EPA Method 1694: Analysis of Pharmaceuticals and Personal Care Products in Water

UCT Part Numbers

ECHLD156-P
ENVIRO-CLEAN® HL DVB
500 mg, 6 mL cartridge

VMF016GL
16 position glass block manifold

VMFSTFR12
Large volume sample transfer tubes

SLDA100ID21-3UM
Selectra® DA HPLC column
(100 × 2.1 mm, 3 µm)

SLDAGDC21-3UM
Selectra® DA guard cartridge
(10 × 2.1 mm, 3 µm)

SLGRDHLDR
Guard cartridge holder

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Summary:

EPA Method 1694 was published in December 2007 as a screening method for the analysis of pharmaceuticals and personal care products (PPCPs) in environmental samples, including water (wastewater, surface water and drinking water) [1]. The method uses solid-phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC-MS/MS) for the analysis of 73 PPCPs in water (a procedure is also outlined for the analysis of PPCPs in soil, sediment, and biosolids). The PPCPs include common prescription drugs, veterinary drugs, over-the-counter medicines, dietary supplements and other consumer products. The method divides the PPCPs into four groups based on their physicochemical properties. Water samples are extracted by means of two SPE procedures, using acidic (pH 2) and basic (pH 10) loading conditions. They are then analyzed by LC-MS/MS using four distinct methods according to their polarity and the extraction conditions of the PCPPs. LC-MS/MS analysis uses different LC conditions (column, mobile phase and gradient) and ionization modes (ESI^+ and ESI^-). EPA Method 1694 is not a regulatory method (i.e. 40 CFR Part 136) and instead is a guide that can be used to screen samples from various sources to profile the occurrence of PPCPs. It can also be used as a starting point for developing a custom PPCP method. Modifications to the method are allowed if they are documented and provide performance equal to or better than that specified in the method.

This application note outlines a SPE procedure using UCT's Enviro-Clean® HL DVB polymeric sorbent for the determination of 64 PPCPs in water according to EPA Method 1694. The SPE procedure was optimized with the aim of obtaining acceptable recoveries of the PCPPs using a single extraction step rather than the multiple extraction procedures outlined in the original EPA method. The LC-MS/MS analysis was also streamlined and uses only two methods (ESI^+ and ESI^-) and a single Selectra® DA HPLC column instead of the four methods and two HPLC columns used in the original method. The unique chemistry of the Selectra® DA column, which contains a polyaromatic stationary phase, provides a high degree of retention and selectivity for aromatic compounds and improved retention of polar compounds.

Sample Pretreatment:

Because the analytes may be bound to suspended particles, the preparation of aqueous samples depends on the presence of visible particles. Aqueous samples absent visible particles can be analyzed directly according to the procedure outlined below. Aqueous samples with visible particles (>1%) should be filtered and the solids extracted and combined with the aqueous portion of the sample prior to the SPE procedure outlined below. Consult EPA Method 1694 for detailed information on sample collection, sample pretreatment, solids extraction, preparation and fortification of the target analytes and isotopically labeled internal standard solutions [1].

- No pH adjustment of the sample is required.
- If residual chlorine is present, add 80 mg/L of sodium thiosulfate.
- Add 500 mg/L of tetrasodium EDTA (removes metal ions that can bind with the analytes).
- Spike sample with appropriate concentrations of internal standard and mix thoroughly (add target analytes for fortified samples).

SPE Procedure:

1. **SPE Conditioning**
   a) 10 mL acetone.
   b) 10 mL reagent water, leaving approximately 2 mL of water on the top of the frit.

2. **Sample Extraction**
   a) Attach a large volume sample transfer tube (**VMFSTFR12**) to the top of each SPE cartridge and place the stainless steel end of the transfer tube directly into the sample bottle.
   b) Adjust the vacuum so that the flow rate is approximate 5-10 mL/min.

3. **Wash Cartridge**
   a) Remove the large volume transfer tubes and rinse the SPE cartridges with 10 mL reagent water (to remove EDTA).
   b) Dry the SPE cartridges under full vacuum (10-15 in. Hg) for 10 min.

4. **Elution**
   a) Elute the analytes with 10 mL methanol/acetone (1:1, v/v). Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion. After the solvent has passed through, apply full vacuum for 30 seconds so that all the elution solvent is collected.

5. **Concentration**
   b) Evaporate extract to near dryness at 40-50°C under a gentle stream of nitrogen.
   c) Reconstitute in 1 ml methanol.
   d) Vortex and transfer the sample to an autosampler vial for LC-MS/MS analysis.

LC-MS/MS Parameters:

Instrumentation	
HPLC system	Thermo Scientific™ Dionex™ Ultimate™ 3000
MS system	Thermo Scientific™ TSQ Vantage™ (MS/MS)
HPLC column	UCT Selectra® DA, 100 × 2.1 mm, 3 µm (p/n: SLDA100ID21-3UM)
Guard column	UCT Selectra® DA, 10 × 2.0 mm, 3 µm (p/n: SLDAGDC21-3UM)
Guard column holder	p/n: SLGRDHLDR
Column temperature	30°C
Flow rate	300 µL/min
Injection volume	5 µL (ESI^+ method) 10 µL (ESI^- method)

Method 1 (ESI^+)		
Time (min)	**Mobile Phase A (%) Water + 0.1% Formic Acid**	**Mobile Phase B (%) Methanol + 0.1% Formic Acid**
0	98	2
1	60	40
4	60	40
6	30	70
10	30	70
12	0	100
15	0	100
15.1	98	2
20	98	2

Method 2 (ESI^-)		
Time (min)	**Mobile Phase A (%) Water + 10mM NH_4OAc**	**Mobile Phase B (%) Acetonitrile**
0	90	10
3	0	100
6	0	100
6.1	90	10
11	90	10

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Retention Times and MRM Transitions

Method 1 (ESI^+)					
Analyte	**RT**	**Polarity**	**Precursor**	**Product 1**	**Product 2**
Metformin	1.15	+	130.1	60.1	71.1
Metformin-D_6	1.15	+	136.1	59.7	76.7
Sulfanilamide	3.45	+	156.0	92.1	108.1
Albuterol	3.81	+	240.1	121.1	148.1
Albuterol-D_3	3.81	+	243.1	124.2	151.2
Acetaminophen	4.12	+	152.1	65.0	110.1
Acetaminophen-D_4	4.12	+	156.1	97.1	114.1
Cotinine	4.15	+	177.1	80.1	98.1
Cotinine-D_3	4.15	+	180.1	80.1	101.1
Cimetidine	4.24	+	253.1	95.1	159.1
Lincomycin	4.31	+	407.2	126.1	359.2
Ranitidine	4.71	+	315.1	102.0	176.0
Codeine	5.09	+	300.1	152.0	165.0
Ampicillin	5.43	+	350.1	106.1	192.0
Trimethoprim	5.45	+	291.1	123.1	230.1
Trimethoprim-$^{13}C_3$	5.45	+	294.1	233.2	264.2
Sulfadiazine	5.63	+	251.0	92.1	156.0
Sulfathiazole	5.84	+	256.0	92.1	156.0
Oxytetracycline	6.00	+	461.1	337.1	426.1
Ormetoprim	6.12	+	275.1	123.1	259.1
Thiabendazole	6.32	+	202.0	131.1	175.1
Thiabendazole-D_6	6.32	+	208.0	137.1	181.1
Dimethylxanthine	6.42	+	181.1	69.1	124.1
Minocycline	6.45	+	458.1	283.1	441.2
Norfloxacin	6.46	+	320.1	233.1	276.1
Tetracycline	6.70	+	445.1	154.0	410.2
Sulfamerazine	6.76	+	265.0	92.1	156.0
Ofloxacin	7.15	+	362.1	261.1	318.2
Ciprofloxacin	7.31	+	332.1	231.1	288.2
Ciprofloxacin-^{15}N-$^{13}C_3$	7.31	+	336.1	235.1	291.2
Sulfamethizole	7.42	+	271.0	92.1	156.0
Sulfamethoxazole	7.44	+	254.1	92.1	148.1
Sulfamethoxazole-$^{13}C_6$	7.44	+	260.0	98.2	162.1
Cefotaxime	7.46	+	456.0	125.0	167.0
Lomefloxacin	7.58	+	352.1	265.1	308.2
Demeclocycline	7.59	+	465.0	430.1	448.1
Azithromycin	7.65	+	749.1	116.0	591.5
Sulfamethazine	7.82	+	279.0	124.1	186.0
Sulfamethazine-$^{13}C_6$	7.82	+	285.1	124.2	186.1
Sulfachloropyridazine	7.89	+	285.0	92.1	156.0
Enrofloxacin	7.85	+	360.1	245.1	316.2
Chlortetracycline	8.15	+	479.0	154.0	444.1
Sarafloxacin	8.21	+	386.1	299.1	342.2

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Digoxigenin	8.40	+	391.2	91.0	355.2
Doxycycline	8.48	+	445.1	321.1	428.2
Diphenhydramine	8.55	+	256.1	115.1	165.1
Sulfadimethoxine	8.73	+	311.0	108.1	156.0
Fluoxetine	8.75	+	310.1	44.1	91.1
Fluoxetine-D_5	8.75	+	315.0	43.7	94.9
Caffeine	8.83	+	195.0	110.0	138.0
Caffeine-$^{13}C_3$	8.83	+	198.1	112.1	140.1
Erythromycin	8.95	+	734.4	158.0	576.4
Erythromycin-$^{13}C_2$	8.95	+	736.4	160.1	578.4
Carbadox	9.08	+	263.0	129.1	231.1
Erythromycin anhydrate	9.19	+	716.4	158.0	558.4
Anhydrotetracycline	9.27	+	427.1	154.0	410.1
Penicillin G	9.21	+	367.1	114.0	160.0
Atrazine-$^{13}C_3$ (IIS)	9.32	+	219.0	106.0	177.0
Clarithromycin	9.42	+	748.4	158.0	590.4
Tylosin	9.64	+	916.4	173.9	772.5
Diltiazem	9.66	+	415.2	150.0	178.0
Penicillin V	9.87	+	383.1	114.0	160.0
Carbamazepine	9.94	+	237.1	192.1	194.1
Roxithromycin	9.98	+	837.4	158.0	679.5
Oxacillin	10.29	+	434.1	144.0	160.0
Digoxin	10.86	+	803.4	803.4	-
Cloxacillin	10.91	+	468.0	160.0	178.0
Oxolinic acid	11.35	+	262.0	160.1	216.0
Miconazole	11.42	+	415.1	122.9	158.9
Flumequine	12.79	+	262.0	126.1	202.0
Virginiamycin	13.85	+	526.2	337.1	355.1

Method 2 (ESI^-)					
Analyte	**RT**	**Polarity**	**Precursor**	**Product 1**	**Product 2**
TCPAA-$^{13}C_6$ (IIS)	3.25	-	258.8	164.9	200.9
Naproxen	3.30	-	229.0	141.0	169.0
Naproxen-D_3	3.30	-	233.0	141.0	169.0
Warfarin	3.40	-	307.0	161.0	250.0
Warfarin-D_5	3.40	-	312.0	161.0	255.1
Ibuprofen	3.60	-	205.1	161.1	-
Ibuprofen-$^{13}C_3$	3.60	-	208.0	163.0	-
Gemfibrozil	4.15	-	249.1	121.0	127.1
Gemfibrozil-D_6	4.15	-	255.1	106.0	121.0
Triclocarban	4.90	-	312.9	126.0	159.9
Triclocarban-$^{13}C_6$	4.90	-	318.9	132.0	159.9
Triclosan	5.00	-	286.9	35.3	161.2
Triclosan-$^{13}C_{12}$	5.00	-	298.8	35.0	148.1

IIS = injection internal standard

Results and Discussion:

Analyte	Fortification Level (ng)	EPA 1694 IPR* Criteria		Deionized Water Results (n=6)		Tap Water Results (n=6)	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Acetaminophen	1500	55 - 108	30	110.8	7.8	111.5	7.7
Albuterol	15	55 - 120	30	82.0	2.6	83.9	2.0
Ampicillin	75	6 - 180	70	88.8	7.3	70.8	24.1
Anhydrotetracycline	250	8 - 127	30	59.3	8.4	61.9	6.0
Azithromycin	75	36 - 108	30	68.5	4.5	92.5	6.8
Caffeine	750	55 - 111	30	106.9	3.0	110.0	3.4
Carbadox	75	36 - 130	30	104.9	4.1	97.2	4.8
Carbamazepine	75	23 - 123	30	112.4	2.8	105.5	2.0
Cefotaxime	300	9 - 168	36	110.7	8.5	90.6	13.9
Chlortetracycline	100	49 - 155	31	63.2	5.9	67.0	5.5
Cimetidine	30	6 - 108	47	42.1	8.0	26.9	8.4
Ciprofloxacin	240	55 - 108	30	105.7	3.4	107.7	2.3
Clarithromycin	75	8 - 139	30	79.6	7.6	94.3	5.7
Cloxacillin	150	6 - 180	30	95.4	1.1	97.6	3.8
Codeine	150	37 - 116	30	95.6	3.3	107.9	2.2
Cotinine	75	55 - 112	30	100.3	1.3	106.6	2.1
Demeclocycline	250	6 - 180	30	68.1	4.4	61.8	3.7
Digoxigenin	300	8 - 165	30	112.3	7.6	108.1	4.0
Digoxin	750	6 - 133	45	99.6	8.9	102.0	6.5
Diltiazem	15	13 - 108	48	102.5	4.0	112.0	2.6
Dimethylxanthine	7500	55 - 124	30	72.9	3.9	112.9	2.8
Diphenhydramine	30	53 - 108	30	73.8	4.1	80.0	7.9
Doxycycline	100	24 - 149	30	76.1	5.5	66.6	2.8
Enrofloxacin	150	55 - 113	30	74.1	6.5	67.0	9.8
Erythromycin	15	N/A	N/A	126.0	15.8	131.2	7.5
Erythromycin anhydrate	15	55 - 142	30	69.2	7.3	94.5	4.9
Flumequine	75	39 - 180	30	97.8	1.2	98.4	2.4
Fluoxetine	75	54 - 112	30	117.3	13.0	105.8	5.9
Gemfibrozil	75	55 - 108	30	99.8	4.0	107.7	8.8
Ibuprofen	750	55 - 108	30	103.8	1.6	101.5	1.8
Lincomycin	150	6 - 108	60	105.9	3.5	99.3	2.4
Lomefloxacin	150	19 - 180	33	89.5	4.2	83.5	8.0
Metformin	1500	55 - 134	30	89.2	1.4	96.2	2.9
Miconazole	75	29 - 108	30	81.3	4.2	74.0	6.1
Minocycline	1000	6 - 159	30	52.6	12.4	42.1	10.2
Naproxen	150	55 - 108	30	106.5	1.5	108.1	1.9
Norfloxacin	750	55 - 121	30	103.8	2.9	99.1	2.4
Ofloxacin	75	55 - 180	30	103.6	3.9	119.8	7.3
Ormetoprim	30	55 - 108	30	99.9	3.1	98.0	1.5
Oxacillin	150	6 - 180	30	94.5	0.5	97.8	4.4
Oxolinic acid	30	46 - 112	30	92.4	2.5	95.7	2.9
Oxytetracyline	100	55 - 165	30	82.9	5.7	65.7	4.4
Penicillin G	150	6 - 180	30	84.6	3.8	89.0	4.3
Penicillin V	150	6 - 180	30	99.1	2.7	102.5	4.0
Ranitidine	30	26 - 144	41	39.3	7.6	26.9	31.4
Roxithromycin	15	42 - 108	30	68.0	7.1	86.6	10.0
Sarafloxacin	660	18 - 180	32	86.3	4.5	77.0	10.9
Sulfachloropyridazine	75	55 - 180	30	68.6	4.5	83.6	5.6
Sulfadiazine	75	6 - 180	30	7.6	19.2	121.4	5.5
Sulfadimethoxine	15	55 - 108	30	83.9	3.7	76.8	6.3
Sulfamerazine	30	55 - 133	30	90.1	4.6	80.6	15.3
Sulfamethazine	30	55 - 128	30	103.2	3.2	104.5	5.3
Sulfamethizole	30	55 - 108	30	43.5	12.7	77.5	15.2
Sulfamethoxazole	30	55 - 108	30	119.6	12.3	110.2	16.2
Sulfanilamide	750	6 - 170	71	22.4	7.5	20.1	9.3
Sulfathiazole	75	45 - 108	30	79.0	5.6	110.4	2.7
Tetracycline	100	55 - 139	30	75.1	5.8	66.4	5.0
Thiabendazole	75	55 - 108	30	108.4	1.5	109.6	2.8
Triclocarban	150	55 - 108	30	104.0	1.9	104.9	3.7
Triclosan	3000	55 - 108	30	100.9	5.0	102.7	3.0
Trimethoprim	75	55 - 114	30	109.7	1.6	110.6	1.9
Tylosin	300	17 - 134	30	60.1	12.6	86.0	8.7
Virginiamycin	150	6 - 170	33	80.6	4.0	90.2	2.5
Warfarin	75	55 - 108	30	101.1	1.7	102.1	2.0

*IPR = Initial precision and recovery

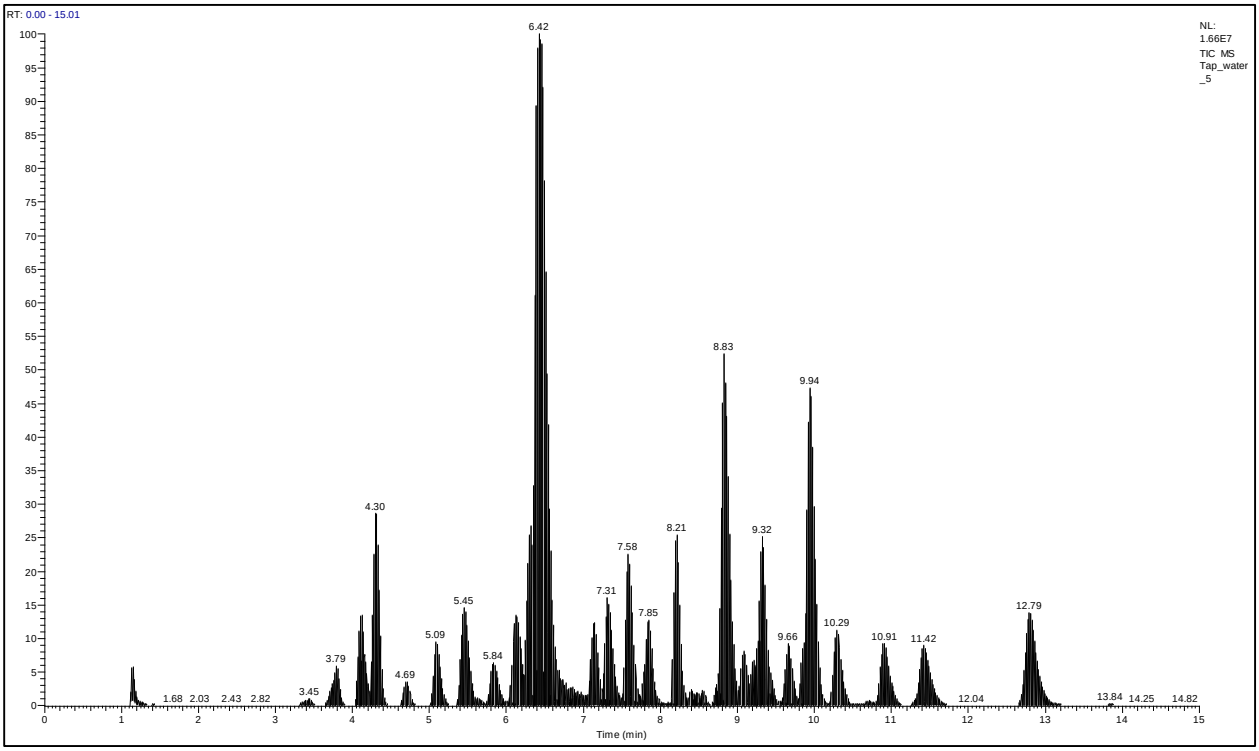

Figure 1: TIC chromatogram of the ESI$^+$ analytes in an extracted tap water sample.

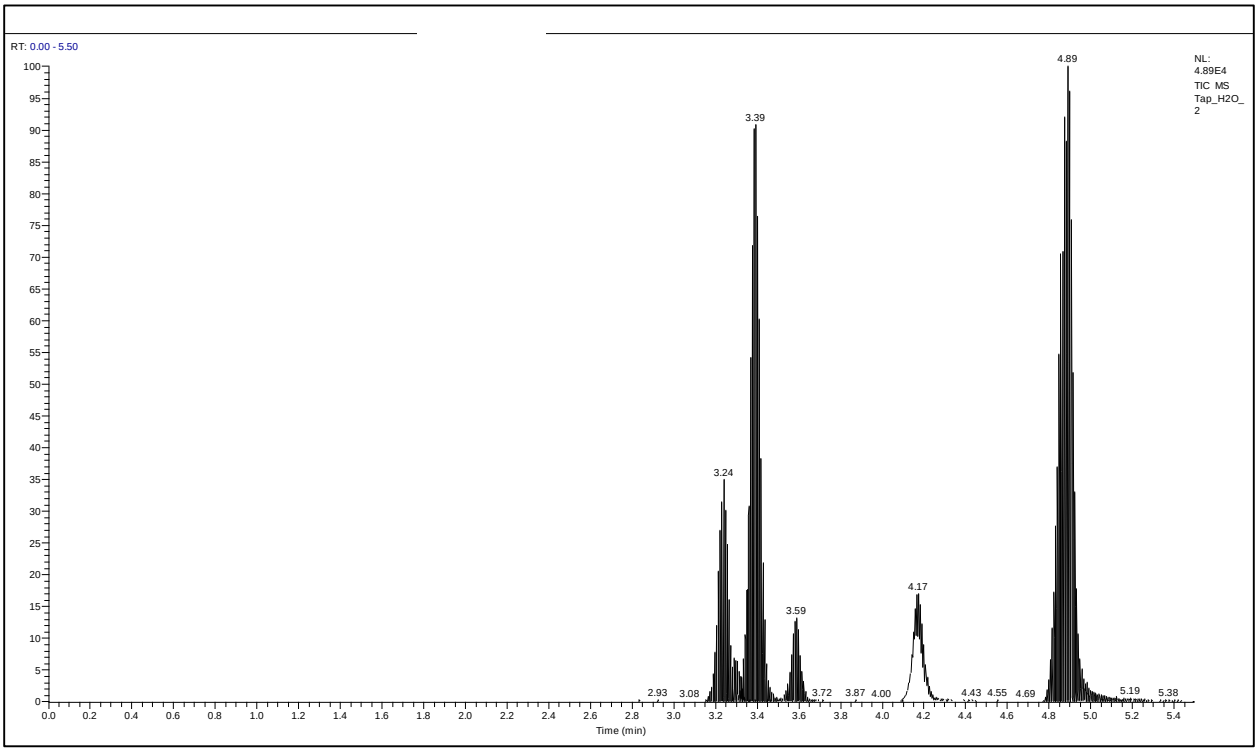

Figure 2: TIC chromatogram of the ESI$^-$ analytes in an extracted tap water sample.

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The original EPA 1694 method divides the PCPPs into four groups with each group having its own separate LC-MS/MS method. Group 1 contains the largest number of analytes; Group 2 is specific to the tetracyclines; Group 3 contains six acidic analytes (ESI^-); and Group 4 contains only four basic analytes. Groups 1, 2 and 3 use reversed phase chromatography on a C18 HPLC column while Group 4 uses HILIC chromatography on a bare silica column. Groups 1, 2 and 4 are analyzed in ESI^+ mode and Group 3 in ESI^- mode. For SPE extraction, Groups 1, 2, and 3 are extracted under acidic (pH 2) conditions and Group 4 is extracted under basic (pH 10) conditions. The main aim of this work was to develop a method for EPA Method 1694 using UCT's Enviro-Clean® HL DVB SPE cartridge and Selectra® DA HPLC column. Another goal was to try and simplify the SPE procedure by doing a single extraction step and try to reduce the number of LC-MS/MS methods required to cover all the PCPPs.

The LC-MS/MS analysis was streamlined from four methods and two HPLC columns to just two methods (ESI^+ and ESI^-) and a single Selectra® DA HPLC column. An attempt was made to cover all the analytes in a single method using fast polarity switching, however it was found that the sensitivity of the Group 3 (ESI^-) compounds was not good when using acidic mobile phase conditions and they required a higher pH mobile phase to be efficiently ionized. However, reducing the number of LC-MS/MS methods from four to two and using a single HPLC column is significantly faster and easier than the original EPA 1694 method. Furthermore, two MRM transitions (a quantifier and a qualifier ion) were used whenever possible rather than a single MRM transition used in the EPA 1694 method

The SPE procedure was optimized with the aim of obtaining acceptable recoveries for all the PCPPs using a single extraction step rather than the multiple extraction procedures outlined in the original EPA method. No sample pH adjustment of the sample was performed, which was the best compromise for the wide range of PPCPs included in the method, including for the pH sensitive β-lactam (susceptible to hydrolysis), macrolide and tetracycline antibiotics. In general, the recovery and RSD values obtained were within EPA Method 1694 requirements for the vast majority of the PPCPs included in this study. Attempts were made to try to improve the recovery of problematic analytes but this ultimately had a negative effect on some of the other PCPPs. Unfortunately, EPA Method 1694 includes a wide range of compounds that are very difficult to recover at 100% due to the wide physicochemical properties (polarity) that they possess and the stability issues experienced by some of the analytes. A comparison against the acidic and alkaline conditions outlined in EPA Method 1694 found that the recoveries of the PCPPs did not improve overall and that no pH adjustment of the water samples is the most suitable approach for a single SPE extraction procedure.

The use of isotopically labeled internal standards is essential for obtaining good recoveries in EPA Method 1694. The inclusion of additional internal standards, if available, can further improve the accuracy and precision for this method, especially for difficult compounds. Furthermore, solvent standards were used for recovery calculations but the use of matrix-matched standards could further help to improve the results.

Conclusion:

This application note outlines a simplified SPE method for the determination of a wide range of PPCPs in environmental water samples according to EPA Method 1694. Water samples are extracted using a single extraction procedure using UCT's Enviro-Clean® HL DVB polymeric SPE cartridge without any sample pH adjustment. LC-MS/MS analysis was conducted with a single HPLC column (Selectra DA®) using only two methods instead of the two HPLC columns and four methods outlined in the original EPA 1694 method. The recovery and RSD values obtained were found to be within the EPA Method 1694 requirements for the vast majority of the 64 PPCPs included in this study. Overall, the streamlined screening method outlined in this application note significantly speeds up the analysis of a PPCPs in water compared to the original EPA 1694 method. The method can also be used as a starting point for a custom PPCP method that is tailored to specific matrices or compounds. In this case, further optimization of the method can be carried out to optimize results.

References:

[1] EPA Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS, December 2007, EPA-821-R-08-002.

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