

**Liquid Chromatography/
Mass Spectrometry**

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Analysis of PPCPs in Drinking Water at Low PPT Levels by Online SPE-UHPLC-MS/MS

veterinary prescription and over-the-counter medications, sunscreens, lotions, soaps, and insect repellants. These commonly encountered products can enter the environment through various sources, including municipal wastewater, polluting ground water, surface water, and even drinking water.¹⁻³ Wastewater treatment plants do not typically possess the right equipment for PPCP removal.³

Identifying and quantifying the presence of chemical pollutants in drinking water has been a growing area of focus, as both the impact of exposure to low levels of PPCPs over time, as well as the impact of exposure to mixtures of these compounds, are not well understood.^{2,3}

The analytical challenge associated with PPCP testing is the wide variety of chemical classes/types that are typically present at parts per trillion (ng/L) concentrations in drinking water.^{4,5} Therefore, developing an optimal analytical method, one that balances effective chromatographic separation and optimal analyte sensitivity, is a daunting task. Historically, this has often required compromises in accommodating all the analytes that one intends to quantify.

Introduction

Pharmaceutical and Personal Care Products (PPCPs) are an emerging environmental concern, and include human and

The traditional analytical methodology for determination of PPCPs consists of an elaborate and time-consuming solid phase extraction (SPE) sample preparation, requiring large sample volumes (often 1 liter) prior to analysis by LC/MS/MS to reach desired detection limits.^{3,6,7} In this study, we demonstrate the application of online SPE coupled to UHPLC-MS/MS for the preconcentration, separation, detection, and quantitation of twelve PPCPs in drinking water at low part per trillion levels.

The automated approach described herein allows for significant and efficient analyte concentration, obviating the need for elaborate and time-consuming sample preparation procedures, and thus improving productivity, reducing solvent usage and laboratory waste. Due to enrichment, compared to other methods, large sample volumes are no longer needed to reach low part per trillion levels for PPCPs in drinking water.

Experimental

Hardware/Software

For online analyte pre-concentration/enrichment and chromatographic separation, a PerkinElmer QSight® SP50 Online SPE System, coupled with a QSight 220 MS/MS detector was utilized. All instrument control, analysis and data processing were performed using the Simplicity 3Q™ software platform.

Online SPE is accomplished through two additional six-port valves in the autosampler, and a high-pressure dispenser (HPD). Per Figure 1, valve A is dedicated to SPE, while valve B allows for the flexible switching from direct injection to online SPE mode. The system was configured with a 10 µL stainless steel needle, 1 mL sample loop, 1 mL syringe and 2 mL buffer tubing. Conditioning and equilibration solvents are delivered via the HPD, both solvents being directed to waste upon passing through the SPE cartridge. The sample is then aspirated into the sample loop using the autosampler syringe, and subsequently transferred via a load solvent from the loop to the SPE cartridge. Analytes are then eluted off the SPE cartridge and onto the analytical column using the LC gradient. There is no separate

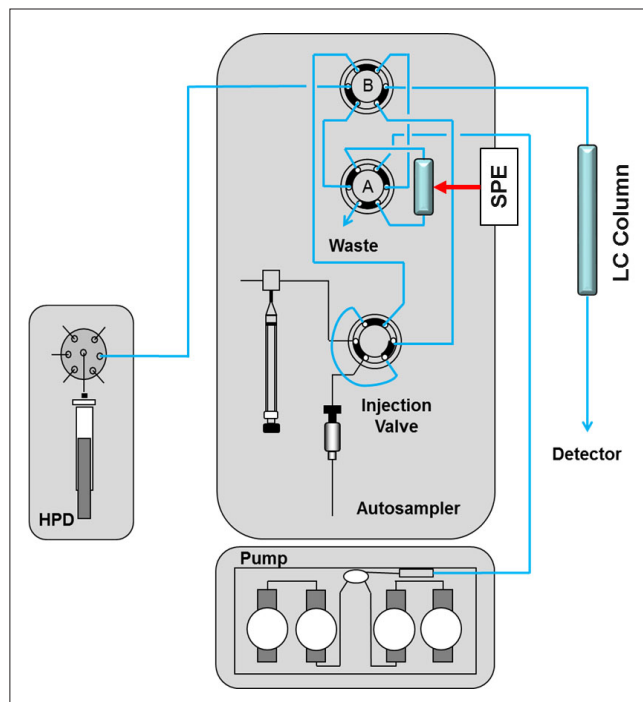


Figure 1. Schematic of QSight® SP50 Online SPE System.

SPE elution step needed, as the focused analytes on the SPE cartridge are eluted right onto the analytical column as part of the chromatographic run.

For this method, sample enrichment was accomplished by loading a total of 3 mL of sample onto the SPE cartridge via a 3 x 1000-µL stacked loading process. The SPE parameters for this method are shown in Table 1.

A 24-vial tray was used, accommodating 10-mL sample vials (N9300922).

Method Parameters

The SPE, LC and MS/MS method parameters are shown in Tables 1-4.

Table 1. SPE Parameters.

SPE Cartridge	PerkinElmer proprietary, 10 µm, 2.1 x 30 mm				
SPE Solvents	SPE Solvent 1 (Conditioning): IPA/ACN/Water 40/40/20 with 1% formic acid SPE Solvent 2 (Equilibration/Sample Loading): Water with 0.1% formic acid				
SPE Program	3x Stacked Injections				
	Step	Step Type	Solvent 1 (mL)	Solvent 2 (mL)	Sample(mL)
	1	Wash/Conditioning	2.0 1.5 mL/min	-	-
	2	Equilibration	-	2.0 1.5 mL/min	-
	3	Sample Loading into 1-mL Loop	-	-	1.5*
	4	Sample Loading onto SPE Cartridge	-	1.25 1.0 mL/min	-
	5	Sample Loading into 1-mL Loop	-	-	1.5*
	6	Sample Loading onto SPE cartridge	-	1.25 1.0 mL/min	-
	7	Sample Loading into 1-mL Loop	-	-	1.5*
	8	Sample Loading onto SPE Cartridge	-	1.25 1.0 mL/min	-
	* The total sample load volume onto SPE cartridge is 3.0 mL (as fixed-loop injection mode is used, 1.5 mL of the 4.5-mL sample aspirated goes to waste).				

Table 2. LC Parameters.

Column	PerkinElmer Brownlee SPP C18, 2.7 μ m, 3.0 x 100 mm (Part# N9308410)				
SPE Solvents	Solvent A: Water with 0.1% Formic Acid				
	Solvent B: acetonitrile with 0.1% Formic Acid				
	Solvent Program:				
	Step	Time (min.)	Flow Rate (mL/min.)	%A	%B
	1	0.0	0.6	100	0
	2	2.0	0.6	60	40
3	7.0	0.6	3	97	
4	7.5	0.6	100	0	
5	11.0	0.6	100	0	
Analysis Time	7.0 min.; Equilibration Time: 4.0 min				
Pressure	3500 psi/233 Bar Maximum				
Oven Temp.	30 °C				

Table 3. MS/MS Parameters.

Compound	ESI Mode	Ret. Time (min)	Time-Managed MRM™ Group	Q1 Mass	Q2 Mass	EV	CCL2	CE
Trimethoprim	+	1.98	1.50 – 2.40 min	291.4	123.2	20	-75	-40
Trimethoprim*	+	1.98	1.50 – 2.40 min	291.4	110.3	20	-56	-48
Enalapril	+	2.60	2.21 – 3.01 min	377.3	234.2	20	-76	-27
Enalapril*	+	2.60	2.21 – 3.01 min	377.3	91.1	20	-124	-88
Erythromycin	+	2.83	2.45 – 3.33 min	716.8	158.2	32	-144	-40
Erythromycin*	+	2.83	2.45 – 3.33 min	716.8	83.1	19	-132	-70
Sulfamethoxazole	+	2.93	2.45 – 3.33 min	254.3	92.2	20	-56	-46
Sulfamethoxazole*	+	2.93	2.45 – 3.33 min	254.3	108.2	20	-56	-46
Fluoxetine	+	3.01	2.64 – 3.44 min	310.4	44.5	20	-56	-48
Carbamazepine	+	3.37	2.97 – 3.87 min	237.2	193.3	25	-55	-49
Carbamazepine*	+	3.37	2.97 – 3.87 min	237.2	179.2	25	-56	-50
Phenytoin	+	3.41	2.97 – 3.87 min	253.2	182.1	20	-56	-25
Phenytoin*	+	3.41	2.97 – 3.87 min	253.2	104.1	20	-100	-50
Naproxen	+	4.23	3.83 – 4.63 min	231.2	185.1	10	-56	-20
Naproxen*	+	4.23	3.83 – 4.63 min	231.2	170.1	10	-60	-35
Diazepam	+	4.51	4.12 – 4.92 min	285.3	154.1	20	-96	-38
Diazepam*	+	4.51	4.12 – 4.92 min	285.3	193.2	20	-108	-43
Diclofenac	+	5.07	4.67 – 5.47 min	296.1	214.2	15	-84	-47
Diclofenac*	+	5.07	4.67 – 5.47 min	296.1	250.0	15	-60	-19
Gemfibrozil	-	5.69	5.30 – 6.10 min	249.0	121.0	-5	36	20
Gemfibrozil*	-	5.69	5.30 – 6.10 min	249.0	127.1	-5	32	13
Triclosan	-	6.17	5.79 – 6.60 min	286.9	35.1	-5	100	90

Table 4. MS/MS Source Parameters

Parameter	Value
Ionization Mode	ESI; Pos/Neg
Drying Gas	120
HSID Temperature (°C)	250
Nebulizer Gas	200
Electrospray Voltage (V)	5000/-4850
Source Temperature	390
Detection Mode	Time-Managed MRM™

Solvents, Standards and Samples

All solvents were LC-MS grade. The PPCP standards (listed in Table 3) were purchased as follows: Pharmaceuticals Mix #1 and #2 were purchased from Restek, Bellefonte, PA; diazepam and phenytoin were purchased from Cerilliant, Round Rock, TX; enalapril and diclofenac were purchased from Sigma-Aldrich® Inc., Milwaukee, WI. The enalapril and diclofenac were prepared as 1 mg/mL stock standards using methanol and stored in the freezer.

A 1-ppm solution was prepared using a methanol diluent and further diluted to a 1-ppb working standard solution using 95:5 water/methanol. The working standard solution was then acidified using formic acid (v/v 0.1%) and allowed to sit at room temperature for two hours to stabilize. Calibrants were then prepared via serial dilution from the working standard solution using 95:5 water/methanol as a diluent. The calibration levels ranged from 1 to 250 ppt for all analytes.

Two tap water samples and three bottled water samples were collected, acidified with formic acid (v/v 0.1%), and allowed to sit at room temperature for two hours to stabilize. Samples were filtered using 0.22-µm nylon filters. All standards and samples were submitted for analysis and run in triplicate.

Results and Discussion

Figure 2 shows the chromatographic separation of the 250-ppt PPCP standard mix.

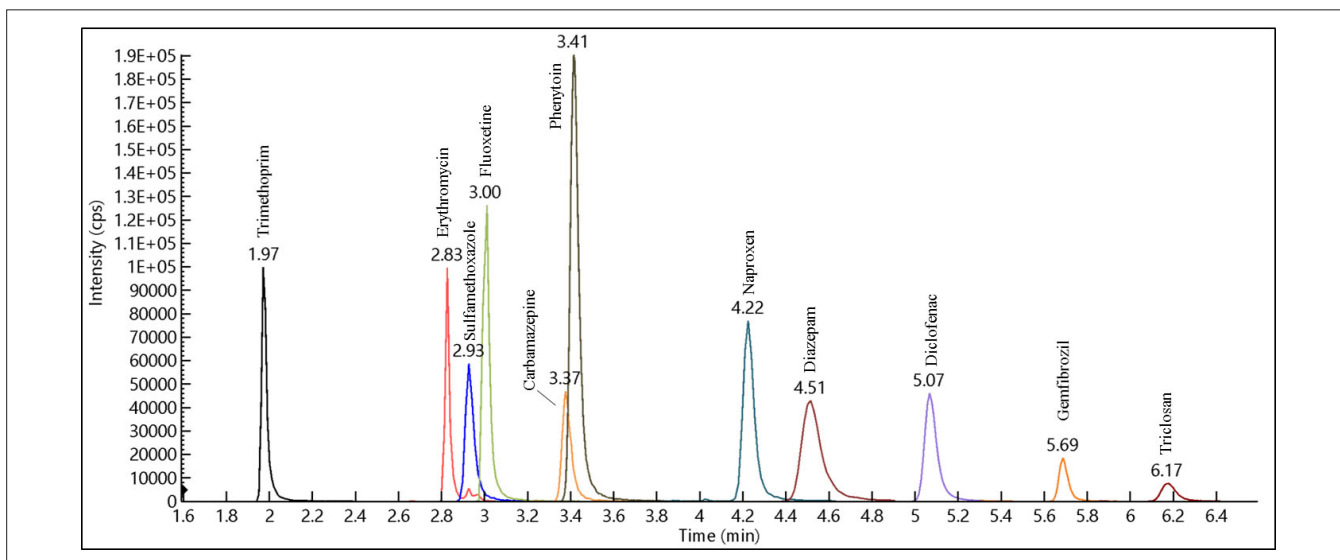


Figure 2. Overlay MRM chromatograms, showing the separation of the 250-ppt standard mix of PPCPs.

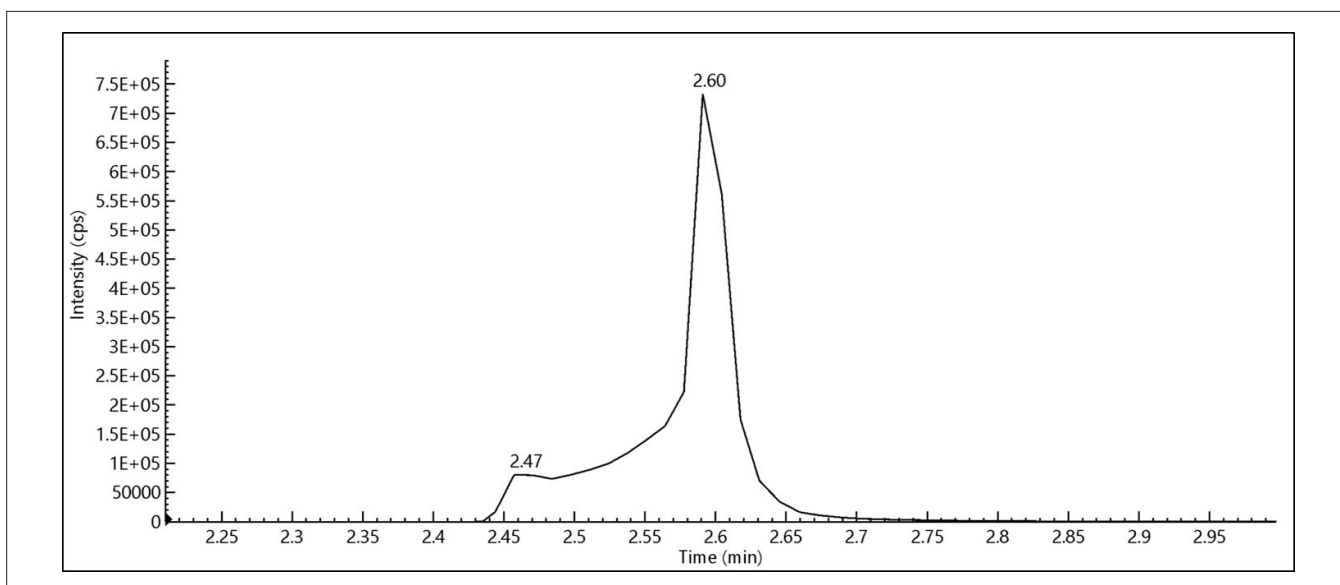


Figure 3. MRM chromatogram of enalapril at 250 ppt.

As can be seen in Figure 3, enalapril presents as a broad, non-uniform peak. Enalapril is present as a mixture of cis and trans conformers.⁶ The non-Gaussian peak shape is due to slow conformational transition between the conformers in solution.⁶ Enalapril was left out of Figure 2 due to its high signal intensity, allowing for better visualization of the separation of the other 11 analytes.

Per Figure 4, chromatographic repeatability was found to be exceptional even at low levels, here shown via the chromatographic overlay of ten replicate 50-ppt standard mix injections.

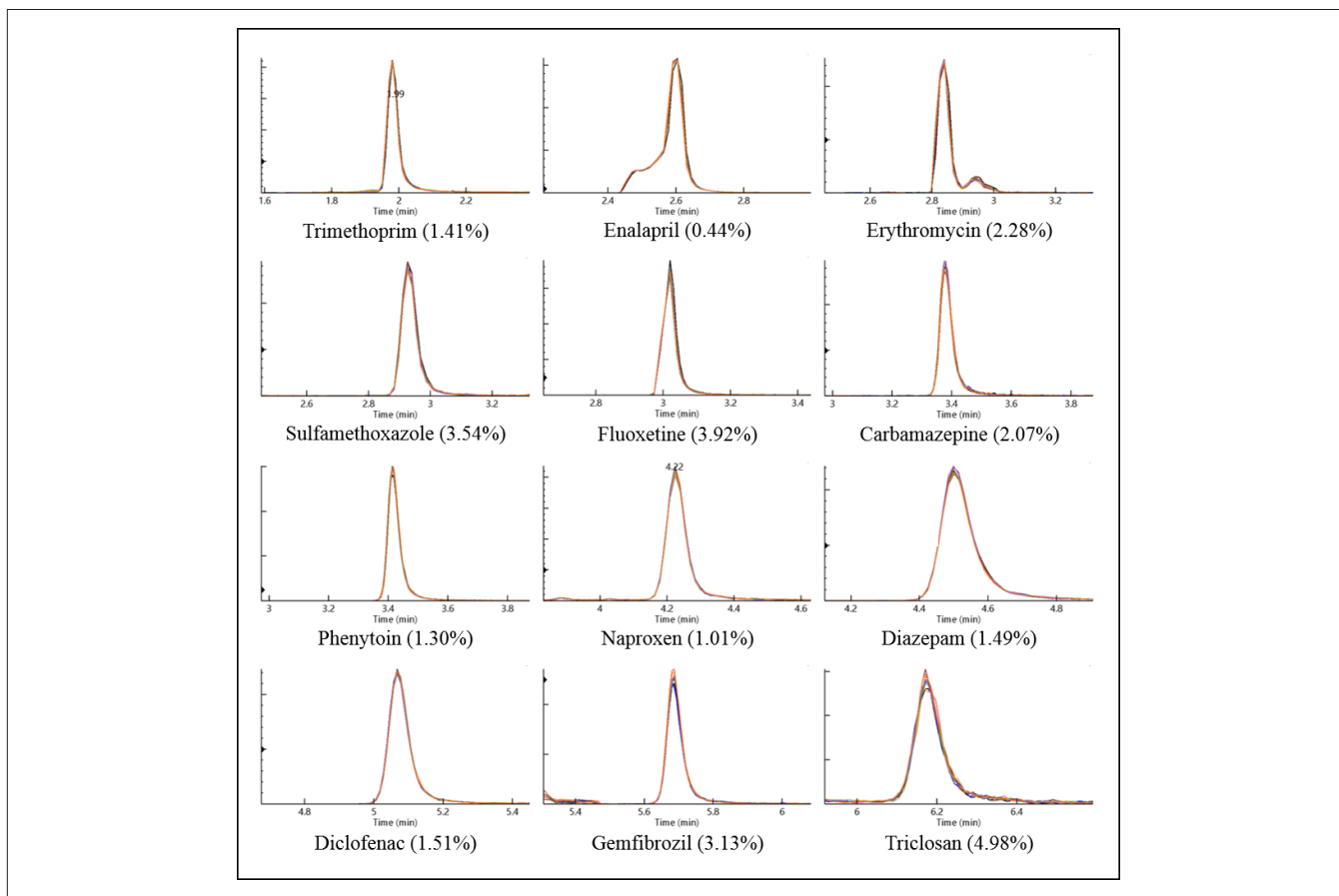


Figure 4. Overlay of ten replicate injections of the 50-ppt PPCP standard mix with peak area %RSDs.

Example linearity plots for select PPCPs are shown in Figure 5, with R^2 values for all analytes above 0.99.

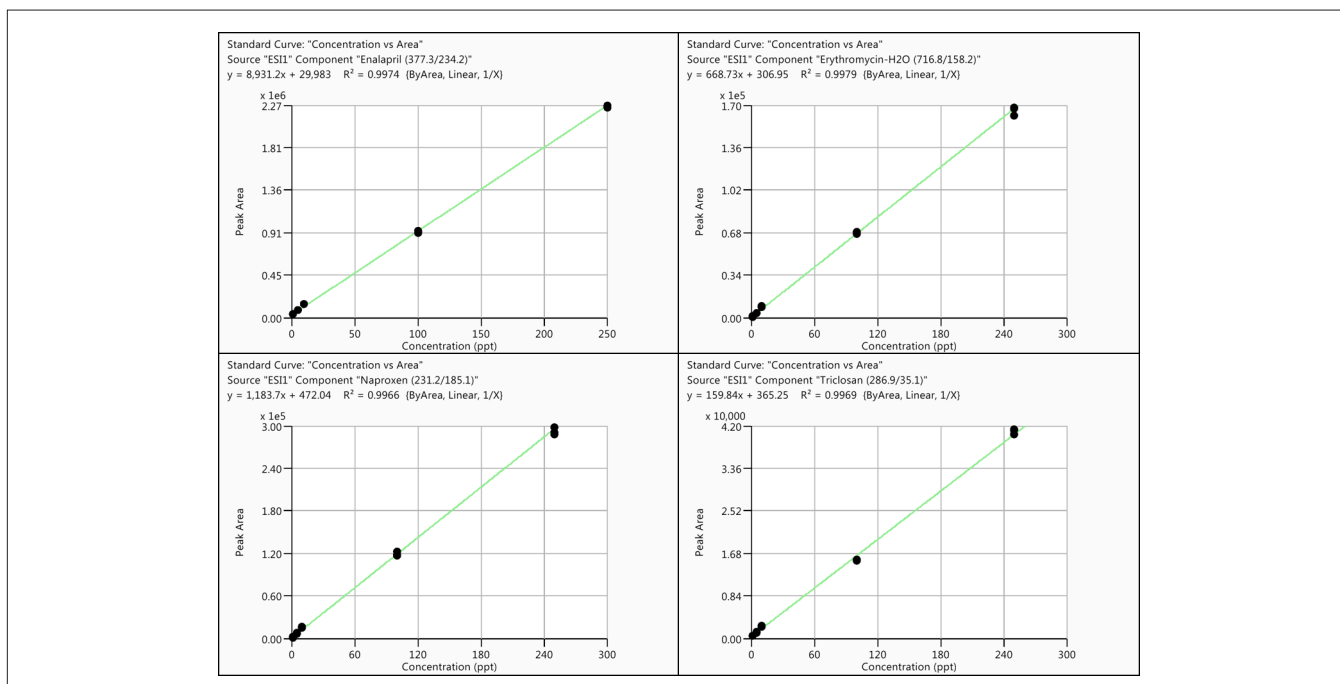


Figure 5. Example linearity plots for enalapril, erythromycin, naproxen, and triclosan.

As listed in Table 5, limits of quantitation (LOQ) were established for each analyte, based upon their averaged Level 1 calibration standard response.

Table 5. LOQs for analytes.

Analyte	Calculated LOQ (ppt)
Trimethoprim	0.17
Enalapril	0.01
Erythromycin	0.42
Sulfamethoxazole	1.02
Fluoxetine	0.08
Carbamazepine	0.39
Phenytoin	0.13
Naproxen	0.54
Diazepam	0.22
Diclofenac	0.09
Gemfibrozil	0.25
Triclosan	0.57

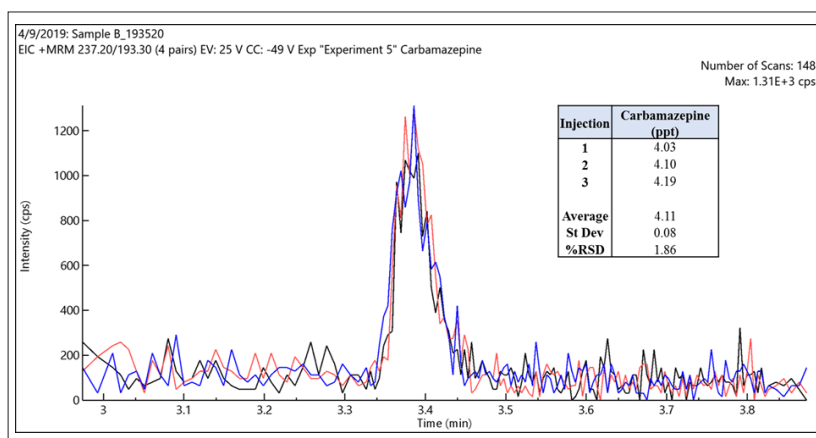


Figure 6. Overlay MRM chromatograms (unsmoothed) from triplicate injections of Tap Water #2 showing carbamazepine.

Samples for analysis consisted of two tap water samples from different sources, as well as samples from three different brands of bottled water. Of those samples, only Tap Water #2 was found to contain any of the analytes of interest. Carbamazepine, an anti-epileptic drug used to prevent and control seizures, was measured in Tap Water #2 at a concentration of 4.11 ppt (Figure 6).

Conclusions

- This work has demonstrated the effective and robust online SPE sample loading, chromatographic separation, and quantitation of PPCPs, using the PerkinElmer QSight SP50 Online SPE System coupled to a QSight 220 MS/MS detector.
- Due to the unique high-capacity SPE cartridge, and the high enrichment factor, large sample sizes are not required, saving time, improving throughput, and reducing laboratory waste.
- As the Simplicity 3Q software automatically provides for work-ahead flow as part of the online SPE/chromatographic process, online SPE only extends the injection-to-injection analysis time by 6.5 minutes per sample.
- This procedure allows for relatively low solvent consumption (≤ 10 mL per sample) as part of the SPE preparation phase.
- The method provides exceptional online sample preparation/pre-concentration and chromatographic repeatability and affords LOQs below 1 ppt for most analytes.
- The method/procedure defined herein can be expected to fulfill the essential task of monitoring for low-level PPCPs in drinking water.

References

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