Automated Solid Phase Extraction of Pharmaceuticals and Personal Care Products from Water Samples using a Novel Robotic Autosampler

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Keywords

- **Sample Preparation**
- **LC-MS/MS**
- **High Throughput Lab Automation**
- **Emerging Contaminants**
- **PPCP**

Abstract

The US Environmental Protection Agency' s (EPA) method 542 was created to monitor drinking water samples for the presence of pharmaceuticals and personal care products (PPCP) using LC-MS/MS [1]. The method requires an extraction volume of one liter of water to meet the parts per trillion (ng/L) reporting limits for the compounds monitored. The large sample volume as well as the requirement to present the subsequent rinsate of the sample bottles to the SPE cartridge, has historically made this method a tedious, manual procedure. Using the LCTech FREESTYLE XANA sampler, the entire solid phase extraction procedure is automated, providing critically needed high throughput extraction for PPCP compounds in water samples. The resulting extracts are then introduced into an LC-MS/MS system such as the Agilent Ultivo LC-MS/MS instrument for detection and quantification.

Introduction

Previous work has shown the LCTech FREESTYLE XANA sampler to successfully provide extracts for the determination of a variety of contaminants including perfluorinated compounds from large volume water samples by LC-MS/MS as regulated by the EPA method 537.1 [2,3,4]. As a result of this study, we were able to show that automated SPE performed by the LCTech FREESTYLE XANA sampler could successfully be used to provide extracts for the determination of PPCP from water samples according to the regulated EPA method 542. PPCP compounds isolated from the water samples using the automated extraction procedure were introduced to an Agilent Technologies 1260 HPLC coupled with an Agilent Ultivo Triple Quadrupole Mass Spectrometer with Jet stream electrospray source. The recovery of the PPCP compounds when extracted from water samples was found to average 109% for all PPCP compounds. Accuracy data averaged 110% (range: 72.7% - 125%) and precision data averaged 2.31% RSD (range: 1.14% - 6.73%) for all PPCP compounds extracted from water samples.

Experimental

Materials

All stock solutions for the analytes listed in Table 1 were purchased from Millapore Sigma. 1.0 mg/mL stock solutions in methanol were prepared for each individual analyte. An intermediate analyte stock solution was prepared by combining the analyte stock solutions with (1:1) methanol: reagent water, resulting in appropriate concentrations of the PPCP compounds for method evaluation.

Deuterated analogues, d_3-triclosan and d_{10}-carbamazepine, were purchased from Cerilliant. A working internal standard solution containing both deuterated internal standards was prepared in (1:1) methanol: reagent water at a concentration of 100 ng/mL. D_3-triclosan was used for the quantitation of the compounds that were detected using negative ionization and d_{10}-carbamazepine was used for the quantitation of the compounds detected using positive ionization.

Calibration standard and QC serum samples were prepared by making appropriate dilutions of the combined intermediate analyte stock solutions using (1:1) methanol: reagent water to reach the concentrations listed in Table 1. Calibration standards were prepared using a dilution ratio strategy from the high concentration sample of 1:5:5:5:1.331. Table 1 lists the concentrations for the highest calibration standard and the limit of quantitation found during this study.

Replicate recovery QC samples at the final concentrations listed in Table 1 were prepared in water using a diluted intermediate stock solution prepared using the analyte stock solutions. All water samples extracted during the method evaluation were prepared using 1 liter of tap water to which 9.4 grams of potassium citrate monobasic, 0.35 grams of EDTA trisodium salt, and 0.100 grams of ascorbic acid had been previously added and dissolved. These preservatives were all purchased from Millapore Sigma.

All other reagents and solvents used were reagent grade.

Instrumentation

All automated solid phase extractions were performed using a LCTech FREESTYLE XANA sampler as shown in Figure 1. All analyses were performed using an Agilent 1260 HPLC with an Agilent Poroshell 120, EC-C18 column, (3.0 x 150 mm, 2.7 µm) and an Agilent Ultivo Triple Quadrupole Mass Spectrometer with Jet stream electrospray source. Sample injections were made using the GERSTEL MPS robotic^{PRO} sampler with LC-MS Tool into a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 10 µL stainless steel sample loop. Sample vials were stored at 10 °C during analytical runs.

Figure 1: LCTech FREESTYLE XANA sampler used during the automated solid phase extraction of large volume water samples.

Automated Solid Phase Extraction for PPCP Water Samples

1. The user places the sample bottle onto the LCTech FREESTYLE XANA sampler, a Waters Oasis HLB (6 mL, 200 mg) SPE cartridge into the SPE cartridge rack, and a 15 mL conical tube into the eluate collection rack.
2. The sampler conditions the SPE cartridge using 2 x 5 mL of (1:1) methanol: acetone.
3. The sampler conditions the SPE cartridge using 2.5 mL of (1:1) methanol: acetone and waits 1 minute.
4. The sampler conditions the SPE cartridge using 2.5 mL of (1:1) methanol: acetone.
5. The sampler conditions the SPE cartridge using 2 x 2.5 mL of water.
6. The sampler adds the 1-liter sample through the SPE cartridge at 10 mL/min.
7. The sampler rinses the sample bottle using 10 mL of water and then adds the rinsate to the SPE cartridge.
8. The sampler elutes the SPE cartridge using 2 x 2.5 mL of (1:1) methanol: acetone collecting the eluant in 15 mL tubes.

Upon completion of the automated SPE method, the eluate from step 8 is transferred to a 2 mL autosampler vial and placed onto the MPS robotic sampler. The MPS robotic sampler is then used to dilute 500 µL of the eluate with 500 µL of water, add 10 µL of the working internal standard, and mix the final extract prior to injecting the sample for LC-MS/MS analysis.

Analysis Conditions LC

Pump	gradient (800 bar) flowrate = 0.2 mL/min
Mobile phase	A – 5 mM ammonium acetate in 10% methanol B – 100% methanol

LC gradient	Time (min)	Flow (mL/min)	% B
	0	0.2	10
	0.5	0.2	10
	0.51	0.2	50
	8.0	0.2	75
	8.01	0.2	100
	10.0	0.2	100
	14.0	0.2	10
	24.0	0.2	10

Run time	25 minutes
Injection volume:	2.0 µL (loop over-fill technique)
Column Temperature:	45 °C

Analysis Conditions MS

Operation	electrospray positive and negative mode
Gas temperature	350 °C
Gas flow (N_2):	5 L/min
Nebulizer pressure:	35 psi
Sheath gas flow (N_2):	11 L/min
Sheath gas temperature:	400 °C
Capillary voltage:	4000 V
Nozzle voltage:	500 V
Delta EMV:	0 V

The mass spectrometer acquisition parameters are shown in Table 1 with qualifier ions. A retention time window value of 1 minute was used for each ion transition being monitored over the course of the dynamic MRM experiments.

Table 1: Mass spectrometer acquisition and calibration parameters.

Compound	Precursor	Frag	Product	CE	Product	CE	Polarity	Retention Time	EPA 542 MRL	EPA 542 MRL at inj.	LOQ	Rec QC	High Std Conc
	[m/z]	[V]	[m/z]	[V]	[m/z]	[V]		[min]	[ng/L]	[ng/mL]	[ng/mL]	[ng/mL]	[ng/mL]
Carbamazepine	237	110	194	15	179	40	ESI+	9.90	2.40	0.240	0.0235	2.00	3.91
Diazepam	285	120	154	30	257.1	25	ESI+	12.76	0.270	0.0270	0.0150	1.28	2.50
Diclofenac	294	80	250	10	214	20	ESI-	10.84	1.10	0.110	0.0768	6.55	12.8
Enalapril	377.1	110	234.1	20	303.1	20	ESI+	8.98	0.600	0.0600	0.0303	2.58	5.05
Fluoxetine	310	80	148	5	117	40	ESI+	11.23	0.980	0.0980	0.0780	6.65	13.0
Gemfibrozil	249	80	121	15	126.9	5	ESI-	13.51	1.40	0.140	0.0750	6.39	12.5
Naproxen	229	80	169	30	170	10	ESI-	8.53	4.50	0.450	0.0750	6.39	12.5
Phenytoin	253	100	182.1	15	104	50	ESI+	9.26	1.40	0.140	0.0230	1.96	3.84
Sulfamethoxazole	254	80	156	15	92	25	ESI+	5.83	0.280	0.0280	0.0221	1.88	3.67
Triclosan	287	80	35	40	141.9	50	ESI-	14.15	3.40	0.340	0.2334	19.9	38.9
Trimethoprim	291.1	120	230.1	25	261.1	25	ESI+	6.79	4.10	0.410	0.0228	1.94	3.80
Erythromycin	716.4	140	158	30	558.3	15	ESI+	12.75	5.00	0.500	0.0300	2.56	5.00
Triclosan-d_3	292	80	35	15	37	15	ESI-	14.13	-				
Carbamazepine-d_{10}	247.1	110	204.1	20	202.1	50	ESI+	9.75	-				

Results and Discussion

Figures 2A and 2B show representative mass chromatogram overlays for the PPCP compounds and deuterated internal standards determined from extracted minimum reporting limit (MRL) samples for both the positive (A) and negative (B) ionization method compounds compared with corresponding extracted blank water samples. As shown, the automated method was found to be free from interferents when compared to the MRL for each compound monitored.

The lower limits of quantitation for the compounds determined using this method are shown in Table 1. As required by EPA method 542, the LOQs were at or below the minimum reporting limits for each compound. Representative calibration curves are shown in Figures 3 A-C. Regression analysis resulted in r^2 values of 0.99 or greater for all PPCP compounds monitored.

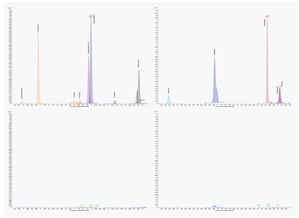

Figure 2: Overlays of representative mass chromatograms from extracted MRL samples with extracted blank tap water samples for positive (A, left) and negative (B, right) ionization method compounds.

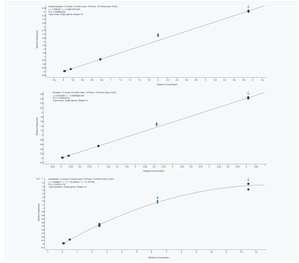

Figure 3A-C: Calibration curve results for carbamazepine (A), enalapril (B), and gemfibrozil (C).

The accuracy and precision of the method were evaluated for all PPCP compounds determined using replicate recovery QC samples. Table 2 shows the resulting accuracy and precision data for all PPCP compounds. Accuracy data averaged 110% (range: 72.7% - 125%) and precision data averaged 2.31% RSD (range: 1.14% - 6.73%) for all PPCP compounds determined in water samples, meeting the requirements of EPA method 542.

Recovery of the PPCP compounds from extracted water samples was assessed by comparing the results of the extracted replicate recovery QC samples to spiked neat standards at the same concentration levels. Table 2 shows the resulting recovery for all PPCP compounds. Recovery data averaged 109% (range: 87.1% - 120%) for all PPCP compounds determined in water samples, meeting the requirements of EPA method 542.

Confirmation of the minimum reporting limits for each PPCP compound monitored was performed by extracting seven replicates of water samples spiked at the MRL of each PPCP compound. Upon quantification of the resulting concentrations, both the upper and lower limits of prediction interval of results (PIR = mean ±HRPIR, where HRPIR – 3.963*standard deviation) were then established. The upper PIR limit was required to be ≤150% and the lower limit PIR was required to be ≥50%. As shown in Table 3, all MRL levels of all PPCP compounds monitored using the automated method were found to be within acceptable limits. This data provides evidence that the LCTech FREESTYLE XANA sampler can be used to determine PPCP compounds in water samples according to EPA method 542.

Table 2: QC sample % accuracy, precision and recovery results.

Compound	% Precision	% Accuracy	% Recovery
Carbamazepine	1.54	119	116
Diazepam	2.42	114	109
Diclofenac	1.69	92.3	110
Enalapril	1.38	112	115
Fluoxetine	2.11	119	109
Gemfibrozil	1.42	105	112
Naproxen	2.12	115	120
Phenytoin	2.02	121	115
Sulfamethoxazole	1.39	106	108
Triclosan	3.80	72.7	87.1
Trimethoprim	1.14	125	92.9
Erythromycin	6.73	121	109

Table 3: Results from the confirmation of minimum reporting limits.

Compound	MRL	mean	SD	%CV	HRPIR	Upper PIR limit	Lower PIR limit
	[ng/mL]	[ng/mL]				[≤ 150%]	[≥ 50%]
Carbamazepine	0.240	0.272	0.01223	4.50	0.0486	134	93.2
Diazepam	0.0270	0.0285	0.00139	4.87	0.00550	126	85.1
Diclofenac	0.110	0.109	0.00278	2.55	0.0110	109	89.0
Enalapril	0.0600	0.0653	0.00449	6.87	0.0178	138	79.2
Fluoxetine	0.0980	0.122	0.00264	2.17	0.0105	135	114
Gemfibrozil	0.140	0.165	0.00578	3.52	0.0229	134	101
Naproxen	0.450	0.544	0.0199	3.65	0.0787	138	103
Phenytoin	0.140	0.169	0.0103	6.11	0.0409	150	91.4
Sufamethoxazole	0.0280	0.0244	0.00190	7.80	0.00754	114	60.2
Triclosan	0.340	0.250	0.0175	7.01	0.0694	93.9	53.0
Trimethoprim	0.410	0.546	0.00331	0.606	0.0131	136	130
Erythromycin	0.500	0.334	0.0184	5.51	0.0730	81.4	52.2

Conclusions

As a result of this study, we were able to show:

- PPCP compounds and internal standards in water samples can be successfully extracted using an automated solid phase extraction method and determined using the Agilent Ultivo Triple Quadrupole Mass Spectrometer.
- This method was readily automated using the LCTech FREESTYLE XANA sampler.
- Linear calibration curves resulting in r^2 values 0.99 or greater were achieved for the PPCP compounds.
- The automated SPE method proved to be accurate and precise. Accuracy data averaged 110% (range: 72.7% - 125%) and precision data averaged 2.31% RSD (range: 1.14% - 6.73%) for all PPCP compounds determined in water samples.
- The recovery of PPCP compounds extracted from water samples was found to average 109% (range: 87.1% - 120%) for all PPCP compounds monitored.
- Confirmation of the minimum reporting limits listed in EPA method 542 method was achieved using the SPE method automated using the LCTech FREESTYLE XANA sampler.

References

[1] United States Environmental Protection Agency, Method 542: *Determination of Pharmaceuticals and Personal Care Products in Drinking Water by Solid Phase Extraction and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry*, https://www.epa.gov/sites/default/files/2016-09/documents/method-542-determination-pharmaceuticals-personal-care-products-drinking-water.pdf, accessed July 2022.

[2] LCTech Application Note No. AN0033, *Determination of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) in Water with FREESTYLE XANA and LC-MS/MS*, **2022**.

[3] LCTech Application Note No. AN0045, *D-EVA – Automated EVAporation of PFAS compliant to US-EPA 537.1*, **2021**.

[4] LCTech Application Note No. AN0017, *Automated Detection of Selected Explosives and Related Compounds*, **2021**.