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Analysis of PFAS in drinking water by large volume direct injection following the EU Drinking Water Directive 2020/2184

Introduction

Per- and polyfluoroalkyl substances (PFAS) represent a diverse group of synthetic fluorinated organic compounds that have been produced and widely used in industrial applications and consumer products since the 1940s. Common applications where PFAS are used include surfactants, fire-fighting foams, nonstick cookware coatings, lubricants, and coatings for food packaging. Because of their wide use, testing of various sources including drinking water is of great importance to mitigate potential risks to our health and the environment.[1] Due to the detrimental impacts PFAS have on human health and the fact that they persist and spread throughout the environment, it is necessary to monitor extremely low levels of these contaminants.[3] To accurately detect and quantify these substances, a highly sensitive method must be utilized.

This application note reports on the LC/MS/MS analysis of PFAS compounds according to the European Union Water Framework Directive 2020/2184. The purpose of this application note is to demonstrate how the PerkinElmer QSight® 420 LC/MS/MS System using a large volume direct injection can determine and quantify the latest 20 PFAS compounds (Figure 1) added in the Directive 2020/2184.[2] The method shows a comprehensive solution including details related to LC and MS methods. Moreover, it provides details on the required steps to prevent any possible contamination arising, by using PerkinElmer PTFE-free injection kit.
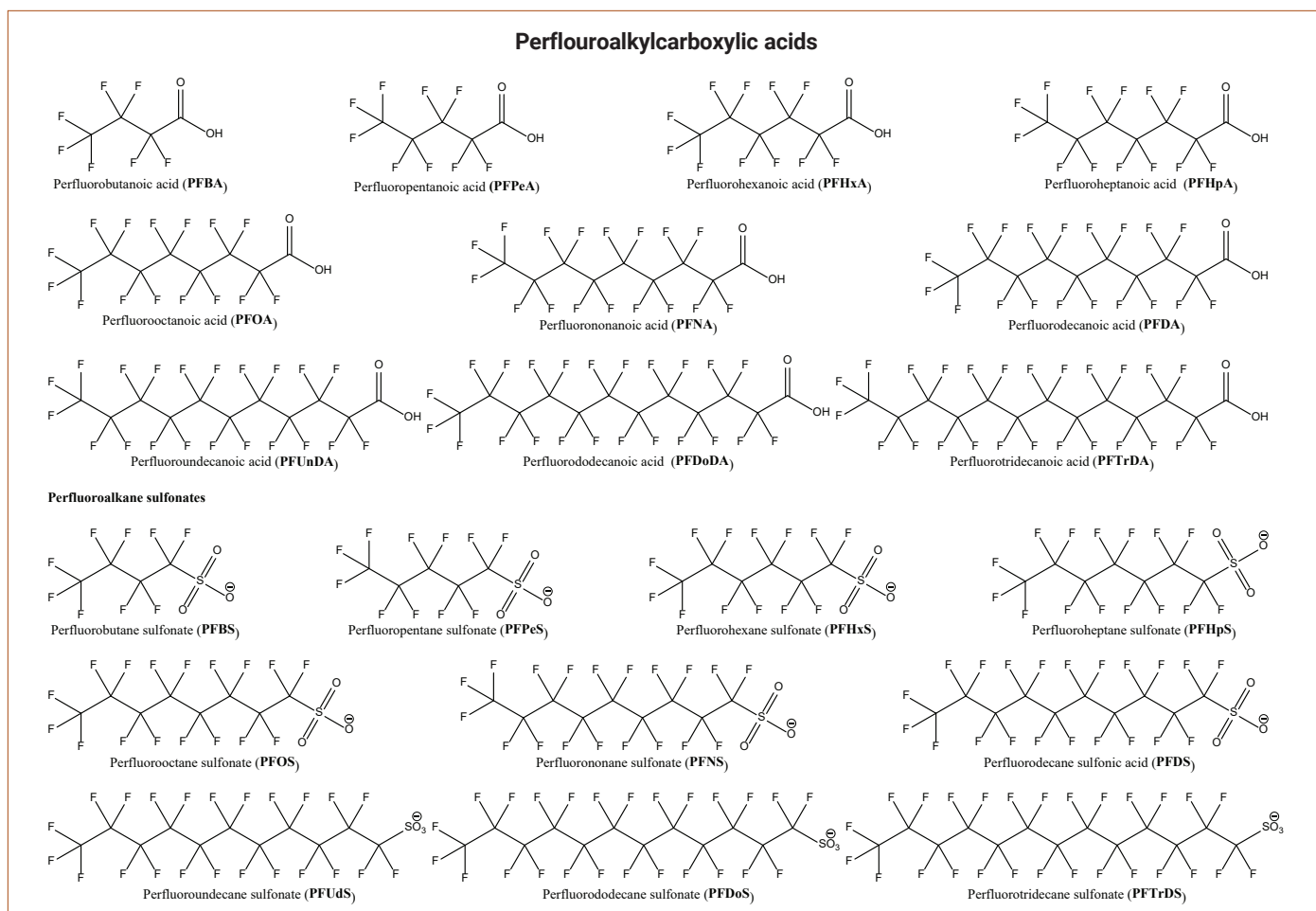
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Figure 1: Chemical structures of the 20 PFAS covered in the latest European Union the Water Framework Directive 2020/2184: 2020.

Experimental

Hardware and Software

The chromatographic separation and subsequent detection were carried out using the PerkinElmer LX50 UHPLC System and the QSight 420 LC/MS/MS System, respectively. The LX50 Autosampler was configured with polyether ether ketone (PEEK) tubing to reduce or eliminate contamination from PFAS compounds and allow for low detections limits of PFAS chemicals. Moreover, a PEEK needle was installed in the autosampler. All instrument control, analysis and data processing were performed using the PerkinElmer Simplicity™ 3Q Software. Chemical structures were drawn using PerkinElmer ChemDraw® Professional software suite.

Solvents, Standards, and Sample Preparation

LC/MS grade methanol, water and formic acid were used for the analysis and were obtained from Carl Roth (Karlsruhe, Germany). Authentic standards were purchased from Campro Scientific GmbH, Berlin, Germany.

For sample preparation, the drinking water samples were obtained from local sources in the Frankfurt am Main area, in Germany. The commercially available bottled mineral water was also purchased in Germany. The samples were extracted with methanol in 50/50 v/v ratio and 0.1% formic acid was added. The same was for the calibration standards, which were diluted with 50/50 methanol: water, with 0.1% formic acid. The stock solutions were kept in the refrigerator until usage and used to make calibration standards and spiking samples.

Regarding the measurements, everyday a freshly pipetted standard curve along with spiked drinking water and mineral water were prepared. The calibration standards and samples were injected on three separate days, while the spiked samples were injected five consecutive times to demonstrate reproducibility and to determine the uncertainty.

Method Parameters

The LC method is shown in Table 1. MS parameters are presented in Table 2 and 3 where the collision energies (CE), entrance voltages (EV) and collision cell lens 2 (CCL2) for each analyte were detected and optimized by direct infusion of the standards for each respective MRM transition. Drying and nebulizer gas flow and temperature settings were optimized by flow injection analysis (FIA) for the MS source conditions.

PerkinElmer QSight LC/MS/MS System.

Table 1: LC parameters.

Step	Time (min)	Flow Rate (mL/min)	% A	% B	Curve
1	0.0	0.8	95	5	
2	3.0	0.8	95	5	Linear
3	4.0	0.8	55	45	Linear
4	10.5	0.8	2	98	Linear
5	14.0	0.8	2	98	Linear
6	14.1	0.8	95	5	Linear
7	18.0	0.8	95	5	Linear
Mobile Phase A	10 mM Ammonium acetate				
Mobile Phase B	Methanol				
Column Oven Temperature	40 °C				
Auto Sampler Temperature	8 °C				
Injection Volume	100 µL				
Column	Brownlee SPP C18 100x4.6 mm, 2.7 µm (N9308416)				
Delay Column	Brownlee SPP C18 50x3 mm, 2.7 µm (PN: N9308408)				

Table 2: MS MRM parameters.

Compound	Component type	RT (min)	Q1	Q2	CC	EV	CCL2
Perfluorobutanoic acid (PFBA)	Quantifier	6.9	213	168.8	14	-3	80
	Qualifier		-	-	-	-	-
	ITSD		217	171.9	14	-3	52
Perfluoropentanoic acid (PFPeA)	Quantifier	8.0	263	219	12	-9	72
	Qualifier		-	-	-	-	-
	ITSD		268	223	12	-8	64
Perfluorobutane sulfonate (PFBS)	Quantifier	8.1	299	80	68	-48	78
	Qualifier		299	99	44	-45	66
	ITSD		301.9	80.1	47	-9	80
Perfluorohexanoic acid (PFHxA)	Quantifier	8.8	313	269	12	-10	72
	Qualifier		313	119	34	-10	100
	ITSD		318	273	14	-14	84
Perfluoropentane sulfonate (PFPeS)	Quantifier	8.8	349	80	80	-51	94
	Qualifier		349	99	43	-60	82
	ITSD*		-	-	-	-	-
Perfluoroheptanoic acid (PFHpA)	Quantifier	9.4	363	319	15	-1	92
	Qualifier		363	169	22	-8	84
	ITSD		367.1	322	15	-3	88
Perfluorohexane sulfonate (PFHxS)	Quantifier	9.4	399	80	111	-54	130
	Qualifier		399	99	44	-53	94
	ITSD		401.8	80.1	89	-29	116
Perfluorooctanoic acid (PFOA)	Quantifier	10.0	413	169	28	-2	80
	Qualifier		413	369	17	-21	88
	ITSD		421	375.9	13	-17	104

Compound	Component type	RT (min)	Q1	Q2	CC	EV	CCL2
Perfluoroheptane sulfonate (PFHpS)	Quantifier	10.0	448.8	80	109	-2	120
	Qualifier		448.8	99	55	-56	108
	ITSD*		-	-	-	-	-
Perfluorononanoic acid (PFNA)	Quantifier	10.4	463	419	15	-20	100
	Qualifier		463	219	25	-12	108
	ITSD		471.9	427	17	-8	112
Perfluorooctane sulfonate (PFOS)	Quantifier	10.4	499	80	110	-68	122
	Qualifier		499	99	55	-77	118
	ITSD		506.8	80	116	-50	156
Perfluorodecanoic acid (PFDA)	Quantifier	10.8	513	469	19	-6	108
	Qualifier		513	219	24	-27	116
	ITSD		518.8	474	17	-16	116
Perfluorononane sulfonate (PFNS)	Quantifier	10.8	549	80	112	-41	136
	Qualifier		549	99	65	-2	128
	ITSD**		-	-	-	-	-
Perfluoroundecanoic acid (PFUnDA)	Quantifier	11.1	563	519	14	-10	122
	Qualifier		563	219	29	-16	146
	ITSD		569.8	524.8	15	-15	132
Perfluorodecane sulfonate (PFDS)	Quantifier	11.1	599	99	65	-69	152
	Qualifier		599	80	109	-45	172
	ITSD***		-	-	-	-	-
Perfluorododecanoic acid (PFDoDA)	Quantifier	11.4	613	569	14	-23	190
	Qualifier		613	169	36	-34	178
	ITSD		614.8	569.7	16	-12	128
Perfluoroundecane sulfonate (PFUdS)	Quantifier	11.4	648.8	79.8	123	0	160
	Qualifier		648.8	99	69	0	160
	ITSD****		-	-	-	-	-
Perfluorododecane sulfonate (PFDoS)	Quantifier	11.6	698.7	79.9	123	0	184
	Qualifier		698.7	98.8	70	0	180
	ITSD*****		-	-	-	-	-
Perfluorotridecanoic acid (PFTrDA)	Quantifier	11.7	663	169	36	-30	122
	Qualifier		663	619	20	-6	130
	ITSD*****		-	-	-	-	-
Perfluorotridecane sulfonate (PFTrDS)	Quantifier	11.7	748.7	79.9	127	0	196
	Qualifier		748.7	98.9	72	0	200
	ITSD*****		-	-	-	-	-

* PFPeS and PFHpS use the internal standard from PFHxS, M3PFHxS
** PFNA uses the internal standard from PFNA, M9PFNA
*** PFDS uses the internal standard from PFDA, M6PFDA
**** PFUdS uses the internal standard from PFUnDA, M7PFUnDA
***** PFDoS, PFTrDA and PFTrDS use the internal standard from PFDoDA, MPFDoDA

Table 3: MS source parameters.

Parameter	Setting Value
Ionization Mode	ESI Negative
Drying Gas Setting	80
HSID Temperature (°C)	275
Nebulizer Gas Setting	400
Electrospray Voltage	-4000
Source Temperature (°C)	400

Results and Discussion

As shown in Figure 1, the structural diversity of the required PFAS in the EU Drinking Water Directive 2020/2184 is quite complex, even though there are only perfluoroalkylcarboxylic acids and perfluoroalkane sulfonates, the various chain lengths pose problems with the varying polarities.

To evaluate the performance of the method, calibration curves were generated for the 20 different PFAS compounds on three separate days. Example chromatograms, from day one, are shown in Figure 2. The calibration standards range from 0.5-100 ng/L and each of the 20 PFAS demonstrated excellent linearity with regression coefficients (R^2) of ≥ 0.99, which is also shown in Table 4. Moreover, to show the separation and peak shape for all 20 PFAS, Figure 3 represents a sample chromatogram at 25 ng/L. This clearly showcases that even with a large volume direct injection, peak symmetry of the smaller PFAS can be sustained as with PFBA, with a retention time of 6.9 minutes and PFPeS with a retention time of 8.01 minutes.
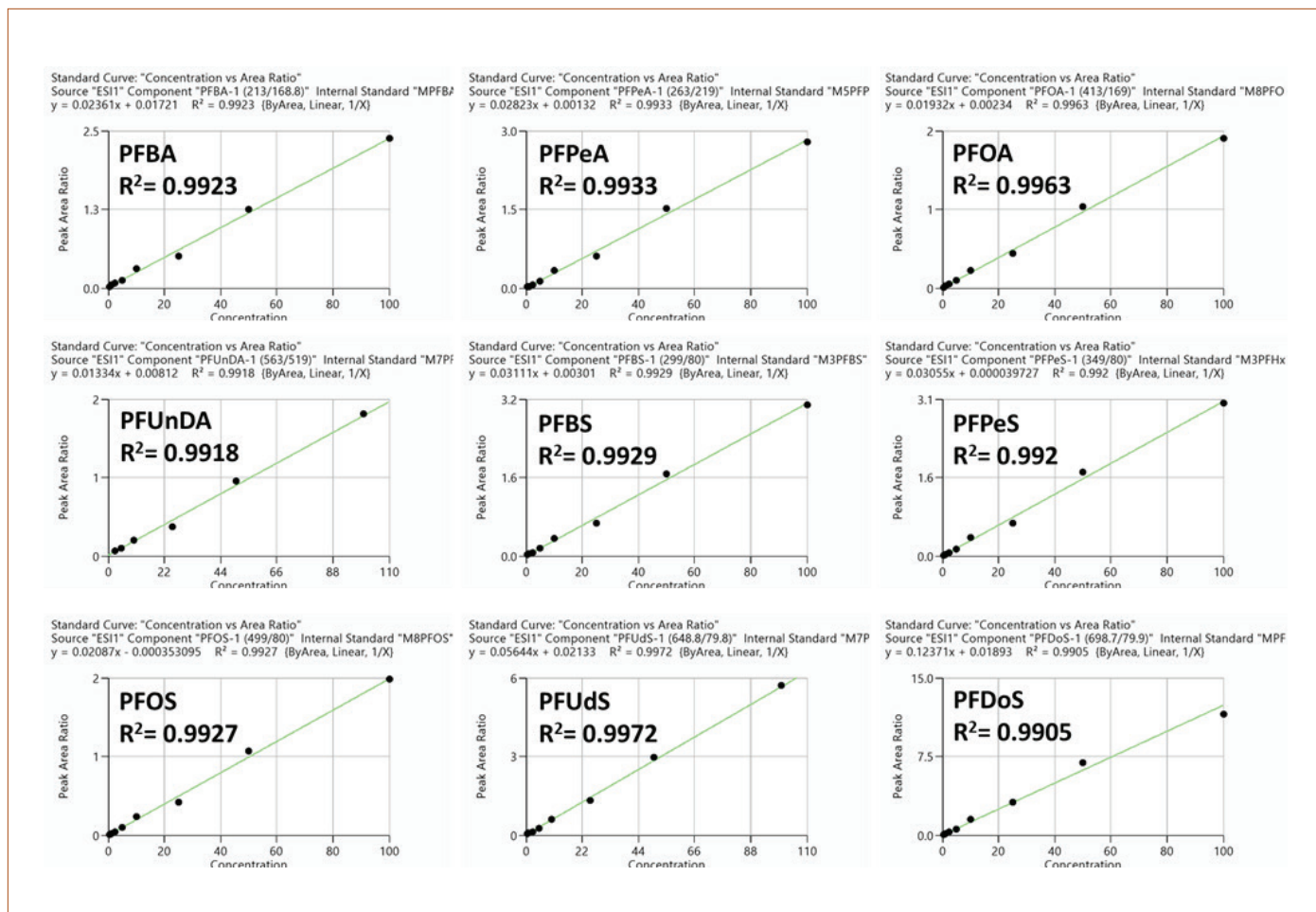

Figure 2: Examples of calibration curves for selected PFAS, additional information can also be found in Table 4.
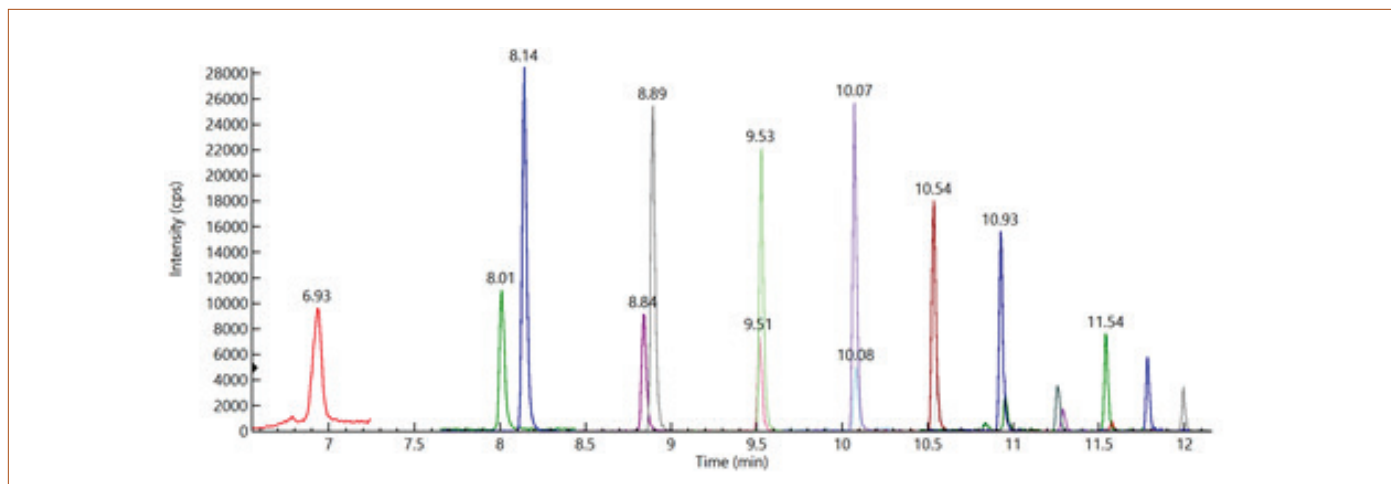
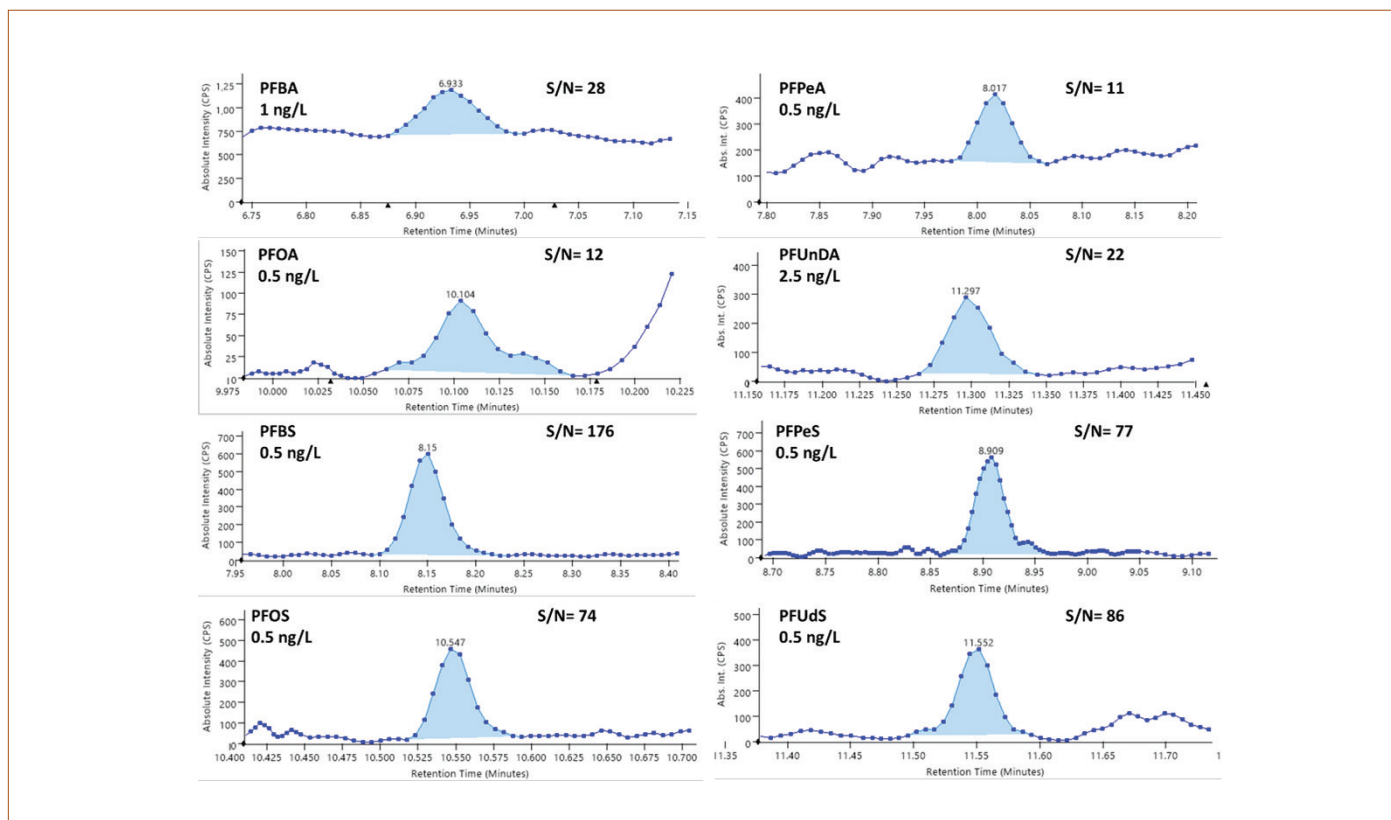*Figure 3: Overlay of the 20 PFAS quantifier fragments at 25 ng/L.*

Figure 4: Example chromatograms at the LOQ for various PFAS along with the calculated S/N.

Table 4: Details the entire results pertaining to the calibration curve, LOQ and recoveries in both drinking water and mineral water. The recovery experiments were done on three separate days with 5 injections of each spiked level per day, giving an n=15.

	Calibration Curve			Drinking Water			Mineral Water		
	LOQ	Linear Range	Linearity	Recovery (%)	Recovery (%)	Recovery (%)	Recovery (%)	Recovery (%)	Recovery (%)
Analyte	(ng/L)	(ng/L)	(R²)	Spiked 2 ng/L	Spiked 10 ng/L	Spiked 10 ng/L	Spiked 2 ng/L	Spiked 10 ng/L	Spiked 100 ng/L
PFBA	1	1 - 100	0,9923	107	117	117	118	113	106
PFPeA	0,5	0.5 - 100	0,9933	114	110	110	114	113	107
PFBS	0,5	0.5 - 100	0,9929	118	113	113	109	115	106
PFHxA	0,5	0.5 - 100	0,9915	106	109	109	112	114	107
PFPeS	0,5	0.5 - 100	0,992	111	110	110	105	112	104
PFHpA	0,5	0.5 - 100	0,9946	113	110	110	117	112	107
PFHxS	0,5	0.5 - 100	0,994	122	114	114	104	116	108
PFOA	0,5	0.5 - 100	0,9963	119	110	110	120	115	108
PFHpS	0,5	0.5 - 100	0,998	115	115	115	114	117	110
PFNA	1	1 - 100	0,9981	103	107	107	104	111	111
PFOS	0,5	0.5 - 100	0,9927	110	111	111	113	114	108
PFDA	2,5	2.5 - 100	0,9979	-	117	117	-	112	113
PFNS	0,5	0.5 - 100	0,996	117	125	125	118	119	116
PFUnDA	2,5	2.5 - 100	0,9918	-	105	105	-	103	100
PFDS	1	1 - 100	0,9946	107	126	126	102	110	117
PFDoDA	5	5 – 100	0,9967	-	93,6	93,6	-	91	96
PFUdS	0,5	0.5 - 100	0,9972	120	114	114	120	109	106
PFDoS	0,5	0.5 - 100	0,9905	107	113	113	102	106	101
PFTrDA	5	5 - 100	0,997	-	102	102	-	100	107
PFTrDS	0,5	0.5 - 100	0,998	94	114	114	91	90	90
QSight LOQ	24,5								
EU DW Parametric value	100								

Additionally Figure 4 shows various chromatograms at the LOQ along with the respective S/N. For each compound at the LOQ, the quantifier fragment has a S/N ≥ 10 and the qualifier a S/N ≥ 3. This further demonstrates the sensitivity of not only the method, but also the QSight LC/MS/MS System. Over 60% of the 20 PFAS have a LOQ of 0.5 ng/L, easily achieving the new limits put by the EU Drinking Water Directive 2020/2184. Table 4 further details the entire dataset for the method, first displaying the LOQs for each compound with a total of 24.5 ng/L putting it four times lower than the parametric value of 100 ng/L.

Moreover, recovery experiments were also conducted on three different days with freshly prepared calibration standards and spiked water samples each injected five times. Table 4 shows all recoveries are within the acceptable limits (70-130%) and furthermore uncertainties were calculated, in which all were well below 50% of the parametric value required from the latest directive.[2] Besides, the recovery data conducted with drinking water, bottled mineral water was also spiked with different levels and showed exceptional recoveries further showing the adaptability of the method to even different water sources.

Conclusion

For the large volume direct injection covering the 20 newly listed PFAS, according to the EU Drinking Water Directive 2020/2184, an LC/MS/MS method was successfully established and performed using PerkinElmer QSight LC/MS/MS System. The method sensitivity and robustness were demonstrated in the three-day experimental setup showing excellent recoveries, linearities and extremely low LOQs for each compound. The linear range for most PFAS was achieved from 0.5-100 ng/L demonstrating the sensitivity of the method and giving a sum of all 20 LOQs to be 24.5 ng/L, which is four times lower than the 100 ng/L parametric value set by the 2020/2184 directive.

The excellent sensitivity was achieved thanks to the the low background noise observed for each these compounds along with minimal contamination. The PEEK injection tubing kit as well as the PEEK lines in the autosampler prevents possible contamination. The use of a delay column separated any contaminants from PFAS compounds of interest in the sample. The QSight LC/MS/MS System enabled high sensitivity as its flow-based design allows for maximum ion formation, while enabling the reduction of any potential chemical noise.

Overall, the combination of sensitivity, versatility, and reliability offered by the QSight LC/MS/MS performance makes it a reliable choice for PFAS analysis in EU drinking water laboratories.

References

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