APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Analysis of Pharmaceutical and Personal Care Products in River Waters via UHPLC-MS/MS

Introduction

Pharmaceutical and Personal Care Products (PPCPs) are an emerging environmental concern and include prescription medications, over-the-counter medications, sunscreens, lotions, soaps, and insect repellant. These commonly encountered products can enter the environment through various sources, including municipal waste water. Identifying and quantifying the presence of the chemical pollutants that these products contribute in surface waters, particularly in rivers and lakes, has been a growing focus.

The analytical challenge is that PPCPs encompass a wide variety of chemical classes/types and are typically present at parts per million (µg/mL) or even parts per trillion (pg/mL) concentrations in surface waters.[1,2] Therefore, developing an optimal analytical method, one that provides effective chromatographic separation, as well as optimal analyte sensitivity, is a daunting task. This often requires some compromises to be made in accommodating all the analytes that one intends to quantify.

In this study we demonstrate the application of UHPLC-MSMS for the separation, detection and quantitation of 31 PPCPs in river waters. UHPLC-MSMS is ideally suited for such an analysis, as UHPLC provides for optimal chromatographic separation, while MSMS provides for optimal sensitivity and specificity. MSMS also allows for positive ID confirmation via an analyte's unique combination of mass transitions and ion ratio.

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Experimental

Hardware/Software

For the chromatographic separations, a PerkinElmer LX50 UHPLC System was used with a PerkinElmer QSight™ 220 MS/MS detector. All instrument control, analysis and data processing was performed using the Simplicity™ 3Q software platform.

Method Parameters

The LC and MS/MS method/source parameters are shown in Tables 1, 2 and 3, respectively.

Solvents and Standards

All solvents, reagents, and diluents used were HPLC-grade or better. The PPCP standards (listed in Table 2) were purchased as follows: Pharmaceuticals Mix #1 and #2 (PharmMix1 and PharmMix2, respectively) were purchased from Restek, Bellefonte, PA; all the other PPCPs were purchased from Sigma-Aldrich® Inc., Milwaukee, WI.

Table 1. LC Method Parameters.

Column	PerkinElmer Brownlee 3.0 x 100-mm C18 SPP, 2.7 µm (Part# N9308410)
Mobile Phase	**Solvent A:** 5 mM ammonium formate in water with 0.1% formic acid **Solvent B:** Acetonitrile with 0.1% formic acid

Step	Time (min)	Flow Rate (mL/min)	%A	%B	Curve
1	Initial	0.6	100	0	
2	0.50	0.6	100	0	
3	2.00	0.6	60	40	Linear
4	7.00	0.6	3	97	Linear
5	7.50	0.6	100	0	Linear
6	11.00	0.6	100	0	

Analysis Time	7 min; re-equilibration time: 4 min
Pressure	3000 psi/207 bar (maximum)
Oven Temp.	35 °C
Injection Volume	50 µL

Table 2. MS/MS Method Parameters.

Molecule	ESI Mode	Ret Time (Min)	Exper. Group	Precursor Ion	Frag. Ion 1 (Quantifier)	EV1	CCL2	CE1	Frag. Ion 2 (Qualifier)	EV1	CCL2	CE1
Acetaminophen	+	2.09	A	152.1	110.0	20	-34	-25	65.3	20	-35	-25
Acetazolamide	+	2.19	A	223.2	181.2	25	-65	-40	73.0	25	-68	-46
Butalbital	-	3.15	D	223.1	42.1	-20	50	25	NA	7.69	25.60	101.24
Caffeine	+	2.27	B	195.0	83.3	20	-35	-25	138.2	20	-60	-50
Carbamazepine	+	3.28	D	237.2	193.3	25	-55	-49	179.2	25	-56	-50
Ciprofloxacin	+	2.23	B	332.5	314.0	20	-55	-25	231.2	20	-111	-49
Chlortetracycline	+	2.52	B	479.5	154.2	20	-140	-50	98.3	20	-160	-70
Codeine	+	2.05	A	300.5	165.3	25	-82	-58	153.2	25	-82	-58
Cotinine	+	1.77	A	177.2	80.3	25	-44	-31	98.2	25	-90	-79
Cyclophosphamide	+	2.89	C	261.3	140.4	25	-45	-29	63.1	25	-55	-50
Diazepam	+	4.22	D	285.4	193.0	25	-81	-42	154.0	25	-90	-80
Diclofenac	+	4.81	D	296.4	214.4	25	-90	-49	133.2	25	-65	-40
1,7-Dimethylxanthine	+	2.08	A	181.2	124.2	25	-35	-29	69.3	25	-40	-43
Diphenhydramine	+	2.74	C	256.5	167.2	25	-55	-40	165.3	25	-70	-48
Doxycycline	+	2.57	B	445.6	428.2	25	-130	-30	267.2	25	-130	-53
Erythromycin	+	2.73	C	735.1	158.3	20	-110	-39	116.3	20	-111	-50
Estrone	+	4.24	D	271.4	133.2	25	-90	-28	157.2	-25	-90	-23
Fluoxetine	+	2.97	C	310.4	44.5	20	-56	-48	NA			
Gemfibrozil	-	5.49	D	249.3	121.2	-20	55	20	127.0	-20	55	20
Ketoprofen	+	3.96	D	255.4	105.2	20	-50	-38	77.3	20	-104	-62
Metformin	+	0.67	A	130.2	71.4	25	-35	-37	43.4	25	-64	-48
Minocycline	+	2.16	A	458.5	352.1	20	-150	-46	283.2	20	-150	-65
Naproxen	+	4.00	D	231.2	170.2	20	-80	-35	153.3	20	-80	-45
Penicillin G	-	3.22	D	333.2	74.1	-20	65	40	192.1	-20	65	40
Penicillin V	+	3.43	C	351.4	160.1	20	-62	-15	114.0	20	-62	-48
Ranitidine	+	1.94	A	315.6	102.2	20	-130	-45	125.2	20	-104	-62
Sulfamethoxazole	+	2.86	C	254.3	92.2	20	-56	-46	108.2	20	-56	-47
Tetracycline	+	2.32	B	445.6	154.1	20	-160	-38	98.1	20	-130	-57
Tramadol	+	2.39	B	264.4	58.0	25	-90	-75	121.0	25	-90	-75
Triclosan	-	5.85	D	287.1	35.2	-20	45	32	NA			
Trimethoprim	+	2.20	B	291.4	123.2	20	-75	-40	110.3	20	-56	-48

EV (V) = Entrance Voltage; CCL2 (V) = Collision Cell Lens 2; CE (V) = Collision Energy; NA = not available

Dwell (Cycle) Time: 10 msec for all analytes; Exper. Groups: A (0.0 – 2.4 min), B (2.0 – 2.8 min), C (2.45- 3.63 min), D (2.9 – 6.0 min).

Table 3. MS/MS Source Parameters.

Parameter	Setting
Ionization Mode	ESI; positive and negative, depending on analyte
Drying Gas	120
HSID Temperature (°C)	320
Nebulizer Gas	275
Electrospray Voltage (V)	4850 (pos. mode) -4850 (neg. mode)
Source Temperature (°C)	420

Standard and Sample Preparation

For all liquid standards, a 1-µg/mL (ppm) stock standard solution was prepared as follows: 500 uL of both PharmMix1 and PharmMix2 (200 µg/mL each, in methanol) were added to a 100-mL volumetric flask. To this flask, 100 uL each of codeine, cotinine, diazepam, butalbital and tramadol (1000 µg/mL each, in methanol) were also added. The flask was then filled to volume with 10% methanol/water.

For all solid standards, 10 mg of each was added to a 1000-mL volumetric flask, which was then filled to volume with methanol (required for solubility of some analytes). This solution was stirred for 20 minutes, to allow all of the solid standards to dissolve completely, and then further diluted 10-fold with water, providing a stock standard solution of 1 µg/mL.

The two stock standard solutions were then combined 1:1, to make a standard mix containing 0.5 µg/mL of each analyte. This was further diluted 1:1 with water, providing a working standard mix (WS) containing 0.25 µg/mL of each analyte in 5% methanol/water. The WS was serially diluted with 5% methanol/water to make calibration standards ranging from 0.025 to 250 ng/mL (ppb).

Two river water samples were collected from local rivers, one from the Hudson River in Cold Spring, New York, and one from the Housatonic River in Stratford, Connecticut. Each sample was first filtered using a 0.45-um nylon filter and then 50 ul of the filtered sample was directly injected on column for analysis.

To check for any carryover, a 5% methanol/water blank was injected after both the standard set and the samples.

All standards and samples were submitted for LC-MS/MS analysis and run in triplicate.

Results and Discussion

Figure 1 shows the total ion chromatogram (TIC) of the WS containing all analyzed PPCPs, of which close to 20 are well resolved. The remaining PPCPS were further resolved via their unique MW transitions.

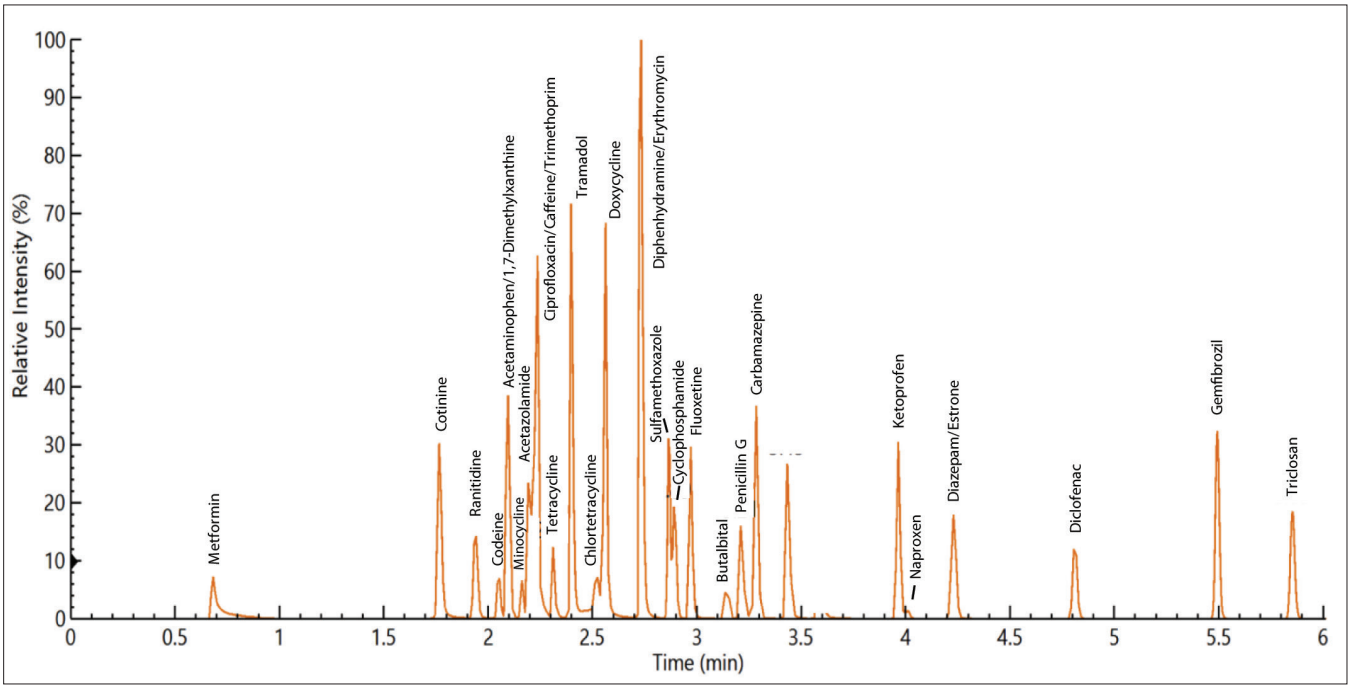

Figure 1. TIC chromatogram of all 31 analytes.

Represented by six of the analyzed PPCPs, the overlaid MRM (multiple reaction monitoring) chromatograms of eight replicate injections are shown in Figure 2, demonstrating exceptional repeatability.

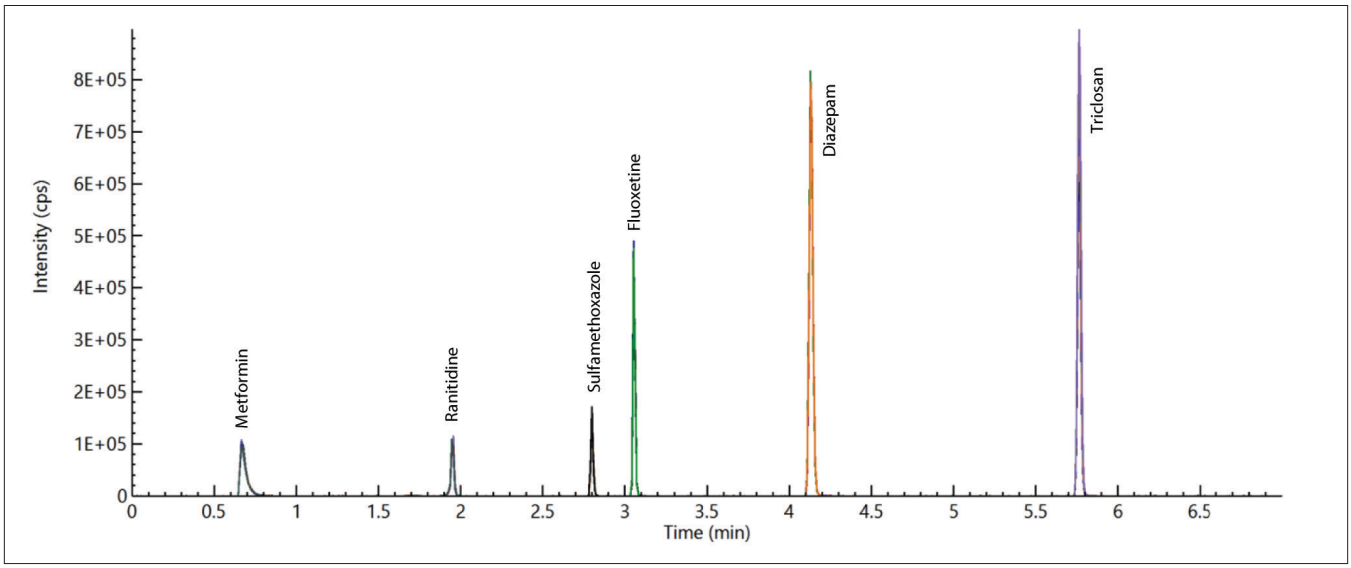

Figure 2. Overlay of eight replicate injections of the 250-ppb PPCP WS. For clarity, the MRMs of six well-distributed analytes were used (metformin, ranitidine, sulfamethoxazole, butalbital, diazepam and triclosan).

Figure 3 shows the calibration plots for six representative PPCPs. The R^2 values for the 31 analytes ranged from 0.9944 to 0.99989. These were based upon at least five calibration levels, dependent on analyte response.

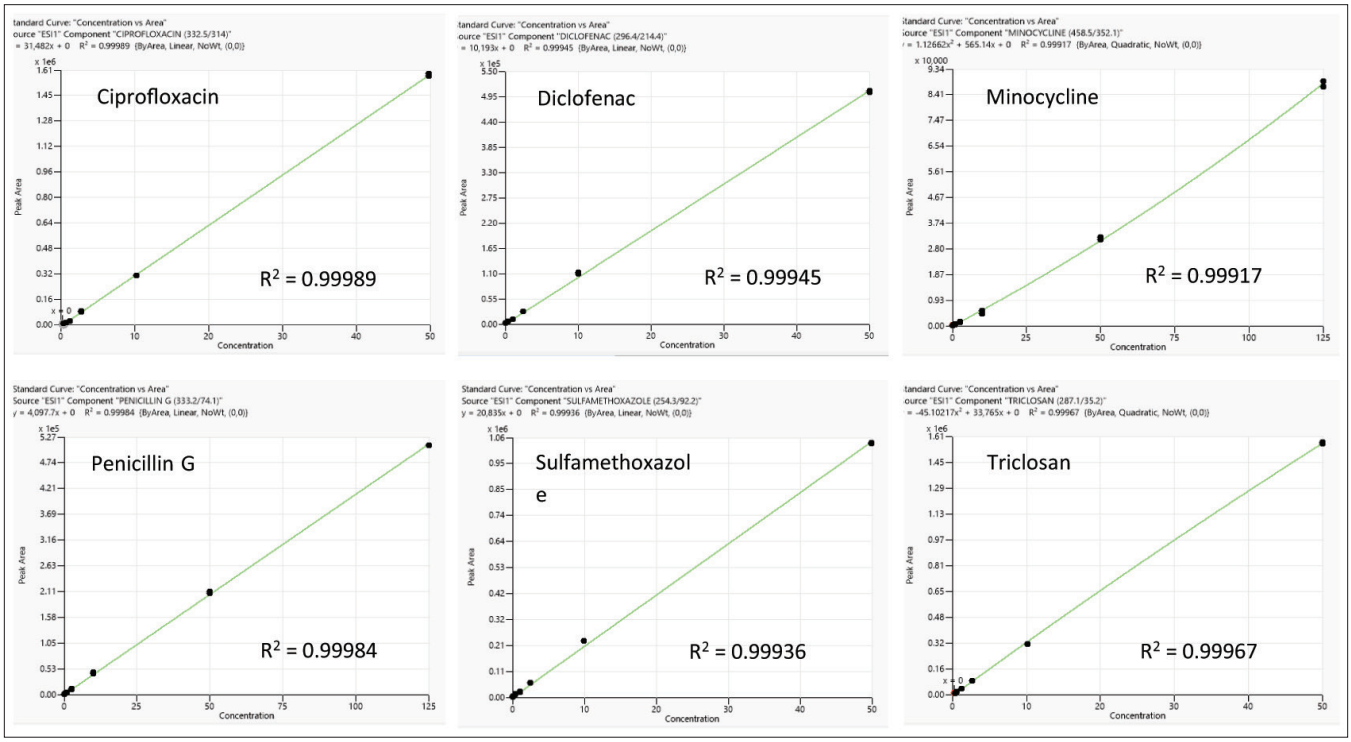

Figure 3. Calibration plots for six representative PPCPs.

The calculated LOQs for the 31 analyzed PPCPS are provided in Table 4, with most values in the low ppt range. This was based upon the S/N calculated from the lowest calibrant used for each analyte. The lowest calibrant depended on the individual response for each analyte. The spread reflects the diversity of the compounds found among PPCPS.

Samples of Housatonic and Hudson River water were then analyzed for PPCPs. Their respective total ion chromatographic (TIC) profiles are shown in Figure 4. The annotations refer to peak retention times.

Table 4. LOQs for the 31 analyzed PPCPs.

Molecule	LOQ* (ppt; pg/mL)
Acetaminophen	32.5
Acetazolamide	15.5
Butalbital	1988
Caffeine	94.2
Carbamazepine	5.3
Ciprofloxacin	11.0
Chlortetracycline	843.5
Codeine	9.9
Cotinine	5.6
Cyclophosphamide	4.4
Diazepam	9.9
Diclofenac	7.6
1,7-Dimethylxanthine	2.3
Diphenhydramine	2.2
Doxycycline	2.9
Erythromycin	153.7
Estrone	202.8
Fluoxetine	16.3
Gemfibrozil	5.0
Ketoprofen	37.3
Metformin	1.9
Minocycline	31.5
Naproxen	272.8
Penicillin G	20.1
Penicillin V	9.7
Ranitidine	7.3
Sulfamethoxazole	7.7
Tetracycline	57.6
Tramadol	3.2
Triclosan	8.8
Trimethoprim	9.2

** Based upon a S/N ≥ 10 (average of three injections).*

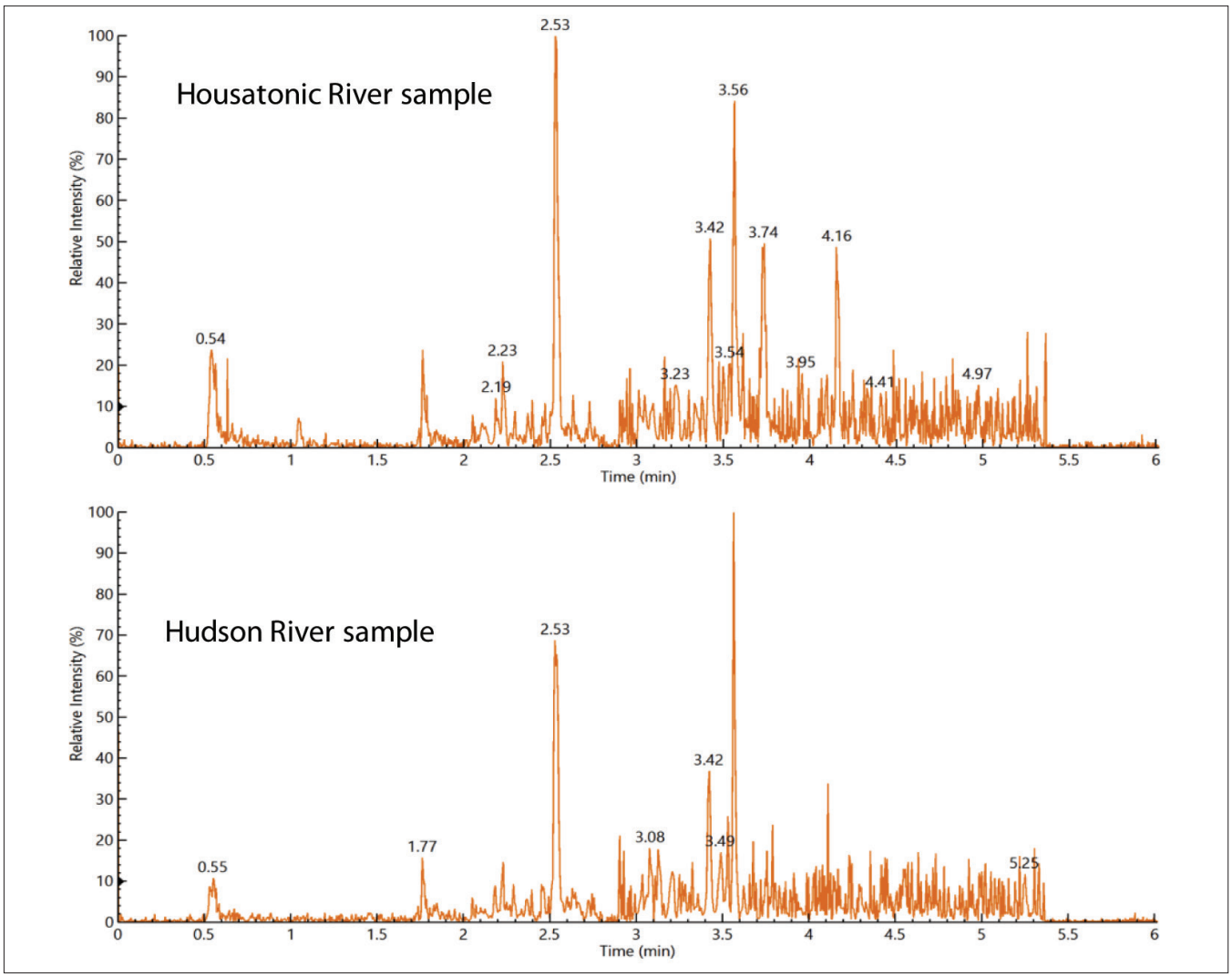

Figure 4. Total ion chromatographic (TIC) profiles of the two river samples.

Figure 5 shows the MRM chromatograms of the quantifiable PPCPs found in the two river waters, as labeled.

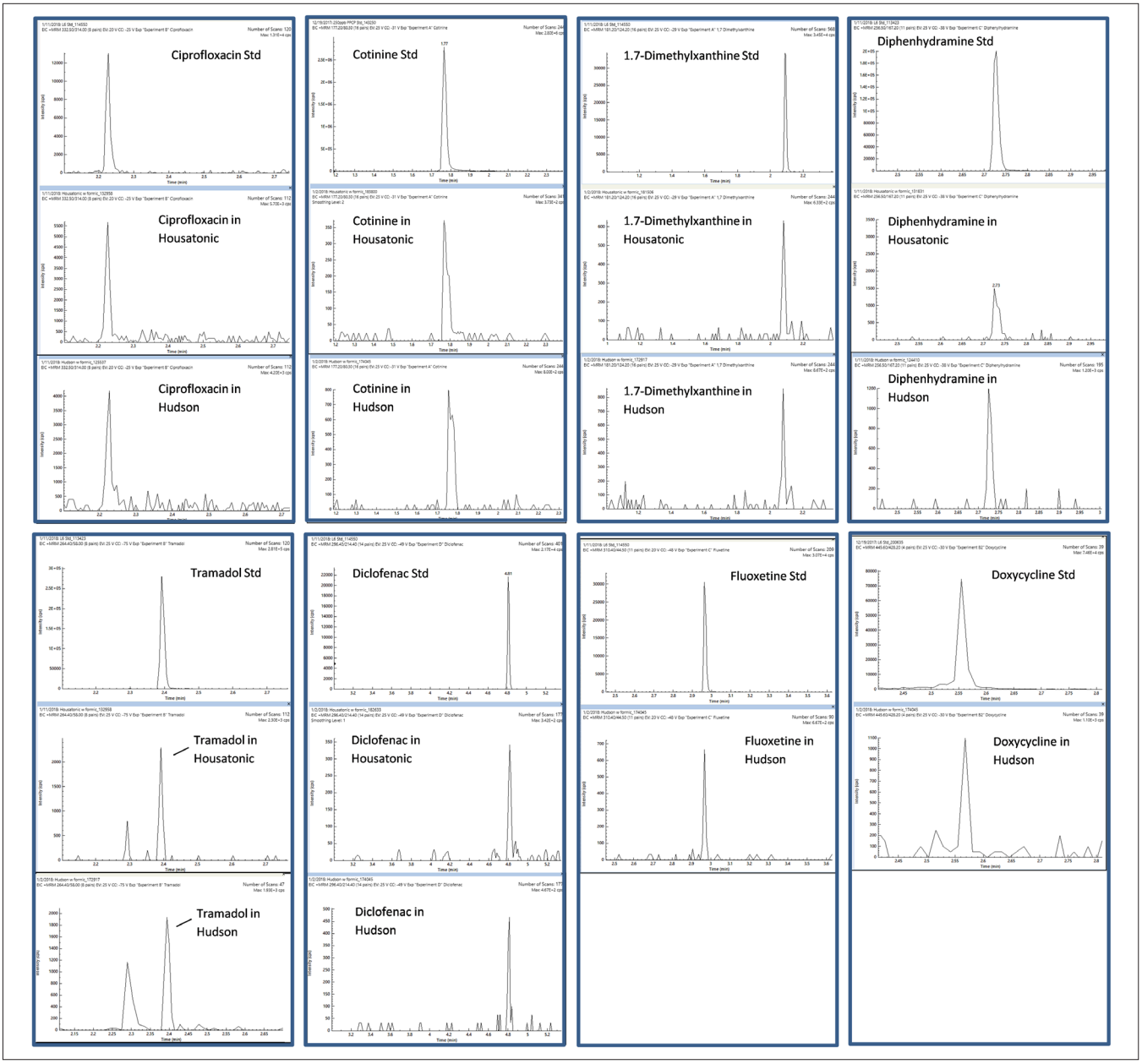

Figure 5. MRM chromatograms of the PPCPs found at quantifiable levels in each of the river water samples.

As shown in Table 5, ppt (pg/mL) levels of tramadol, cotinine, ciprofloxacin, 1,7-dimethylxanthine, diphenhydramine and diclofenac were found in both river samples. Quantifiable levels of doxycycline and fluoxetine were only found in the Hudson River sample, while fluoxetine was found at a trace level in the Housatonic River sample. Acetaminophen, sulfamethoxazole and gemfibrozil were also found in the Hudson River sample, but only at trace levels. None of the other analyzed PPCPs were detected in either river sample.

Table 5. Detected amounts of PPCPs present in the two river waters, each run in triplicate.

	Avg. Concentration (ppt; pg/mL)					
	Acetaminophen	**Ciprofloxacin**	**Cotinine**	**1,7-Dimethylxanthine**	**Diphenhydramine**	**Doxycycline**
Housatonic	Not Detected	121.0	199.9	60.2	18.8	Not Detected
Hudson	Trace	76.2	101.2	61.8	37.0	23.8

	Avg. Concentration (ppt; pg/mL)				
	Fluoxetine	**Gemfibrozil**	**Sulfamethoxazole**	**Tramadol**	**Diclofenac**
Housatonic	Trace	Not Detected	Not Detected	18.5	38.6
Hudson	84.0	Trace	Trace	11.6	118.0

To check for possible analyte carryover or background interference, a blank sample, consisting of 5% methanol/water was also run in triplicate, both after the calibration set and after the samples. No carryover was observed for any of the analytes.

For analyte ID confirmation, the qualifier/quantifier ion ratios were used, with a 20% tolerance limit. For many analytes, these were applicable down to the lowest calibrant concentration (25 ppt). An example of ion ratios for diphenhydramine at the individual calibration levels, as well as for actual samples, is shown in Table 6. For those analytes with significantly weaker qualifier ions, the ratios were applicable down to low ppb levels. For butalbital, fluoxetine and triclosan, a suitable/robust qualifier transition was not identified.

Conclusion

- A reverse phase LC-MS/MS method has been developed and demonstrated to be effective for the analysis of 31 PPCPs in river waters, using a PerkinElmer LX50 UHPLC/QSight 220 MS/MS system.
- The described method/procedure provides a fast, reliable direct-injection PPCP analysis in under six minutes, with a sample turn-around time of 11 minutes and LOQs at low ppt levels for most analytes.

Table 6. Ion ratios for diphenhydramine at the calibration levels used and for the actual samples. (Quantifier transition: 256.5/167.2; Qualifier transition: 256.5/165.3).

Calibrant/Sample	Ion Ratio (By Area; Avg. of Three Injections)
L1 (25 ppt)	0.43
L2	0.40
L3	0.38
L4	0.41
L5	0.40
L6	0.40
L7	0.40
L8 (50 ppb)	0.40
Housatonic River Sample	0.35
Hudson River Sample	0.42

References

1. Daughton, C.G., Jones-Lepp, T.L., Pharmaceuticals and personal care products (PPCPs) in the environment. Scientific and Regulatory Issues, ACS Symp. Ser. 791, Oxford University Press, Washington, U.S. (2001).
2. Daughton, C.G., Environ. Impact Assess. Rev. 24 (2004) 711.

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