lab work to incorporate the plan into the research project by all research personnel.

Any equipment and experimental procedures that are used to provide numerical data must be calibrated to the accuracy requirements for its use. Records are to be kept of all calibrations. Calibration schedules are generally established for all aspects of physical and chemical measurements and these must be strictly followed. Physical standards and measuring devices must have currently valid calibrations, traceable to national standards. Most chemical standards are acquired from commercial suppliers, and they should be of the highest purity available. When necessary, unavailable standards should be synthesized using the best methodology available.

As a general rule, experiments should be replicated to assure reproducibility. All data reported should include a statement of its uncertainty, and the means for the determination and assignment of such limits. Standard reference materials are used for this purpose where possible. Statistically established confidence limits and an analysis of sources of systematic error are to be used in the absence of experimental demonstration of limits of inaccuracy.

All data will be subject to review by the faculty QC officer before release. The analysts involved will certify reports as well as all who review them. All analysts and QC officers must attest that the data and associated information contained in the report are believed to be correct and that all quality assurance requirements have been fulfilled, unless exceptions are approved and noted. Careful and detailed laboratory records will be maintained by each analyst, including source of reagents, meticulously detailed procedures (referring to an SOP, and any departures or clarifications), instrument and conditions of analysis, failed experiments, etc. Data output will be archived.

Regular meetings will be held to review the results and project progress, and to plan further experiments. The results will be analyzed promptly and summarized by means of internal reports or formal reports for external review. The experimental and analytical procedures will be reviewed for their performances and changes will be made as necessary. The quality assurance program as described in this document must be strictly observed.

Quality Assurance Objectives

The precision, accuracy and method detection limits will be evaluated, and where there are existing methods, held within the control limits set forth in the accepted references (e.g.,APHA et al., 1999; USEPA-EMSL, 1990; ASTM, 1994). In addition to the analysis of sample replicates, a minimum of 10 percent of the time is typically involved in analytical determinations that are devoted to quality control. The precision of each test is determined through analysis of sample replicates. These are commonly presented in the form of control charts (e.g. Section 1020B of APHA et al., 1999).

The accuracy of each analysis will be determined by measuring spike recoveries in the matrix of interest. The relative errors will be calculated and will be considered acceptable if they fall within the control limits determined for the particular test. For new methods developed at UMass or for modifications of existing methods, we will have to establish criteria on acceptable control limits. In general, a test will not be deemed useful if its precision or accuracy is found to be equal to or greater than 20% of the highest values observed. Where possible, external performance standards will also be run. This serves as a measure of accuracy both for the analysis and for standard preparation.

Data generated by the QA program will be incorporated into a Quality Control (QC) archive that is used to monitor the fluctuations in precision and accuracy so that chance or assignable causes of error can be determined. Control charts such as X-charts for simple successive samples or cumulative sum techniques may be employed to record both precision and accuracy data (Taylor, 1987).

General Procedures

General sample collection and handling will be in accordance with the guidelines of Section 1060 of Standard Methods (APHA et al., 1999). All previously established analytical methods used in laboratory research will follow approved methods in the standard compilations (e.g., ,APHA et al., 1999; USEPA-EMSL, 1990, or ASTM, 1994).

Reagent grade chemicals or higher quality when needed will be used throughout the research. Super-Q water (purified by reverse osmosis, deionization, and carbon adsorption) will be used for preparation of reagents, sample blanks, and dilution water. Where necessary, this water will be further purified using batch UV irradiation. All glassware used in the experiments and in analytical analyses will be thoroughly cleaned with a chromium-free sequence of detergent, oxidant and acid to prevent interferences from trace contaminants.

Procedures specific to Chromatographic Analysis

Quantitative chromatographic analyses must always be standardized by the use of carefully prepared solutions of known standards. In general, non-aqueous primary stocks are kept in a -10^{o}C freezer and discarded after two months. Duplicate primary stocks are prepared regularly, as a check against degradation of the primary stock. Data quality objectives for GC analysis is assured by: (1) use of blanks; (2) use of an internal standard;

(3) analysis of duplicates; (4) determination of spike recovery; (5) analysis of a matrix standard; (6) monitoring of response factors; and (7) monitoring for spurious peaks.

Three types of blanks should be run daily or with each set of samples: (1) reagent blanks, composed of the extracting solvent(s) and internal standard; (2) laboratory water blanks or a zero standard; and (3) field blanks. This last type of blank is prepared by transporting laboratory reagent to the study site, and transferring it to a labeled sample vial at the time of general sample collection. In some laboratory experimentation, the laboratory water blank can also serve as a "field blank". Peaks co-eluting with the analyte may appear in either the reagent blanks or the laboratory water blanks. Efforts must be made to minimize these (e.g., use of highest quality reagents, avoidance of possible sources of contamination). Some small interfering peaks or background analyte contamination may be unavoidable. If the evidence suggests that contamination is from the reagents (solvent & internal standards), the concentrations measured in the reagent blank should be subtracted from the concentrations determined for the analytical samples. If the source is unclear (possibly from the laboratory environment), it should not be subtracted. If the laboratory water blank shows higher apparent analyte concentrations than the reagent blank, there is probably some contamination from the laboratory water. This additional contaminant level should not be subtracted from analytical samples, unless those samples were prepared with laboratory water.

An internal standard is used to control for solvent evaporation and variable injection volume. Most samples will be run in duplicate. If they differ by more than the acceptable range, additional GC or LC injections will be made. If there still exists a significant problem, either the original sample will be re-extracted or the data will be discarded.

Spike recoveries are determined for each analyte/method. With some DBP studies, precursor matrix standards may be prepared and analyzed for the full suite of analytes. These are generally test-specific, but it is also traditional in our laboratory to make use of a bulk sample of raw Wachusett Reservoir water. This would be treated with the oxidant of interest under well-defined conditions. Control charts are prepared and continually updated for matrix spikes and standards. Data falling outside of the control limits require that the method be re-tested in order to bring it back under control. Calibration response factors will be monitored and compared from one day to the next. Significant changes in either these response factors or in the spike recoveries will be considered cause for method re-evaluation. Finally, general QA requires that all chromatograms be manually inspected for spurious peaks. When such peaks are observed, potential sources must be investigated. If the problem cannot be corrected, the data may have to be discarded.

Instrument usage must be monitored by means of instrument log books. A sign-up book or calendar is advisable for scheduling purposes. However, once an instrument is to be used, the analyst must document this use in an instrument-dedicated log book. The exact dates, times (starting and ending) and approximate number and type of samples must be recorded. General maintenance activities must be documented here (e.g., new septa, cleaning of injection liner). The analyst should also indicate any irregularities in the instrument's operation or in the physical environment (e.g., high room temperature)

This outlines our general QA philosophy for chromatographic and other methods. Many specific details relating to the individual procedures may be found in the cited references, and other particulars will have to be adopted as new methods are developed.

Data Quality Indicators

A wide range of data quality indicators are normally calculated for the purpose of assessing method performance. Some of these are defined below.

Precision

Precision may be expressed as the relative percent difference (RPD) from duplicate measurements (C_1 and C_2) of the same sample:

$$RPD = \frac{|C_1 - C_2| x100\%}{(C_1 + C_2)/2}$$

When three or more replicates are available, the relative standard deviation (RSD) should be used:

$$RSD = \left(\frac{s}{\bar{y}}\right) x100\%$$

where the standard deviation (s) is determined from:

$$s = \sqrt{\sum_{i-1}^{n} \frac{(y_i - \bar{y})^2}{n-1}}$$

Accuracy

Accuracy is best assessed by analysis of a standard reference material (SRM) prepared in the matrix of interest. It is quite rare that such materials are available, so two possible compromise may be used instead. These are the laboratory-prepared matrix spikes, and the independent SRM prepared in a standard matrix. One or both may be analyzed and the percent recovery (%R) calculated as a measure of accuracy.

$$\%R = \left(\frac{S - U}{C_{sa}}\right) x100\%$$

where:

S = measured concentration in spiked aliquot

U = measured concentration in unspiked aliquot

C_{sa} = actual concentration of spike added

$$\%R = \left(\frac{C_m}{C_{srm}} \right) x100\%$$

and:

C_m = measured concentration of SRM

C_{srm} = actual concentration of SRM

Method Detection Limit (MDL)

The method detection limit (MDL) will be defined as:

$$MDL = s_7 \bullet t_{(n-1,1-\alpha=0.99)}$$

where:

s_7 = standard deviation of 7 replicate analyses where the mean is no more than 10 times the MDL

$t_{(n-1,1-\alpha=0.99)}$ = Students' t-value for a one-sided 99% confidence level and a standard deviation estimate with n-1 degrees of freedom.

Linearity

The calibration curve linearity (L) is defined as the ratio of the slope using the highest standards (S_U) divided by the slope determined from the lowest standards (S_L) as follows:

$$L = \frac{S_U}{S_L}$$

The highest standards shall be all those that fall within the top 50% of the calibration range including the 50% standard if it is used. If only one standard falls within that range, the S_U shall be calculated based on the top two standards. The lowest standards are all those that fall within the bottom 50% of the calibration range including the 50% standard if it is used. Least squares linear regression is used to determine slopes.

Sampling Custody

In most cases analyses will be performed immediately upon return from the field or after preparation of samples in the laboratory. Problems with sample custody are minimized, because the same people who receive (or sometimes, collect) the samples also analyze them. In general sample collection, handling, and preservation will be in accordance with the guidelines of Section 1060 of Standard Methods (APHA et al., 1999). All samples must be fully labeled with the sample identity, date, and name of researcher.

Sample Collection and Storage

Samples are collected and stored in clean borosilicate (e.g., Pyrex, Kimax) glass containers. Containers must be capped with either Teflon-lined septa or ground glass stoppers. Exceptions are made for large volume samples which may be stored and shipped in clean polyethylene carboys. Glass containers are cleaned with detergent, followed by 5% sulfuric acid soak, and final rinsing with reagent-grade water. These containers are dried in a 150ºC oven.

Samples for PPCP analysis must be kept in the dark, and in a refrigerator from the time of disinfectant quench until the start of analysis. PPCP are biodegradable, so a biocide must be added if the samples are to be kept for more than 2 days prior to extraction. Organic extracts can be kept in a freezer (~-10C) for up to 14 days. The liquid level must be marked on all vials at the time of capping, so that solvent loss can be noted.

Handling and Storage of Standards and Reagents

Solvent used for extraction is purchased from Aldrich Chemical Company as a LC/MS grade product. They are mixed as needed with the internal standard in batches of 1-L. This solvent+IS is then stored in the original solvent bottle, but clearly marked as having been fortified with the IS. It is used until the volume reaches 20 % of the original. At this point the solvent+IS is discarded (due to concern over excessive volatilization and changes in the IS concentration). Whenever new solvent+IS is mixed, the IS peak are is evaluated by injection of a solvent blank. If this falls outside of the control limits (±30 of the long term average), the solvent+IS is discarded and a new one is prepared.

Data Reduction, Validation and Reporting

To ensure the accuracy and permanency of collected data, all research data are recorded with permanent ink in bound notebooks and all QC data (precision, accuracy) are recorded in instrument log notebooks. Summary QC graphs and tables are reviewed at least quarterly by the Faculty QC officer to observe noteworthy trends or inconsistencies. These are maintained in loose leaf notebooks for subsequent use in preparing progress reports, final reports, and theses. Major concerns and conclusions are reported to the external Project Officer via the progress reports.

Pages from the laboratory data books are regularly duplicated so that a file copy of raw data can be placed in safe storage in the event that the book is lost or destroyed. At the end of the project, all bound data books and any loose leaf data will be stored by the project team for at least ten years. Summary data files will be put on magnetic media so that statistical analysis of the data can be done. Our laboratory has several personal computers that can be used for this purpose.

Procedures specific to PPCP Analysis

General Analytical QC

Many types of QC procedures are required as indicated under US EPA method 1694. The guidelines below are prepared assuming that samples are run in groups, whereby a "daily" frequency refers to once every day that the analytical method is being used.

Table 9. Summary of QC Elements as Applied to PPCP Analysis

Types of Samples or Standards	§ in 1694	Purpose	Frequency	Timing	QC data
Solvent Blank		Assess general LC operation, cleanliness of column, and possible ghost peaks	1 for every 25 samples	Beginning of each day and scattered throughout	
Laboratory Performance Check Standard (LPC)[15]		To establish basic LC performance for separation and sensitivity	1 standard per day	Beginning of each day	S/N, peak gaussian factor (PGF), Resolution
Initial Demonstration of Capability (IDC)		To show that an analyst's technique and equipment are adequate for PPCP analysis	4-7 standards when first learning method, otherwise not done	Not done unless learning	Mean % recovery and standard deviation
Method Detection Limit (MDL)		To determine the lowest concentration level that the analyst can report	7 sandards run	Usually done once per year	MDL and EDL
Laboratory Reagent Blank (LRB)[16]		Test lab conditions and quench for interferents	1 per day, if no FRB	Near Beginning of day	Max peak size within analyte windows
Field Reagent Blank (FRB)[17]		Test all field conditions for interferents	1 per day, if sampling occurred outside of the lab	Near end of day	Max peak size within analyte windows
Spiked sample, or Laboratory Fortified Sample Matrix (LFM)		To test analyte recovery in the sample matrix	1 for every 10 samples	Mixed throughout day	% recovery, mean and standard deviation

[15] usually one of the calibration standards serves this purpose
[16] laboratory water treated exactly as a field sample (e.g.,buffering, chlorination, quenching, etc.)
[17] laboratory water transported to field, transferred to sampling bottle and then treated exactly as a field sample

Calibration Standards[18]		To provide a basis for determining the concentrations in unknowns	≥ 6 levels including zero	At beginning of day, and re-checked later	Calibration curves (PAR vs. conc.), including slopes and intercepts; surrogate PARs; mean IS areas
Continuing Calibration Check Standards (CCC)[19]		To verify the accuracy of the calibration standards	At least 1 per day	Usually mid-day	
Unchlorinated blank		Evaluate contaminants in background water	1 per run	mid-day	Interfering peak areas; surrogate recovery
Positive chlorination control		Evaluate all conditions including chorination & quench	1 per run	mid-day	Recovery based on known yield
Unknowns or "samples"		This is what you really want to measure	As many as desired	Mixed throughout day	Surrogate recoveries vs. calibration standards, IS areas
		Laboratory Replicates	As many as 1 per sample, depending on study	Throughout	Analytical Precision
		Field Replicates	As many as 1 per sample, depending on study	Throughout	Aggregate sampling handling, treatment and analytical precision

Table 10 shows a recommended sequence for a typical run of about 30 samples. The first two samples require immediate attention, as they are simple indicators of unacceptable QC. When these chromatograms show abnormally elevated noise levels or drifting baseline, the operator must intervene before proceeding. The problem must be diagnosed, solved and the sequence restarted at vial #1.

Table 10: Typical Vial Sequence for a 30-sample Run

Vial #	Sample type	QC objectives
1	Solvent Blank	To check on LC condition
2	Zero Standard	To check for gross contamination of water or lab environment
3-7	Remaining calibration standards	Calibration
8	Laboratory Reagent Blank	Contamination
9-13	**Analytical Samples (5)**	
14	Calibration check standard	Check on accuracy of calibration samples
15-19	**Analytical Samples (5)**	
20	Spiked sample	Spike recovery
21-25	**Analytical Samples (5)**	
26	Field Blank/Lab Blank	Contamination
27	Spiked sample	Spike recovery

[18] Prepared from the currently-used calibration stock II (less than 1 month old)
[19] Prepared from the previously-used calibration stock II

28	Positive Chlorination Control	Recovery
29-33	**Analytical Samples (5)**	
34	Unchlorinated sample	Check on interferences
35-39	**Analytical Samples (5)**	
40	Spiked sample	Spike recovery
41-45	Analytical Samples (5)	
46-48	Calibration standards (0, highest, and one intermediate)	Final check to verify that calibration hasn't changed during run

Special QC Tests

The following are "special" tests that are not part of the normal QC protocol. They are used when first learning this analytical method (e.g., IDC), and they may be used when there are suspected problems or there is a need for method performance evaluation.

Initial Demonstration of Capability (IDC)

This is normally performed by each analyst when he/she is first learning to measure PPCPs. It is designed to be a double-blind test. The QC officer prepares 4 aqueous samples of differing analyte concentrations. This is done by starting with about 10 liters of a real natural water (free from oxidant residual). The QC officer then divides the bulk sample into 4 equal aliquots. Three of these are spiked with known amounts of each analyte (typically from an aqueous stock) and the exact concentrations are recorded and only known to the QC officer. The range of concentrations selected should be appropriate to the range of interest to the analyst.

Procedure

The analyst conducting the IDC is then asked to analyze the set of 4 aqueous samples prepared by the QC officer. Each is divided into 1-L volumes so that separate base and acid extractions may be performed. These should be carried through the entire method including addition of labelled standards compounds, SPE and LC/MS. Along with these 4 aqueous samples, a set of aqueous standards (typically 4 levels plus a blank) and a set of instrument standards (typically 8 levels) must be analyzed.

Performance

IDC results are expected to meet the LFM QC criteria from Table xx.

Method Detection Limit (MDL)

Procedure

Performance

There are no firm performance criteria for MDL. Some typical values are listed in **Error! Reference source not found.**.

Table 11. Reported MDLs for PPCP Analysis in Water

Analyte	**MDL** (μg/L)		
	EPA 1694		

QC Criteria

Quality control data must be analyzed as soon as possible. The best practice is to have the QC data tabulated and evaluated as the run is underway. However, it is recognized that there will be times when this is impossible (e.g., when injections are being made overnight by the autosampler). QC and calibration data must always be analyzed and reported within 24 hours of completion of a run (see section on **Error! Reference source not found.**, page **Error! Bookmark not defined.**). Quantitative criteria (**Error! Reference source not found.**) must be applied, and violations must be immediately reported to the faculty QC officer. The graduate and faculty QC officer along with the analyst will then work out a plan for returning the analysis to acceptable levels of QC. **Error! Reference source not found.** lists some typical corrective action, however the actions taken may differ depending on the particular circumstances. Excursions from QC criteria can be quite complex, and many analytical characteristics and conditions must be considered before a decision can be made on the most effective steps to be taken.

In several cases, quantitative criteria are based on long term trends, and these must be monitored by means of appropriate control charts. Standard slopes, % analyte recoveries, calibration check controls and mean surrogate recoveries are documented over time in this way. All summarized QC data (tabular and graphical) must be kept in a notebook in the room 301 of Elab II. A duplicate set must be deposited with the faculty QC officer (D. Reckhow).

Table 12: Quantitative Criteria for Judging Data Acceptability

Types of Samples or Standards	Frequency	Timing	QC data Acceptance Criteria	Typical Corrective Action
Spiked sample, or Laboratory Fortified Sample Matrix (LFM)	1 for every 10 samples	Mixed throughout day	Mean % recovery = 85%-115%, for PPCPs, 70%-130% for ?	❖ Re-run matrix spikes ❖ Re-examine entire run for errors ❖ Possibly change SOP
Calibration Standards[20]	7 levels including zero		Calibration slopes (PAR vs. conc.), = ±30% of long-term average for PPCPs, = ±50% of long-term average for ?	❖ Run new set of standards ❖ Prepare new PPCP stock ❖ Examine LC for problems, needed maintenance
			Calibration linearity must not fall below 0.5	❖ Use a lower calibration range until L≥0.5, and dilute samples if necessary ❖ Perform maintenance on column and other components
			Average Surrogate area for a run = ±30% of long-term average	❖ Examine chromatograms ❖ Re-run standards, with special attention to derivatization conditions
Continuing Calibration Check Standards (CCC)[21]			Calculated Conc. = ±25% of expected value	❖ 1. Prepare new calibration check standard ❖ 2. Prepare new standard curve based on new stock
Unknowns or "samples"	As many as desired	Mixed throughout day	Average Surrogate area for a run <70% of long-term average	❖ Examine derivatization procedure ❖ Examine surrogate area for matrix spikes & standards ❖ Re-run some samples with more severe methylation
			Average IS area for a run = ±30% of long-term average	❖ Prepare new solvent & IS
			Surrogate area for a sample = ±50% of entire-run average	❖ Re-run samples
			IS area for a sample = ±25% of entire-run average	❖ Inspect samples for possible evaporation

[20] Prepared from the currently-used calibration stock II (less than 1 month old)
[21] Prepared from the previously-used calibration stock II

				❖ Inspect chromatograms for interfering peaks or poor integration ❖ Re-calculate based on peak areas only ❖ Re-run samples
			RSD or RPD for laboratory replicate analyses ≤20% or AD ≤5 µg/L, whichever is less restrictive	❖ Re-run samples and/or discard outliers[22] until precision can be brought under control
			RSD or RPD for laboratory replicate analyses ≤30% or AD ≤10 µg/L, whichever is less restrictive	❖ Re-evaluate sampling and field protocols until precision can be brought under control
			Estimated concentration in unknowns must not exceed highest standard	❖ Re-run samples with higher level standards ❖ Dilute and re-run samples ❖ If within 150% of max standard, concentrations may be flagged as tentative

[22] using Dixon's Q Test, or some logic test (e.g., monotonic increase with timed data series).

Appendix

Papers Focusing on PPCP Analysis

Citation	Notes	Abstract
San Jose Creek WQL, Method 18CD SOP	Preferred method from 4167 study	This method covers the determination of 13 pharmaceuticals and personal care products (PPCPs) in wastewater and groundwater. The procedure concentrates samples, removes some interferences, and then measures analyte concentrations by two separate LC/MS/MS methods.
Vanderford, B.J., Drewes, J.E., Hoppe-Jones, C., Eaton, A., Haghani, A., Guo, Y.C., Snyder, S., Ternes, T., Schleusener, M. and Wood, C., J. (2012) Evaluation of Analytical Methods for EDCs and PPCPs via Interlaboratory Comparison, Water Research Foundation, Denver, CO.	The 4167 round robin study Includes Individual Compound Reports: 1,2, 3; Data; 1, 2, 3; Responses: 1, 2, 3	
EPA (2007) Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS.	SPE protocol used by UMass through 2011	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.
EPA (2007) Method 1698: Steroids and Hormones in Water, Soil, Sediment, and Biosolids by HRGC/HRMS.		EPA Method 1698 determines steroids and hormones in environmental samples by isotope dilution and internal standard high resolution gas chromatography combined with high resolution mass spectrometry (HRGC/HRMS). EPA Method 1698 was developed for use with aqueous, solid, and biosolids matrices.

Kasprzyk-Hordern, B., Dinsdale, R.M. and Guwy, A.J. (2008)Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography-electrospray tandem mass spectrometry. Analytical and Bioanalytical Chemistry 391(4), 1293-1308.	Basis for UMass LC/MS protocol through 2011	The main aim of the presented research is to introduce a new technique, ultra performance liquid chromatography-positive/negative electrospray tandem mass spectrometry (UPLC-ESI/MS/MS), for the development of new simultaneous multiresidue methods (over 50 compounds). These methods were used for the determination of multiple classes of pharmaceuticals (acidic, basic and neutral compounds: analgesic/anti-inflammatory drugs, antibiotics, antiepileptics, beta-adrenoceptor blocking drugs, lipid regulating agents, etc.), personal care products (sunscreen agents, preservatives, disinfectant/antiseptics) and illicit drugs (amphetamine, cocaine and benzoylecgonine) in surface water and wastewater. The usage of the novel UPLC system with a 1.7 mu m particle-packed column allowed for good resolution of analytes with the utilisation of low mobile phase flow rates (0.05-0.07 mL min(-1)) and short retention times (method times of up to 25 min), delivering a fast and cost-effective method. SPE with the usage of Oasis MCX strong cation-exchange mixed-mode polymeric sorbent was chosen for sample clean-up and concentration. The influence of mobile phase composition, matrix-assisted ion suppression in ESI-MS and SPE recovery on the sensitivity of the method was extensively studied. The method limits of quantification were at low nanogram per litre levels and ranged from tenths of ng L-1 to tens of ng L-1 in surface water and from single ng L-1 to a few hundreds of ng L-1 in the case of wastewater. The instrumental and method intraday and interday repeatabilities were on average less than 5%. The method was successfully applied for the determination of pharmaceuticals in the River Taff (South Wales) and a wastewater treatment plant (WWTP Cilfy-nydd). Several pharmaceuticals and personal care products were determined in river water at levels ranging from single ng L-1 to single mu g L-1.
Vanderford, B.J. and Snyder, S.A. (2006) Analysis of pharmaceuticals in water by isotope dilution liquid chromatography/tandem mass spectrometry. Environmental Science &	early isotope dilution method	A method has been developed for the trace analysis of 15 pharmaceuticals, four metabolites of pharmaceuticals, three potential endocrine disruptors, and one personal care product in various waters. The method employs solid-phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC-MS/MS), using electrospray ionization (ESI) in both positive and negative modes.

Technology 40(23), 7312-7320.		Unlike many previous LC-MS/MS methods, which suffer from matrix suppression, this method uses isotope dilution for each compound to correct for matrix suppression, as well as SPE losses and instrument variability. The method was tested in five matrices, and results indicate that the method is very robust. Matrix spike recoveries for all compounds were between 88 and 106% for wastewater influent, 85 and 108% for wastewater effluent, 72 and 105% for surface water impacted by wastewater, 96 and 113% for surface water, and 91 and 116% for drinking water. The method reporting limits for all compounds were between 0.25 and 1.0 ng/ L, based on 500 mL of sample extracted and a final extract volume of 500 mu L. Occurrence of the compounds in all five matrices is also reported.