Application Note

Environmental

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Automated Online SPE-UHPLC/MS/MS Analysis of Emerging Pollutants in Water

Simultaneous quantification of contaminants in environmental water matrices

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Abstract

In this application note, an automated online solid phase extraction (SPE) method coupled to ultrahigh-performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS) is described for simultaneous determination of emerging organic contaminants (EOCs) in environmental water matrices. A total of 87 EOCs, including 58 pharmaceuticals and personal care products (PPCPs), 22 perfluoroalkyl substances (PFASs), and seven organophosphorous flame retardants (PFRs), were selected as the target analytes. Through optimization of the online SPE sample enrichment parameters and the LC/MS separation and detection conditions, the method was evaluated for performance across all 87 analytes in environmental water matrices including drinking water, surface water, and wastewater effluent. The optimized method delivered very good linearity, analytical sensitivity (LOQs <10 ng/L for almost all analytes), accuracy, and precision, and can be reliably applied for high-throughput screening of these EOCs in environmental water matrices.

Introduction

Pharmaceuticals and personal care products (PPCPs), perfluoroalkyl substances (PFASs), and organophosphorous flame retardants (PFRs) are three classes of organic substances widely used in daily life, agricultural, and industrial activities. With a lack of regulation and effective supervision, these substances may enter the environment through direct wastewater discharge or ineffective wastewater treatment. These activities could be harmful towards the drinking water resources residents depend on, and the environment in general. Timely and accurate monitoring of organic substance contamination of environmental water bodies is critical for alerting the public and evaluating water-processing reliability.

Much of the previous literature has focused on detection of one or several classes of organic contaminants in water. Conventional offline SPE is the major approach used for analyte enrichment, but is both time and labor-consuming. Online SPE enrichment coupled to LC/MS/MS analysis has been demonstrated to be a promising approach for the analysis of organic contaminants in water, and has been reported for the analysis of specific classes of contaminants such as antibiotics, pharmaceuticals, and PFASs in water in the past decade.[1-3] Additional reports demonstrated the potential of a combined online SPE-LC/MS/MS approach for simultaneous analysis of several classes of analytes in environmental water matrices.[4,5] Such a method allows analysis of water samples with minimal manual intervention, and is cost-effective considering savings in time, solvents, and consumables. However, the dramatic variation of different classes of analytes in terms of physicochemical properties often limits the coverage of the analytes using a single method. Therefore, the key to this method is to select a universal online SPE cartridge to enrich as many types of analytes as possible under suitable conditions. This application note describes a PLRP cartridge-based online SPE method combined with UHPLC/MS/MS for simultaneous screening of 87 organic substances based on a recent report.[6] The three major classes of EOCs (PPCPs, PFASs, PFRs) in diverse environmental water matrices are covered.

Experimental

Materials and methods

Chemical standards of the analyzed compounds and isotope-labeled internal standards were purchased from Sigma-Aldrich (Steinheim, Germany), Dr. Ehrenstorfer (Augsburg, Germany), or Toronto Research Chemicals (Toronto, Canada).

A set of 87 analytes was studied, including: 58 PPCPs (e.g, sulfonamides, quinolones, and β-lactams), 22 PFASs (e.g. perfluoroalkyl carboxylic acids (PFCAs, C_4 to C_{13}), and perfluoroalkane sulfonates (PFSAs, C_4, C_6, C_8, and C_{10}), and seven PFRs. An additional 37 isotopically labeled internal standards (ILIS) were applied for ILIS dilution calibration to avoid quantitation bias. All the analytes and the ILISs are listed in the appendix (Table 1), and the ILIS for each analyte is also specified in it.

HPLC-grade solvents from J. T. Baker (USA) were used for all analyses, and a Milli-Q unit (Millipore, USA) was used to produce ultrapure water. Stock solutions were prepared in methanol (MeOH) and stored at 4 °C in the dark. Working solutions were obtained by serial dilution of stock solutions with ultrapure water.

Online SPE setup

Six online SPE cartridge types were sourced from Agilent Technologies (Santa Clara, CA, USA):

- Agilent ZORBAX Bonus-RP (p/n 821125-928)
- Agilent ZORBAX Eclipse Plus C18 (p/n 821125-936)
- Agilent Bond Elut Plexa PCX (factory-customized)
- Agilent ZORBAX Eclipse Plus Phenyl-Hexyl (p/n 821125-938)
- Agilent PLRP-S (p/n 5982-1271)
- Agilent ZORBAX SB-Aq (p/n 821125-933)

All the cartridges had specifications of 2.1 × 12.5 mm, 5 µm particle size, except Bond Elut Plexa PCX and PLRP-S, which had particle sizes of 15 to 20 µm.

The online SPE LC system featured an Agilent InfinityLab Quick Change 2-position/10-port switching valve with two trapping columns housed in the column compartment controlled by a valve driver. Initially, the valve position was set to 1 → 2, the first trapping cartridge (SPE1) was in loading mode, and the second SPE cartridge (SPE2) was set to elution mode (Figure 1A). The quaternary LC pump, which was connected to the autosampler, flushed the sample to SPE1 for enrichment of the analytes. SPE2, containing enriched analytes through the previous run, was eluted in front of the analytical column by the binary LC pump. After switching to the 1 → 10 position, the binary pump delivered the gradient mobile phase to elute the enriched analytes from SPE1 in backflush mode and separated the analytes in the analytical column (Figure 1B). At the same time, SPE2 was cleaned and reconditioned by the quaternary pump to prepare for loading in the next run. This setup allowed alternate enrichment of the analytes on SPE1 and SPE2, increasing the throughput of analysis.

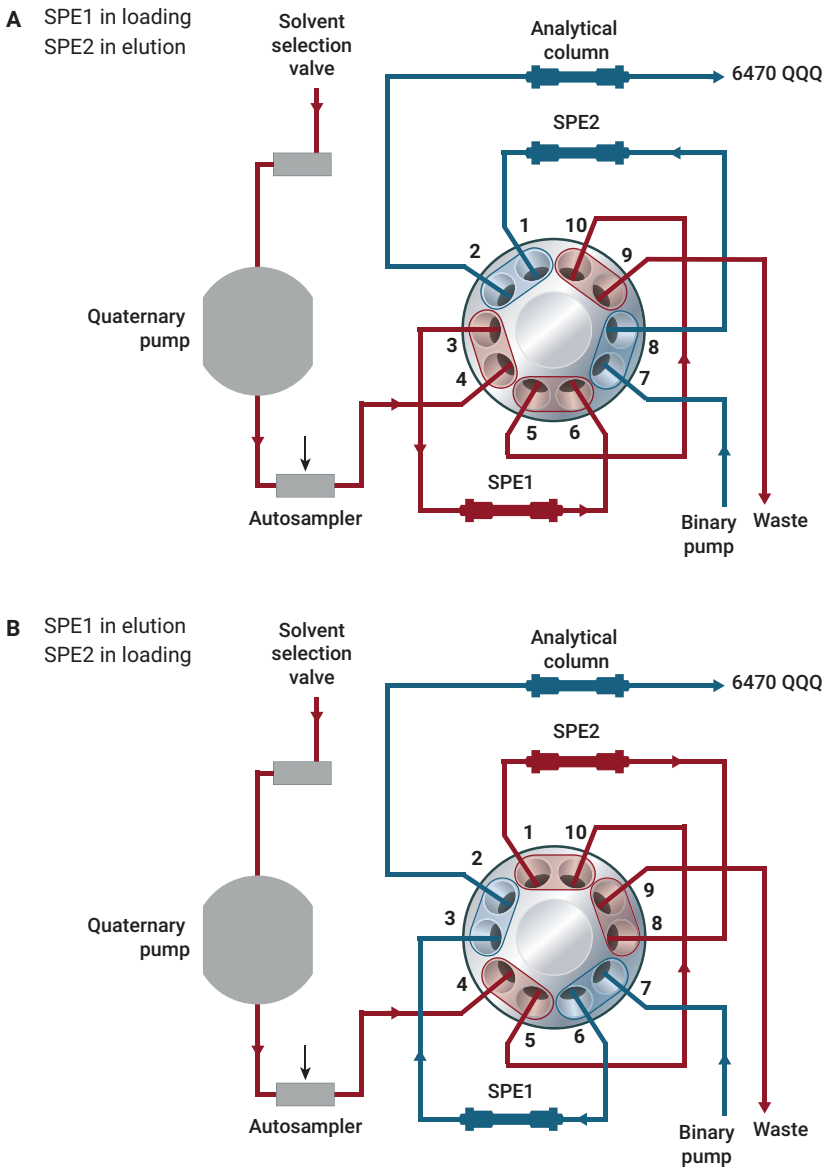

Figure 1. Valve positions for alternating loading and elution of the two SPE cartridges.

Online SPE conditions

Parameter	Value
Enrichment Pump	Agilent 1260 Infinity II quaternary pump
Autosampler	Agilent 1260 Infinity II multisampler
SPE Cartridge	Agilent PLRP-S, 2.1 × 12.5 mm, 20 µm (p/n 5982-1271)
Sample pH	7
Injection Volume	1.8 mL
Loading/Washing Solvent	Methanol/0.05% formic acid aqueous solution (2:98, pH 4)
Maximum Pressure Limit	400 bar
Loading/Washing Speed	1 mL/min
Cleaning Solvent	Methanol/acetonitrile/isopropanol (1:1:1)
Cleaning Speed	0.6 mL/min
Valve	Agilent InfinityLab Quick Change 2-position/10-port valve
Valve Switch Time	4 min
Delay Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 3.5 µm (p/n 959943-902) (between the quaternary pump and the autosampler for removing the PFCs interference from the system)
Gradient Profile	0 to 5 min: 98% water containing 0.05% formic acid (A), 2% pure methanol (B), flow rate: 1 mL/min 5 to 5.01 min: change to 100% cleaning solvent; flow rate:0.6 mL/min

LC separation conditions

Parameter	Value
LC	Agilent 1260 Infinity II LC
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)
Delay Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 3.5 µm (p/n 959943-902) (between the mixture of the binary pump and the Quick Exchange valve for removing the PFCs interference from the system)
Mobile Phase	A) 0.05% Formic acid aqueous solution B) Acetonitrile
Column Temperature	30 °C
Flow Rate	0.3 mL/min
Maximum Pressure Limit	600 bar
Gradient Profile	0 to 4 min: 5% acetonitrile 4 to 9 min: 5 to 40% acetonitrile 9 to 16 min: 40 to 100% acetonitrile 16 to 21 min: 100% acetonitrile
Post Time	9 min

MS/MS conditions

Parameter	Value
MS	Agilent 6470A triple quadrupole LC/MS
Ionization Mode	Positive and negative ESI
Capillary Voltage	3500 V (Positive/Negative)
Nozzle Voltage	500 V (Positive/Negative)
Nebulizer Gas (N_2) Pressure	45 psi
Drying Gas (N_2) Temperature	300 °C
Drying Gas Flow Rate	7 L/min
Sheath Gas (N_2) Temperature	350 °C
Sheath Gas Flow Rate	7 L/min
Scanning Mode	Dynamic MRM
Cell Accelerator Voltage	4 V
MRM Parameters	Obtained by Agilent MassHunter Optimizer, listed in appendix (Table 1)
Software	Agilent MassHunter Acquisition/Qualitative Analysis/Quantitative Analysis software packages

Results and discussion

Selection of SPE cartridges for optimal recovery

Six types of online SPE cartridges were evaluated for their suitability for use in the SPE method, and samples at three pH levels (3, 7, and 10) were used in testing. As the target analytes vary significantly in terms of physicochemical properties, a total of 20 representative analytes from each group were selected for demonstrating the recovery performance for each cartridge.

Results show that PLRP-S offers the best recovery of most analytes, even with varying pH levels; for this reason, PLRP-S was selected for use in the method (Figure 2).

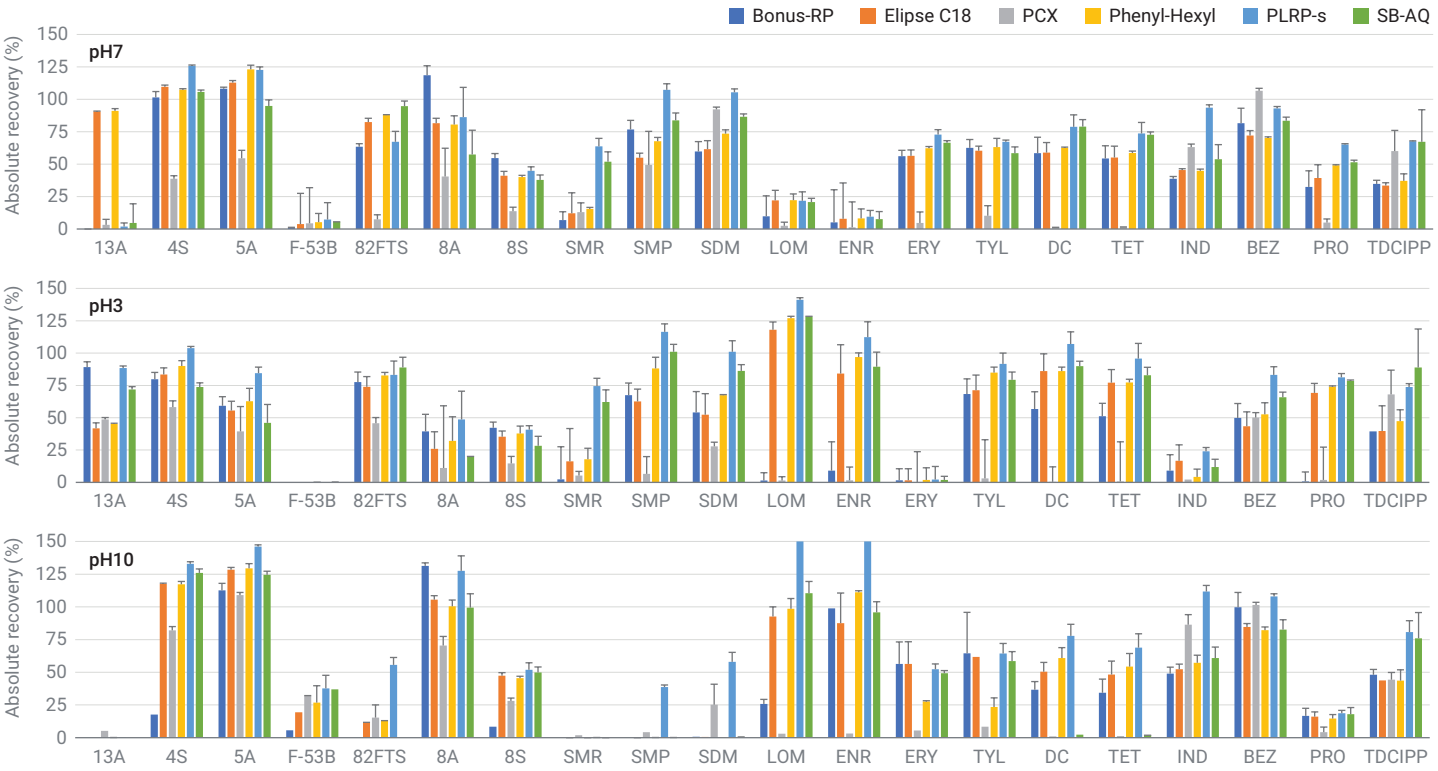

Figure 2. Absolute recovery for the representative analytes with six different SPE cartridges at three pHs.

Solvent selection for both SPE loading/washing and analytical column separation

To achieve better method performance for most of the analytes, multiple parameters for both LC separation and online SPE extraction were tested, respectively, using representative analytes from each group. An orthogonal design of experiments was applied for the mobile phase additives and the sample pH in front of the analytical column. This was done using UHPLC system without online SPE configuration. It was found that 0.05% formic acid as aqueous additive and a sample pH of 4 provide the overall best response for all representative analytes (Figure 3A). For this reason, these conditions were selected for the analytical column.

Using online SPE configuration, the loading/washing solvent and the sample pH before loading onto the SPE cartridge were also investigated. As shown in Figure 3B, it was found that 0.05% formic acid solution as loading/washing solvent and pH 10 for the sample solution provided a better response than all other combinations (purple line in Figure 3B). The second best was the combination of 0.05% formic acid as loading/washing solvent and pH 7 for the sample solution (red line in Figure 3B). This result was consistent with Figure 2, in which a majority of analytes showed acceptable absolute recovery at pH7 using a PLRP-S cartridge. Sample pH at 7 can provide sufficient intensity for all the representative analytes and is more convenient for practical operation. Therefore, 0.05% formic acid solution was selected as loading/washing solution for the SPE column, and pH 7 was selected for sample pH before loading. As the washing solution brings the sample enriched on the SPE cartridge to the front of the analytical column, the optimal loading/washing solution aligns with the ideal requirements for sample pH in front of the analytical column.

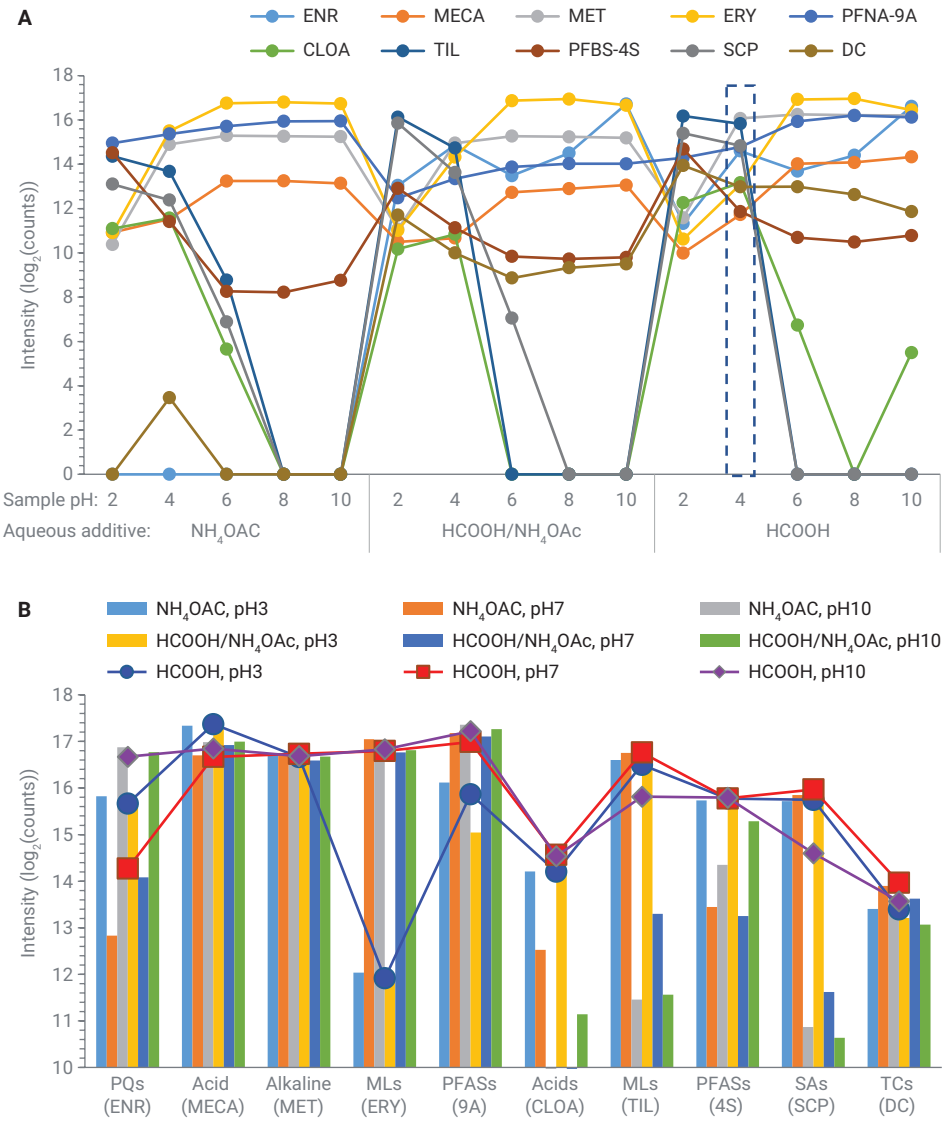

Figure 3. Optimization of multiple parameters for LC separation conditions and online SPE conditions. (A) Combined effect of mobile phase additive (5 mM NH_4OAc, 0.05% HCOOH, and 0.05% HCOOH/5 mM NH_4OAc) and sample pH (2 to 10) on the representative analytes' response; (B) the combined effect of aqueous loading/washing solvent additive (5 mM NH_4OAc, 0.05% HCOOH, and 0.05% HCOOH/5 mM NH_4OAc) and sample pH (3, 7, and 10) on the representative analytes' response. **Note:** series labels in B are for loading/washing solvent-sample pH combinations.

Other parameters, including the organic solvent, the flow rate of enrichment pump, and the valve switching time (the latter two related to washing volume), were also optimized to ensure better recovery of the analytes. The optimized conditions were shown in the experimental section.

Separation of analytes under optimized conditions

Under the optimized online SPE conditions and the LC separation, 87 analytes were eluted off the analytical column with retention times ranging from 7 to 20 minutes. Among the 87 analytes, there were three pairs of isomers and seven pairs of isobaric analytes. Though the retention times for some pairs are very close, each such pair has characteristic MRM transitions, enabling their individual identification. The typical overlapped MRM chromatograms are shown in Figure 4.

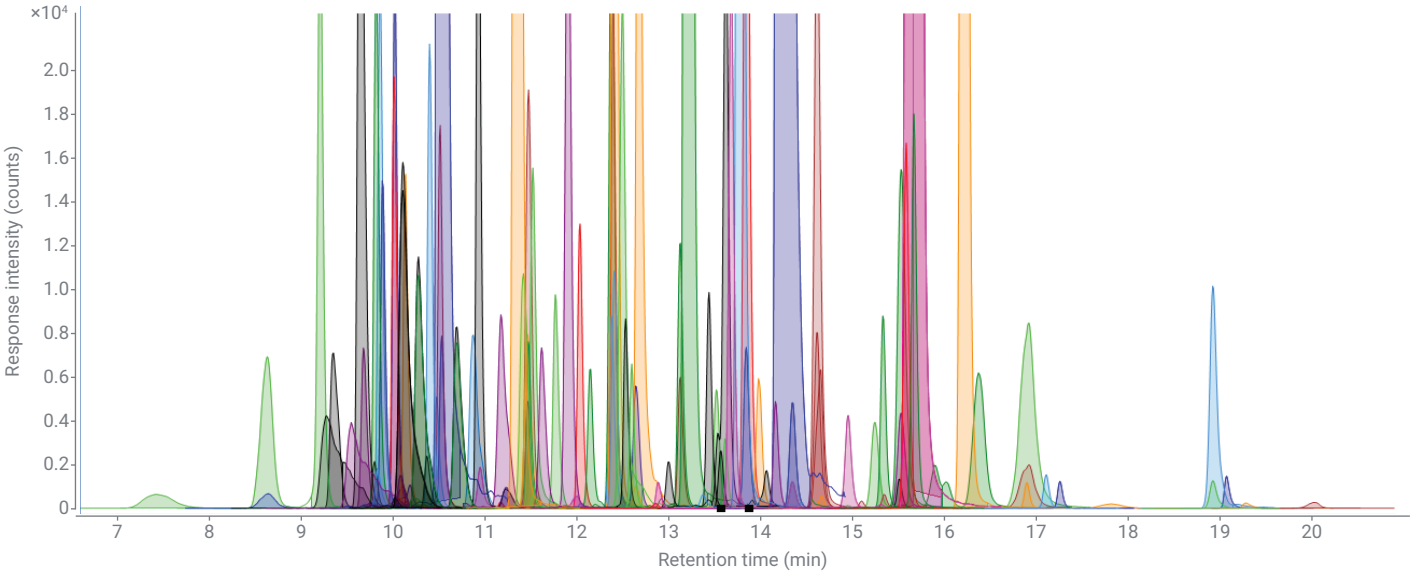

Figure 4. Overlapped MRM chromatograms for all 87 analytes in pure water at 100 ng/L.

Method performance evaluation

To minimize the matrix effect on the quantitation accuracy, the isotopically labeled internal standards dilution method using 37 IS compounds mixture solution at a level of 25 ng/L was selected for quantitation. The linearity of the method was evaluated in pure water spiked with all target analytes with concentrations ranging from 1.0 to 200 ng/L. As shown in Figure 5A, 85 out of 87 analytes had linear regression coefficients (R^2) higher than 0.98, indicating that these analytes can be screened quantitatively. The exceptions were diclofenac acid (DLOA) with R^2 of 0.9570 and N,N-diethyl-meta-toluamide (DEET) with R^2 of 0.9772. The nonideal linearity was due to the high background for both analytes in pure water, so these could only be qualitatively screened.

The sensitivity of the method was evaluated in three environmental water matrices, including drinking water (DW), surface water (SW), and wastewater effluent (WWE). As shown in Figure 5B, the limits of quantitation (LOQ) in DW are all below 10 ng/L for all 85 analytes that could be quantitatively measured (excluding DLOA and DEET); for SW, 7 out of 85 analytes have LOQs between 10 and 20 ng/L, and the remaining are below 10 ng/L; for WWE, up to 15 analytes have LOQs between 10 and 20 ng/L, with the remaining analytes having LOQs lower than 10 ng/L (Figure 5B). Among these analytes, up to 76%, 65%, and 48% of the 85 analytes exhibited LOQs of ≤5 ng/L in DW, SW, and WWE, respectively. These results suggest that the method is sensitive for enviornmental testing.

The method accuracy and precision were also evaluated by measuring the response of spiking samples at levels of 25 and 100 ng/L for each analyte in three types of water matrices. For all three water matrices, more than 87% of analytes exhibit recovery values within 60 to 130% (Figure 5C), and the corresponding RSDs are within 20%. The analytes with lower recovery are mainly long-chain PFASs, PFRs, and several polar compounds. This demonstrates that the method is also accurate and reliable for quantitating the majority of these compounds.

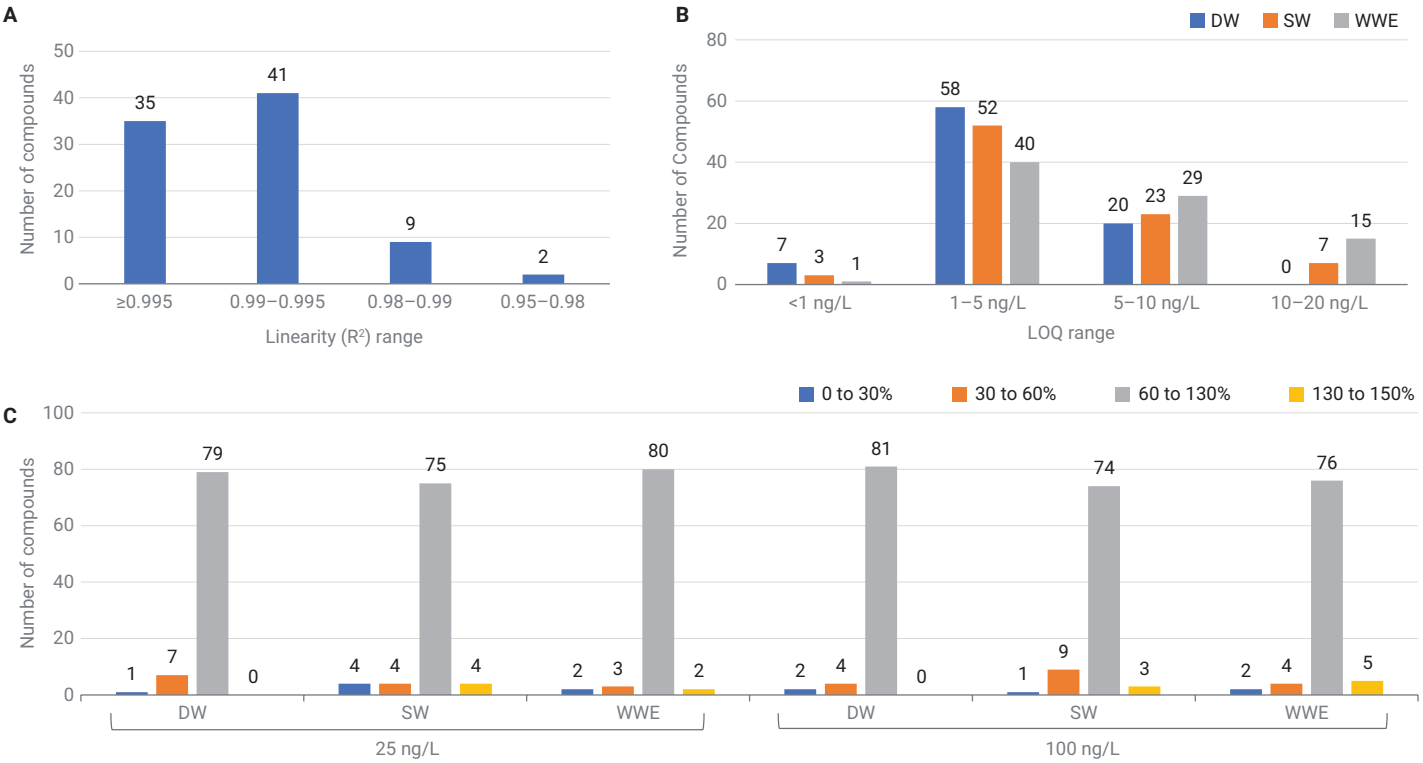

Figure 5. Method performance evaluation in terms of the following parameters. (A) Linearity from 1 to 200 ng/L in pure water; (B) limit of quantitation in DW, SW, and WWE; (C) recovery at spiking level of 25 and 100 ng/L (n = 3) in DW, SW, and WWE.

Conclusion

An automated online SPE method coupled with UHPLC/MS/MS has been described for the simultaneous determination of 87 EOCs including 22 PFASs, 58 PPCPs, and seven PFRs in environmental water matrices. The PLRP-S SPE cartridge was selected for analyte enrichment due to its excellent retaining capability and recovery for a wide spectrum of analytes under the optimized conditions. Multiple parameters for LC separation and online SPE enrichment have been evaluated to achieve the overall best performance for all the analytes. The optimized method has very good linearity, a very low limit of quantitation, and satisfactory recovery and precision in the three environmental matrices tested for a majority of analytes included. These results suggest that the method can reliably be applied in real water sample screening. The optimized strategy can be extended to online SPE analysis of other groups of organic contaminants in water.

References

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Appendix

Table 1. The compounds information, their retention times, and the data acquisition parameters for multiple reaction monitoring using LC/QQQ.

Compound	Abbreviation	RT (min)	Precursor Ion (*m/z*)	Fragmentor (V)	Product ions (*m/z*) Quant./Qual.	CE (V) Quant./Qual.	IS	Polarity
Perfluorobutanoic acid	4A	10.09	212.9	60	168.9	8	$^{13}C_4$-4A	Neg
$^{13}C_4$-Perfluorobutanoic acid	$^{13}C_4$-4A	10.09	217.0	60	171.9	5	IS	Neg
Perfluoropentanoic acid	5A	11.55	262.9	61	218.9	5	$^{13}C_5$-5A	Neg
$^{13}C_5$-Perfluoropentanoic acid	$^{13}C_5$-5A	11.55	268.0	60	222.9	5	IS	Neg
Perfluorohexanoic acid	6A	12.36	312.9	60	268.9/119.0	5/21	$^{13}C_2$-6A	Neg
$^{13}C_2$-Perfluorohexanoic acid	$^{13}C_2$-6A	12.36	315.0	60	269.9	5	IS	Neg
Perfluoroheptanoic acid	7A	13.11	362.8	60	168.9/319.0	17/5	$^{13}C_4$-7A	Neg
$^{13}C_4$-Perfluoroheptanoic acid	$^{13}C_4$-7A	13.11	367.1	60	321.9	5	IS	Neg
Perfluorooctanoate	8A	13.82	413.1	65	368.8/168.9	5/17	$^{13}C_4$-8A	Neg
$^{13}C_4$-Perfluorooctanoate	$^{13}C_4$-8A	13.82	417.1	50	371.8	5	IS	Neg
Perfluorononanoic acid	9A	14.60	462.9	60	418.8/218.9	5/17	$^{13}C_5$-9A	Neg
$^{13}C_5$-Perfluorononanoic acid	$^{13}C_5$-9A	14.60	468.1	60	422.9	5	IS	Neg
Perfluorodecanoic acid	10A	15.51	513.1	50	468.8/268.9	9/17	$^{13}C_2$-10A	Neg
$^{13}C_2$-Perfluorodecanoic acid	$^{13}C_2$-10A	15.51	515.1	60	469.9	5	IS	Neg
Perfluoroundecanoic acid	11A	16.90	563.1	88	518.9/493.2	9/29	$^{13}C_2$-11A	Neg
$^{13}C_2$-Perfluoroundecanoic acid	$^{13}C_2$-11A	16.90	565.0	100	519.8	8	IS	Neg
Perfluorododecanoic acid	12A	18.90	613.1	103	568.9/169	9/15	$^{13}C_2$-12A	Neg
$^{13}C_2$-Perfluorododecanoic acid	$^{13}C_2$-12A	18.90	615.0	120	519.8	8	IS	Neg
Perfluorotridecanoic acid	13A	19.27	663.1	93	618.9/168.9	9/29	$^{13}C_2$-12A	Neg
Perfluorobutanesulfonate	4S	12.60	298.8	128	80.0/98.9	37/33	$^{13}C_3$-4S	Neg
$^{13}C_3$-Perfluorobutanesulfonate	$^{13}C_3$-4S	12.60	301.8	100	79.9	32	IS	Neg
Perfluoropentanesulfonate	5S	13.47	348.8	136	80.0/98.9	45/37	$^{13}C_3$-6S	Neg
Perfluorohexanesulfonate	6S	14.30	398.8	161	80.0/98.9	45/41	$^{13}C_3$-6S	Neg
$^{13}C_3$-Perfluorohexanesulfonate	$^{13}C_3$-6S	14.30	401.8	156	80.0/98.9	49/41	IS	Neg
Perfluoroheptanesulfonate	7S	15.19	448.8	166	80.0/98.9	49/45	$^{13}C_4$-8A	Neg
Perfluorooctanesulfonate	8S	16.30	498.8	172	80.0/98.9	50/45	$^{13}C_4$-8A	Neg
Perfluorodecanesulfonate	10S	19.05	598.8	196	80.0/98.9	61/53	$^{13}C_2$-11A	Neg
3,3,4,4,5,5,6,6,6-Nonafluoro-1-hexanesulfonic acid	4:2FTS	12.02	327.1	123	306.9/81.0	21/29	$^{13}C_3$-4S	Neg
1H,1H,2H,2H-Perfluorooctanesulfonic acid	6:2FTS	13.42	427.2	128	406.9/81.0	25/37	$^{13}C_4$-7A	Neg
1H,1H,2H,2H-Perfluorodecanesulfonic acid	8:2FTS	13.82	527.2	171	506.8/81.0	29/41	$^{13}C_3$-6S	Neg
N-ethylperfluorooctanesulfonamide	EtFOSA	17.22	526.2	128	168.9/218.9	29/25	$^{13}C_2$-11A	Neg
N-methylperfluorooctanesulfonamide (MeFOSA)	MeFOSA	16.87	512.2	128	168.9/218.9	29/25	$^{13}C_2$-11A	Neg
Chlorinated polyfluorinated ether sulfonate	62F-53B	17.68	530.7	136	83.0	29	$^{13}C_2$-11A	Neg
Tilmicosin	TIL	11.16	869.3	260	174.3/696.9	50/46	D_3-TIL	Pos
D_3-Tilmicosin	D_3-TIL	11.16	872.6	270	177.0/696.4	50/46	IS	Pos
Clarithromycin	CTM	12.53	748.4	165	158.0/590.3	29/21	D_7-ROX	Pos
Erythromycin	ERY	11.78	734.4	155	158.0/576.3	29/17	D_7-ROX	Pos
Roxithromycin	ROX	12.60	837.4	165	679.3/558.3	21/25	D_7-ROX	Pos
D_7-Roxithromycin	D_7-ROX	12.60	844.4	170	686.4/558.3	21/25	IS	Pos
Tylosin	TYL	11.98	916.4	240	772.3	33	D_7-ROX	Pos
Clindamycin	CLD	10.93	425.1	145	126.1/377.1	29/21	D_3-CLD	Pos
D_3-Clindamycin	D_3-CLD	10.93	428.1	150	129.1/380.1	29/21	IS	Pos
Lincomycin	LCM	9.22	407.1	148	126.1/359.1	33/21	D_3-LCM	Pos

Compound	Abbreviation	RT (min)	Precursor Ion (*m/z*)	Fragmentor (V)	Product ions (*m/z*) Quant./Qual.	CE (V) Quant./Qual.	IS	Polarity
D_3-Lincomycin	D_3-LCM	9.22	410.1	150	129.1	33	IS	Pos
Flumequine	FLU	12.66	262.0	120	244.0/201.9	21/37	$^{13}C_3$-FLU	Pos
$^{13}C_3$-Flumequine	$^{13}C_3$-FLU	12.66	265.0	120	247.0/204.9	17/37	IS	Pos
Oxolinic acid	OXA	11.51	262.0	100	244.0/215.9	17/33	$^{13}C_3$-FLU	Pos
Nalidixic acid	NA	12.48	233.0	89	215.0/186.9	13/29	D_5-DA	Pos
D_5-Nalidixic acid	D_5-DA	12.48	238.0	106	220.0/188.0	13/29	IS	Pos
Difloxacin	DIF	10.53	400.0	140	356.1/299.0	21/33	D_5-LOM	Pos
Danofloxacn	DAN	10.02	358.1	135	340.1/82.1	25/49	D_5-LOM	Pos
Marbofloxacin	MAR	9.69	363.0	130	320.0/345.1	13/21	D_5-LOM	Pos
Sarafloxacin	SAR	10.47	386.0	130	368.1/342.1	25/21	D_5-LOM	Pos
Lomefloxacin	LOM	10.02	352.0	130	265.0/308.1	25/17	D_5-LOM	Pos
D_5-Lomefloxacin	D_5-LOM	10.02	357.1	135	270.1/313.1	25/17	IS	Pos
Ciprofloxacin	CIP	9.89	332.1	130	314.1/288.0	21/41	D_8-CIP	Pos
D_8- Ciprofloxacin	D_8-CIP	10.33	340.1	134	322.1/296.0	25/29	IS	Pos
Sparfloxacin	SPA	10.51	393.1	140	349.1/292.1	21/29	D_5-LOM	Pos
Pefloxaxin	PEF	9.87	334.1	125	316.1/290.1	25/17	D_5-LOM	Pos
Enrofloxacin	ENR	10.14	360.1	130	342.1/316.1	25/21	D_8-OFL	Pos
Norfloxacin	NOR	9.80	320.0	130	302.1/276.1	25/17	D_8-OFL	Pos
Ofloxacin	OFL	9.82	362.0	140	318.1/261.0	21/29	D_8-OFL	Pos
D_8-Ofloxacin	D_8-OFL	9.82	370.1	135	326.1/265.0	21/33	IS	Pos
Sulfachloropyridazine	SCP	10.87	285.0	105	155.9/92.0	13/29	$^{13}C_6$-SCP	Pos
$^{13}C_6$-Sulfachloropyridazine	$^{13}C_6$-SCP	10.87	291.0	100	161.9/98.0	13/33	IS	Pos
Sulfadiazine	SD	8.81	250.9	100	155.9/92.1	13/33	D_4-SD	Pos
D4-Sulfadiazine	D_4-SD	8.81	254.9	105	160.0/96.0	17/33	IS	Pos
Sulfamethoxazole	SMX	11.17	254.0	104	92.0/155.9	29/17	$^{13}C_6$-SIX	Pos
Sulfamonomethoxine	SMM	10.70	281.0	115	155.9/92.0	17/37	D_4-SMM	Pos
D_4-Sulfamonomethoxine	D_4-SMM	10.70	285.0	120	155.9/96.1	17/33	IS	Pos
Sulfathiazole	STZ	9.27	255.9	105	155.9/92.0	13/29	D_4-STZ	Pos
D_4-Sulfathiazole	D_4-STZ	9.27	259.8	105	159.9/96.1	17/33	IS	Neg
Sulfamerazine	SMR	9.55	262.9	115	92.0/155.9	33/17	D_4-STZ	Pos
Sulfisoxazole	SIX	11.41	267.9	105	155.9/92.0	13/33	$^{13}C_6$-SIX	Pos
$^{13}C_6$-Sulfisoxazole	$^{13}C_6$-SIX	11.41	274.0	115	161.9/98.0	13/29	IS	Pos
Sulfisomidin	SAAM	10.12	279.0	125	185.9/124.0	17/25	$^{13}C_6$-SCP	Pos
Sulfamethoxypyridazine	SMP	10.28	280.9	115	155.9/92.0	17/33	D_3-SMP	Pos
D_3-Sulfamethoxypyridazine	D_3-SMP	10.28	284.0	110	155.9/92.0	17/33	IS	Pos
Sulfamethazine	SMZ	10.12	278.9	120	185.9/92.0	17/33	D_3-SMP	Pos
Sulfadimethoxine	SDM	11.89	310.9	115	156.0/92.0	21/41	D_4-SDM	Pos
D_4-Sulfadimethoxine	D_4-SDM	11.89	315.0	125	156.0/96.0	25/37	IS	Pos
Trimethoprim	TMP	9.65	291.1	145	230.0/264.0	25/29	D_3-TMP	Pos
D_3-Trimethoprim	D_3-TMP	9.65	294.1	140	230.0/264.0	25/29	IS	Pos
Doxycycline	DC	10.14	445.0	130	154.0/428.5	13/18	D_3-DC	Pos
D_3-Doxycycline	D_3-DC	11.14	448.1	130	430.9/202.6	17/53	IS	Pos
Methacycline	MEC	10.14	443.0	110	426.3/201.0	15/40	D_3-DC	Pos
Oxytetracycoine	OTC	9.84	461.1	115	426.1/443.0	19/10	D_6-TET	Pos
Chlorotetracycline	CTC	10.37	479.0	135	462.0/444.0	14/22	D_6-TET	Pos
Tetracycline	TET	10.14	445.1	120	154.0/410.1	29/21	D_6-TET	Pos

Compound	Abbreviation	RT (min)	Precursor Ion (*m/z*)	Fragmentor (V)	Product ions (*m/z*) Quant./Qual.	CE (V) Quant./Qual.	IS	Polarity
D_6-Tetracycline	D_6-TET	10.14	451.3	115	416.1/160.0	21/29	IS	Pos
Florfenicol	FF	11.20	357.8	95	337.9/185.0	5/17	D_3-FF	Neg
D_3-Florfenicol	D_3-FF	11.20	360.8	95	340.9/188.0	8/20	IS	Neg
Chloramphenicol	CAP	11.52	320.9	110	256.9/152.0	17/9	D_7-PEN-G	Neg
Thiamphenicol	THI	11.22	355.8	110	185.0/291.9	21/9	D_7-PEN-G	Neg
Indomethacine	IND	14.65	356.0	85	312.0/297.0	5/17	D_4-IND	Neg
D_4-Indomethacine	D_4-IND	14.65	360.1	70	316.0/301.0	5/17	IS	Neg
Diclofenac acid	DLOA	14.61	293.9	76	249.9/213.9	9/21	D_4-DLOA	Neg
D_4-Diclofenac acid	D_4-DLOA	14.61	297.9	79	253.9/217.0	9/21	IS	Neg
Mefenamic acid	MECA	15.32	240.0	105	196.0/192.0	17/29	D_4-BEZ	Neg
Phenacetin	PHE	11.46	180.0	136	110.0/138.0	21/17	D_7-PEN-G	Neg
D_7-Penicilline G	D_7-PEN-G	12.10	342.1	170	218.0/98.0	13/61	IS	Pos
Gemfibrozil	GEM	15.50	249.0	76	121.0/113.0	21/5	D_4-CLOA	Neg
Bezafibrate	BEZ	13.60	360.0	103	274.0/153.9	17/33	D_4-BEZ	Neg
D_4-Bezafibrate	D_4-BEZ	13.60	364.0	105	278.0/158.0	17/33	IS	Neg
Clofibric acid	CLOA	13.55	212.9	73	126.9/85.0	17/5	D_4-CLOA	Neg
D_4-Clofibric acid	D_4-CLOA	13.55	216.9	78	131.0/85.0	17/5	IS	Neg
Metoprolol	MET	10.40	268.1	127	74.1/116.0	25/21	D_7-PRO	Pos
Propraolol	PRO	11.46	260.1	122	116.0/56.1	17/33	D_7-PRO	Pos
D_7-Propraolol	D_7-PRO	11.46	267.1	125	123.1/79.1	21/25	IS	Pos
Sulpiride	SUL	8.63	342.1	140	112.0/213.9	29/37	IS	Pos
Tiamulin	TIA	12.38	494.3	137	192.0/119.0	21/45	D_3-LCM	Pos
Carbamazepin	CMP	12.40	237.0	125	194.0/178.9	21/41	D_{10}-CMP	Pos
D_{10}-Carbamazepin	D_{10}-CMP	12.40	247.1	125	204.1/202.0	25/45	IS	Pos
Caffeine	CAF	9.36	194.9	120	138.0/42.2	21/45	––	Pos
N,N-diethyl-meta-toluamide	DEET	13.20	192.1	120	119.0/91.0	17/37	D_6-DEET	Pos
D_6-N,N-diethyl-meta-toluamide	D_6-DEET	13.20	198.1	135	119.0/91.0	21/37	IS	Pos
Penicilline G	PEN-G	12.13	335.1	165	216.9/91.0	10/58	D_7-PEN-G	Pos
Olaquindox	OLA	7.42	264.0	115	143.0/202.9	37/17	D_4-OLA	Pos
D_4-Olaquindox	D_4-OLA	7.42	268.1	125	143.0/216.0	41/25	IS	Pos
Monensin	MON	19.99	693.4	230	675.4/479.3	41/61	D_{27}-TNBP	Pos
Tris (2-butoxyethyl) phosphate	TBOEP	16.21	399.2	122	299.1/199.0	13/13	D_{21}-TPP	Pos
Tris (1,3-dichloro-2-propyl) phosphate	TDCIPP	15.33	430.8	143	98.9/208.9	33/17	D_{15}-DCIPP	Pos
D_{15}-Tris (1,3-dichloro-2-propyl) phosphate	D_{15}-DCIPP	15.33	446.0	143	102.0	33	IS	Pos
Triethyl phosphate	TEP	11.35	183.0	93	98.9/81.0	21/50	D_{15}-TEP	Pos
D_{15}-Triethyl phosphate	D_{15}-TEP	11.35	198.0	93	102.0/82.0	21/50	IS	Pos
Tri-isobutylphosphate	TIBP	15.71	267.1	90	98.9/211.0	17/5	D_{27}-TNBP	Pos
Trimethylolpropane phosphate	TMPP	17.11	369.0	170	165.0/91.1	49/45	D_{27}-TNBP	Pos
Tri-*n*-butyl phosphate	TNBP	15.61	267.1	94	99.0/211.0	21/5	D_{27}-TNBP	Pos
D_{27}- Tri-*n*-butyl phosphate	D_{27}-TNBP	15.61	294.0	94	166.0/102.0	9/21	IS	Pos
Tri-*n*-propyl phosphate	TPP	13.76	225.0	85	99.0/183.0	17/5	D_{21}-TPP	Pos
D_{21}- Tri-*n*-propyl phosphate	D_{21}-TPP	13.76	246.0	85	150.0/102.0	9/21	IS	Pos

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