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Application News

No. AD-0127

Water Analysis / LCMS-8060

A Highly Sensitive MRM-Based Method for Detection and Quantitation of Seven Pharmaceuticals and Personal Care Products (PPCPs) in Surface Water

❑ Introduction

The presence of pharmaceutical and personal care products (PPCPs) in drinking water has become a growing public concern due to their continuous input and persistence to the environment [1,2]. They include many drug compounds from medicines and chemical ingredients from daily personal care products such as soaps, toothpastes and cosmetics. Many PPCPs act as endocrine disruptors and thus altering the normal functions of hormones resulting in reproductive defects and health issues [2]. The main sources of PPCPs in surface water are wastewaters from industries and domestic sewages. It has been reported that the levels of PPCPs in sewage treatment plants are in the range of low ng/L to µg/L. Risk assessments of PPCPs are evaluated, and regulations for control and management of PPCPs into surface water have been established in many countries [1,2]. For monitoring PPCPs in surface water and wastewater, mass spectrometry methods on LC-Q-TOF and triple quadrupole LC/MS/MS are used widely for their high sensitivity and superior identification capability [3,4]. To ensure the sensitivity for detection of low ng/L level or parts per trillion (ppt), off-line or on-line pre-concentration of water samples is often required [5]. In this Application News, a highly-sensitive LC/MS/MS method is described, which has been developed on a high sensitivity model of triple quadrupole LCMS-8060, aiming at direct determination of seven PPCPs (see Table 2) of low ppt levels in surface water samples like treated water and reservoir water without pre-concentration.

❑ Experimental

Analytical conditions and sample preparation

A LCMS-8060 triple quadrupole system coupled with Nexera UHPLC was employed in this work. A pentafluorophenyl (PFP) column from Phenomenex was used and a fast gradient elution program was optimized for analysis of the seven PPCPs. Details of the UHPLC conditions and MS/MS parameters are shown in Table 1. Stock solutions of the seven PPCPs standards (see Table 2) were prepared and diluted in series with Milli-Q water to obtain calibrants. A treated water sample (S1) from a wastewater treatment, a reservoir water sample (S2) and a few control samples were obtained from a third-party laboratory. These water samples were analyzed by injecting into the LC/MS/MS directly without any sample pre-treatment or enrichment.

Table 1: Analytical conditions of PPCPs on LCMS-8060

Column	Kinetex 2.6u PFP 100A (100 mm L. x 2.10mm I.D.)
Mobile Phase	A: Water 0.1% formic acid B: Acetonitrile
Elution Program	Gradient elution, 5%B (0.00-0.50 min), 75%B (4.54 min), 95% B (4.55-6.50min), 5%B (6.60-8.00 min)
Flow Rate	0.3 mL/min
Oven Temp.	40ºC
Injection	10 µL
Interface	ESI Heated
MS Mode	MRM, Positive and Negative mode
Block Temp.	400ºC
DL Temp.	250ºC
Interface Temp.	300ºC
Nebulizing gas	N_2, 3.0 L/min
Drying gas	N_2, 5.0 L/min
Heating Gas	Zero air, 15.0 L/min

❑ Results and Discussion

A. Highly sensitive MRM method for seven PPCPs

The seven PPCPs used in this study include four nonsteroidal anti-inflammatory drugs, one fibrate drug and two antibacterial agents. The compound names and information are compiled into Table 2. The two antibacterial agents, dichlorofenac and triclosan, are commonly used in personal care products such as toothpaste, soaps, detergents and lotions. MRM optimization of the compounds were performed and two MRM transitions for each compound were selected with one for quantitation and the other for confirmation. However, as shown in Table 2, the relative intensities of the reference MRM transitions of four compounds are very low at 2%~8%, which has significant limitations as confirmation of the compounds at low concentration levels.

Table 2: Summary of MRM transitions, calibration range, linearity and detection sensitivity of seven PPCPs on LCMS-8060

Compound Name & Formula	Type of PPCP	MRM Parameter				Quantitation Method					
		Precursor	Product	CE (V)	Intensity	RT (min)	Range (ng/L)	R^2	LOD (ng/L)	LOQ (ng/L)	%RSD (n=3)*
Ketoprofen ($C_{16}H_{14}O_3$)	Anti-inflammatory drug	255.0	105.1	-23	100	4.15	10~1000	0.9998	13.5	38.5	4.1
			77.1	-47	76						
Naproxen ($C_{14}H_{14}O_3$)	Anti-inflammatory drug	(-)229.2	(-)170.1	15	100	4.31	5~500	0.9963	2.2	6.7	7.2
			(-)169.1	32	84						
Ibuprofen ($C_{13}H_{18}O_2$)	Anti-inflammatory drug	(-)205.2	(-)161.3	9	100	4.70	10~500	0.9962	9.0	27.2	6.1
			(-)117.1	22	2						
Gemfibrozil ($C_{15}H_{22}O_3$)	Fibrate Drug	(-)249.2	(-)121.1	20	100	5.10	1~500	0.9960	1.0	2.9	3.9
			(-)106.1	43	2						
Dichlorofenac ($C_{14}H_{11}Cl_2NO_2$)	Anti-inflammatory drug	296.1	215.0	-21	100	4.57	5~1000	0.9995	4.7	14.2	3.0
			214.0	-33	96						
Triclocarban ($C_{13}H_9Cl_3N_2O$)	Antibacterials	(-)313.1	(-)160.1	14	100	5.47	5~500	0.9924	2.2	6.8	1.7
			(-)126.1	23	8						
Triclosan ($C_{12}H_7Cl_3O_2$)	Antibacterials	(-)287.0	(-)35.1	10	100	5.33	10~500	0.9936	6.1	18.9	7.3
			(-)142.0	35	6						

** At concentrations nearest LOQs*

A gradient elution of 8 minutes was optimized and MRM chromatograms of the seven compounds are illustrated in Figure 1. Based on the quantifying MRM transitions, linear calibration curves were established using the calibrant series of 1, 5, 10, 20, 50, 100, 250, 500 and 1000 ng/L in pure water. The calibration curves constructed with weighting method of 1/C are shown in Figure 2. The range and linearity (coefficient R^2) of the method are summarized in Table 2.

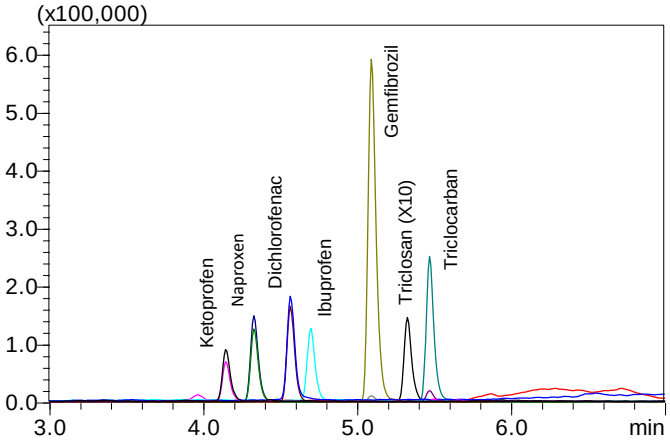

Figure 1: MRM chromatograms of seven PPCPs in a mixed standard sample of 500 ng/L.

Furthermore, the repeatability of the quantitation method at the levels nearest to the LOQs of every compounds are determined with triplicate injections (n=3). As can be seen in Table 2, the %RSD falls in a range of 1.7%~7.2%, indicating that the quantitation method is very repeatable and reliable. The LODs and LOQs of the method were estimated from the results of the lowest concentration standards following the rules of S/N >/=10 for determining LOQs and S/N >/=3 for LODs. The LODs obtained with an injection of 10 µL are 1.0 ng/L for gemfibrozil, 2.2 ng/L for naproxen and triclocarban, 4.7~9.0 ng/L for dichlorofenac, triclocarbon and ibuprofen, and 13.5 ng/L for ketoprofen.

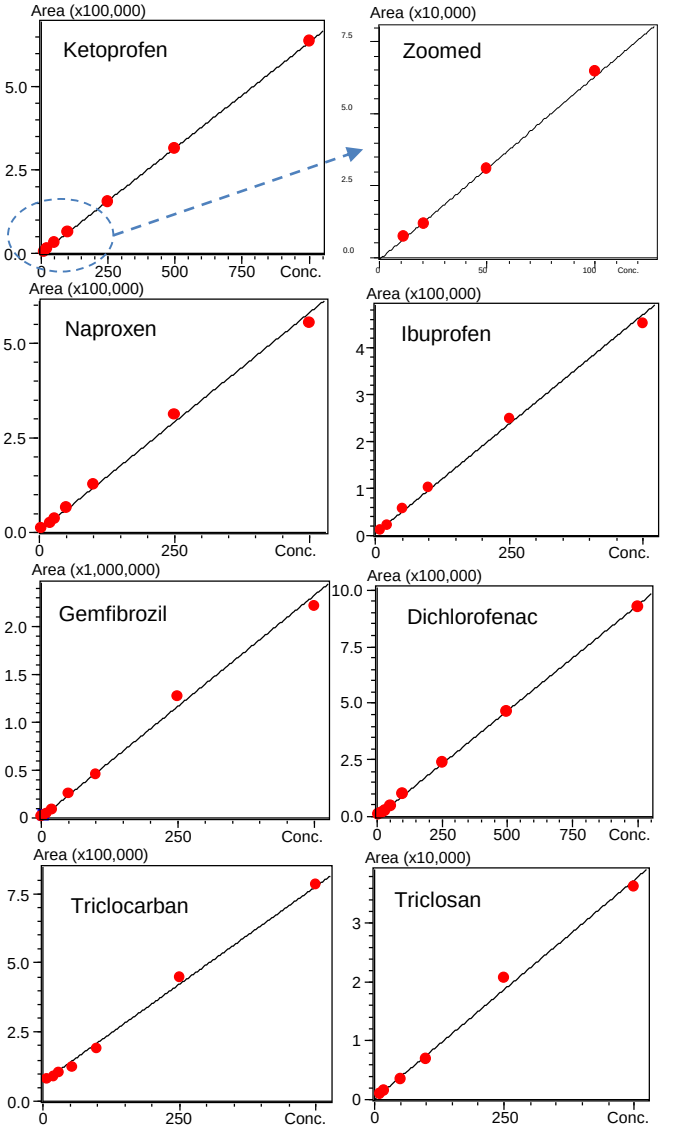

Figure 2: Calibration curves of seven PPCP standards by MRM method on LCMS-8060 with an injection volume of 10 µL.

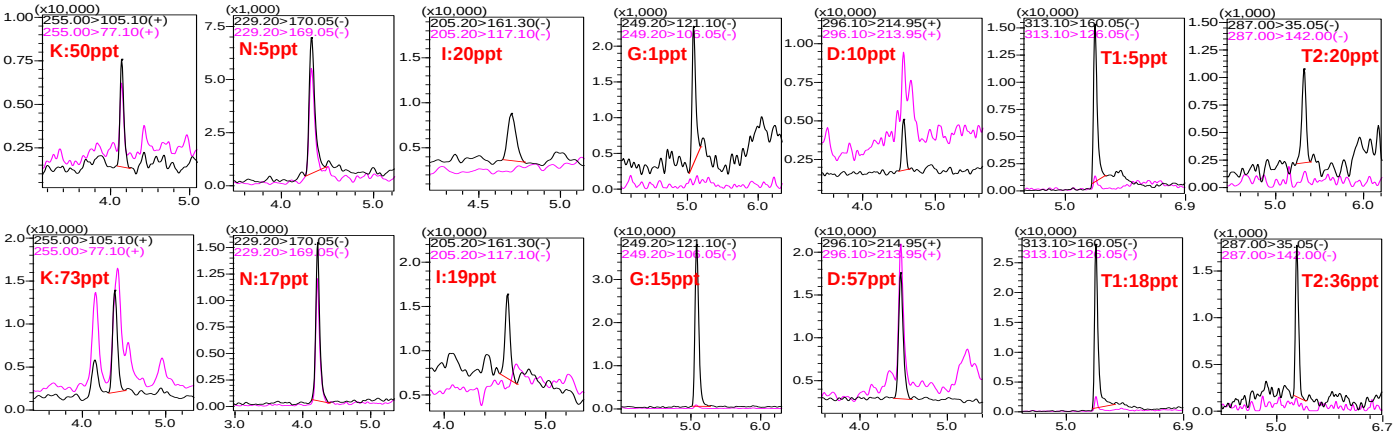

Figure 3: A comparison of individual MRM peaks of seven PPCPs spiked in Milli-Q water at nearest LOQ levels (Top) and in the reservoir water sample S4 (Bottom) at few tens ng/L (ppt).

B. Analysis of treated water and reservoir water

The MRM method established was applied to several surface water samples. Sample S1 is a treated water from sewage treatment unit and S2 is a reservoir water from a local source. In addition, spiked samples S3 and S4 were prepared by a third-party laboratory, which were used for verification of the detection and quantitation reliability of the method without pre-concentration or enrichment of the samples.

Table 3: Quantitation results of PPCPs in water samples. (N.D. = Not Detected)

Name (Abbr.)	RT (min)	Determined Concentration (ng/L)			
		S1	S2	S3	S4
Ketoprofen (K)	4.15	N.D.	N.D.	55.4	72.9
Naproxen (N)	4.31	N.D.	N.D.	33.5	17.0
Ibuprofen (I)	4.70	N.D.	N.D.	36.4	19.1
Gemfibrozil (G)	5.10	0.54	0.49	36.6	14.5
Dichlorofenac (D)	4.57	N.D.	N.D.	53.9	56.5
Triclocarban (T1)	5.47	N.D.	N.D.	34.0	18.2
Triclosan (T2)	5.33	10.9	10.9	64.8	35.9

Milli-Q water was used as blank and always injected before every water samples to confirm a clean baseline and free of sample carryover. The analysis results shown in Table 3 indicate that the two water samples S1 and S2 are free of the targeted PPCPs except triclosan, which the concentrations are 10.9 ng/L (Figure 4, top). A very small peak of gemfibrozil was observed (Figure 4, bottom) and the corresponding level is about 0.5 ng/L, which is below the LOD of the method. Thus, its presence in the samples is suspected only. The individual MRM peaks of the spiked sample S4 are compared with those of mixed standards in Milli-Q water in Figure 3, which is served as a reference verification of the method for detection and quantitation of the seven targets.

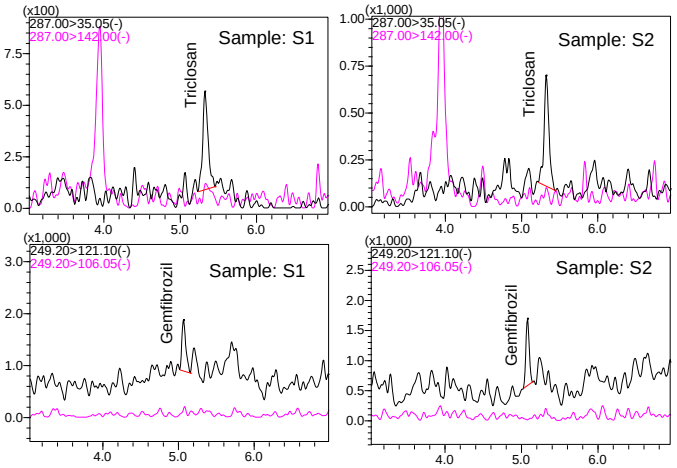

Figure 4: Triclosan peak (top) and gemfibrozil (bottom) in treated water (S1) and reservoir water (S2).

❑ Conclusions

A high sensitivity MRM method for quantitative determination of seven PPCPs in water samples was developed. Without sample pre-concentration or enrichment, the LODs of the MRM method achieved are 1.0 ng/L for gemfibrozil, 2.2 ng/L for naproxen, triclocarban and Ibuprofen, 4.7~9.0 ng/L for dichlorofenac and triclosan, and 13.5 ng/L for ketoprofen.

❑ References

1. U.S. EPA Method 1694: *Pharmaceuticals and Personal Care Products in Water, Soil, Sediment and Biosolids by LC/MS/MS* (2007).
2. Liu J, Wong M, *"Pharmaceuticals and personal care products: A review on environmental contamination in China"*, Env Int. 59 (2013): 208-224.
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