A robust and sensitive routine analysis method of 40 PFAS compounds in surface water using the SCIEX 7500 system

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This technical note describes a sensitive and robust method for the analysis of per- and polyfluoroalkyl substances (PFAS) in surface water which is based on EU Directive 2020/2184 and WAC/IV/A/025 and is accredited under the ISO 17025. Using the SCIEX 7500 system, a comprehensive method for 40 PFAS compounds was developed with limits of detection (LODs) ranging from 0.5 to 5 ng/L and limits of quantitation (LOQs) ranging from 1 to 10 ng/L in surface water. The validation study showed good accuracy, precision and 4-week shelf life for all PFAS compounds. A thorough quality control program was implemented to ensure consistent data quality during the routine analysis. For example, the Shewhart chart for PFBS showed good accuracy and instrument stability for the 60 ng/L standard (see Figure 1). The use of the calculated columns and custom flagging rules in the SCIEX OS software resulted in fast, efficient data processing and review.

Key benefits for PFAS analysis in surface water using the 7500 system

- **Accreditation achieved with the flexible scope of ISO 17025:** The method was based on EU directive 2020/2184 and WAC/IV/A/025
- **Comprehensive method for PFAS in surface water:** The analyte panel covered 40 PFAS compounds, including 6 compounds that were reported as their total (branched + linear) and linear isomers
- **High levels of sensitivity:** Surface water LOD_w values between 0.5 – 5 ng/L and LOQ_w values between 1 – 10 ng/L
- **Efficient data processing and review:** SCIEX OS software calculated columns and automated flagging rules were integrated into the comprehensive quality control program to streamline data analysis, achieving a 50% reduction in data analysis time

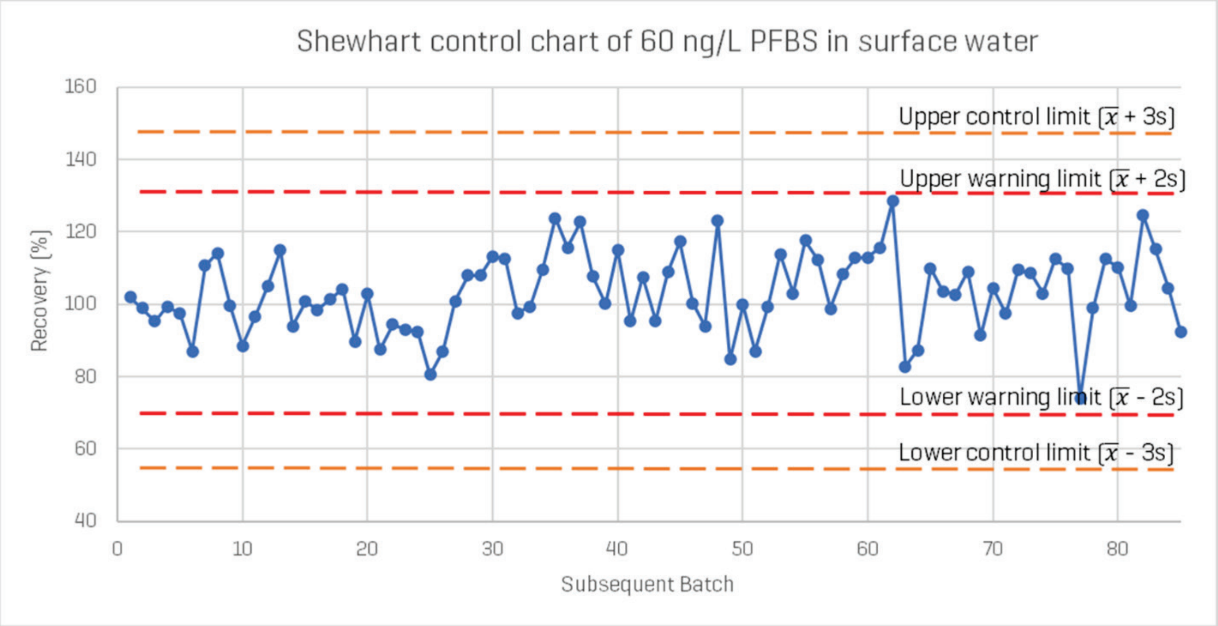


Figure 1: Shewhart control chart of 60 ng/L PFBS in surface water.

Introduction

PFAS are synthetic chemicals known for their persistence in the environment and potential toxicity, thereby posing environmental and health risks. In response, EU member states, including Belgium, have implemented legislation to regulate PFAS levels in surface water. Belgium's comprehensive regulations, detailed in the Belgian compendium of water sampling, measurement, and analysis WAC/IV/A/025,[1] and aligned with the EU Directive 2020/2184,[2] mandate the monitoring of 42 PFAS compounds in water. Among these, 33 compounds are quantitatively measured, 9 should be measured at an increased reporting limit of 50 ng/L, and an additional 6 are also reported as the total of their linear and branched isomers.

Building on previous work,[3, 4] this method was expanded to include 40 PFAS compounds, incorporating additional analytes such as 10:2 FTS and 4:2 FTS. This method reduced PFAS contamination during the sample and standard preparation to achieve the required low reporting limits. Further, the calculated columns and custom flagging rules features in the SCIEX OS software were used to enhance the data analysis efficiency. This included a comprehensive quality control program which ensured reliable and fast data processing. This method has obtained accreditation under the flexible scope of ISO 17025.

Methods

Managing external PFAS contamination: To minimize external PFAS contamination the laboratory work was conducted in a laminar flow cabinet cleaned with acetone before and after use. All solvents and consumables were initially screened to ensure that background PFAS levels were below the LOD. All consumables and lab equipment (including pipette tips, centrifuge tubes, and glassware) were cleaned three times with a 50:50 (v/v) methanol/acetonitrile rinse, followed by a last rinse with the final solvent for which the consumable was intended. The LC system was equipped with a delay and pre-column, and each batch was preceded by a 3-hour equilibration (50:50, v/v, mobile phase A/B, flow 0.05 mL/min, 1 µL injection volume) to limit contamination from the mobile phase and hardware.

Standard preparation: Standards were initially diluted in methanol, and the final calibration samples were prepared in 55.5:22.2:22.2:0.1 (v/v/v/v) LC-MS water/methanol/acetonitrile/formic acid. Calibration curves ranged from 1 to 120 ng/L and the internal standard concentration was 20 ng/L.

Sample preparation: Surface water samples were preserved within 24 hours by adding 4690 µL of 50:50 (v/v) acetonitrile/methanol with 0.225% (v/v) formic acid to 6000 µL of the sample and stored at 4 °C. Prior to analysis, 2475 µL of the preserved sample was spiked with 25 µL of the internal standard mix (final concentration = 20 ng/L). Samples were vortexed and then centrifugated for 5 min at 4500 rpm. A 1000 µL aliquot of the supernatant was transferred to a 1.5 mL polypropylene vial, capped, and placed in the LC-MS/MS autosampler at 8 °C. Samples were analyzed in the following order: injection solvent, calibration standards, procedural blank, independent control standard, technical PFOS mixture sample, ten samples, two calibration drift control samples, ten samples, spiked sample, LOD_w verification, and two calibration drift control samples.

Liquid chromatography: Chromatography was performed using a SCIEX ExionLC system modified to remove the fluoropolymer tubing. The columns used were the Gemini C18 (3 µm, 100 x 2.0 mm, Phenomenex, 00D-4439-B0), SecurityGuard ULTRA Holder and cartridge (Phenomenex, AJ0-9000 and AJ0-8951), and Luna Omega PS C18 (3 µm, 100 x 2.1 mm, Phenomenex, 00D-4758-AN). The overlaid XICs chromatogram from the 120 ng/L standard is shown in **Figure 2**. Building on previous technical notes,[3,4] the gradient was optimized by modifying mobile phase B to be 50:50 (v/v) acetonitrile/methanol and the method was expanded from 26 PFAS compounds with 3 branched isomers to 40 compounds with an additional 6 branched isomers which are quantitated as the total of branched and linear isomers. The novel compounds were: 10:2 FTS, 4:2 FTS, 6:2 diPAP, 6:2/8:2 diPAP, 8:2 FTS, EtPFOSA, branched EtPFOSA, MePFBSA, MePFBSAA, MePFOSA, branched MePFOSA, MePFOSAA, PFBSA, PFECHS, PFHxDA, PFHxSA, PFOSA, branched PFOSA, and PFTeDA.

Mass spectrometry: Samples were analyzed using a SCIEX Triple Quad 7500 system with the OptiFlow Pro ion source. Source and gas parameters are listed in **Table 1**, compound-specific transitions and parameters can be requested via sciexnow@SCIEX.com.

Data processing: Data acquisition and processing were performed using the SCIEX OS software (version 3.3). The processing method was constructed to allow for custom calculations and automated results flagging.

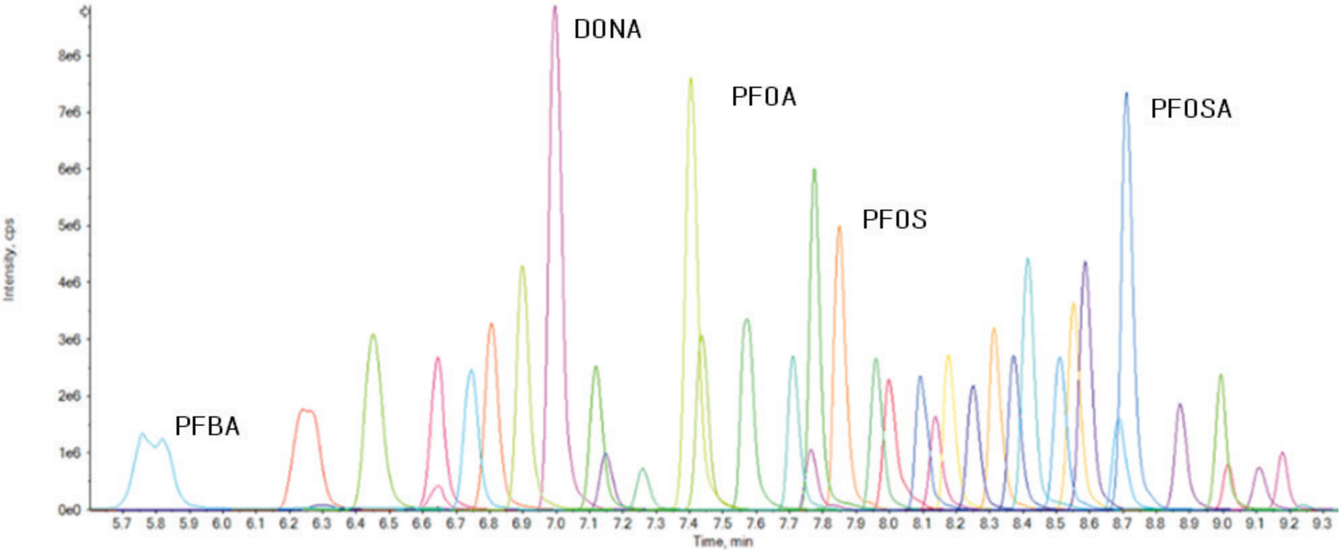


Figure 2: Overlaid extracted ion chromatogram (XIC) for a 120 ng/L mixed PFAS standard.

Table 1: Optimized source and gas parameters for PFAS analysis in surface water using 7500 system.

Parameter	Value
Polarity	Negative
Ion source gas 1	42 psi
Ion source gas 2	60 psi
Curtain gas	46 psi
Source temperature	555 °C
Ion spray voltage	1570 V
CAD gas	10 psi

Instrumental detection limits and linear dynamic range in the injection solvent samples

The validation was conducted in accordance with the guidelines outlined in document WAC/VI/A/001,[5] in conjunction with the in-house validation procedure.

Instrumental detection limit (LOD$_{instr}$): The LOD$_{instr}$ was determined by spiking the injection solvent with concentrations ranging from 0.001 to 5.0 ng/L. The lowest concentration meeting the identification criteria set by Commission Decision N° 2002/657/EC was recorded as LOD$_{instr}$. Individual LOD$_{instr}$ concentrations are presented in **Table 2**.

Linearity: The linear dynamic range for each compound was established using ≥ 6 concentrations prepared in injection solvent ranging from the LOD$_{instr}$ to 120 ng/L. The F-test ($r^2 > 0.99$, 1/x weighting) showed most PFAS compounds were linear whereas 6:2 FTS, 8:2 FTS, EtPFOSAA, PFBA, PFDS, PFECHS, PFHxS, PFHxSA, PFNS, and PFOA demonstrated quadratic performance (**Table 2**).

Method validation in surface water

Limit of detection (LOD$_w$) and quantitation (LOQ$_w$) in surface water: The LOD$_w$ and LOQ$_w$ were determined using five different spiked surface water samples, each analyzed in duplicate on five different days (n = 5). The surface waters were spiked with all compounds at 2 ng/L. The LOD$_w$ was calculated as three times the inter-sample, inter-day standard deviation, and LOQ$_w$ was defined as twice LOD$_w$. The average background concentration of the injection solvent was added to the average LOD$_w$ and LOQ$_w$ values to establish the final LOD$_w$ and LOQ$_w$.[5] The LOD$_w$ ranged from 0.5 to 5 ng/L and the LOQ$_w$ ranged from 1 to 10 ng/L (**Table 2**).

Reproducibility: Intra-laboratory reproducibility was assessed using five different surface water samples analyzed in duplicate and spiked at 20 ng/L for most PFAS analytes. The exceptions were for the indicative compounds (PFTrDA, PFDoDS, PFUnDS, PFTrDS, 10:2 FTS, 6:2 diPAP, and 6:2/8:2 diPAP) which were spiked at 50 ng/L. This approach was performed on 5 different days. The coefficient of variation (CV) was below 20% for all components, meeting the method requirements (**Table 2**).

Accuracy: Five surface water samples were spiked at 10 and 20 ng/L, while the listed indicative compounds were spiked at 50 and 80 ng/L and analyzed on five separate days. For 96% of the measurements, the measurement uncertainty (U) was less than 30%, meeting method requirements.[6] The accuracy was monitored in each batch and must be between 70% and 130%.

Sample shelf life: Five surface water samples were preserved, spiked at 60 ng/L and measured for 6 consecutive weeks to determine the analyte stability (shelf life). Weekly results were compared to the day 0 mean concentration, x_1, and the associated uncertainty, U.[6] The shelf life was accepted if the weekly mean concentration (x_i) fell inside the range of the day 0 mean concentration and uncertainty (x_1 +/- U). For this analysis, the shelf life was established to be 4 weeks for all PFAS compounds (**Table 2**).

Comprehensive quality control program: Use of calculated columns and flagging rules in SCIEX OS

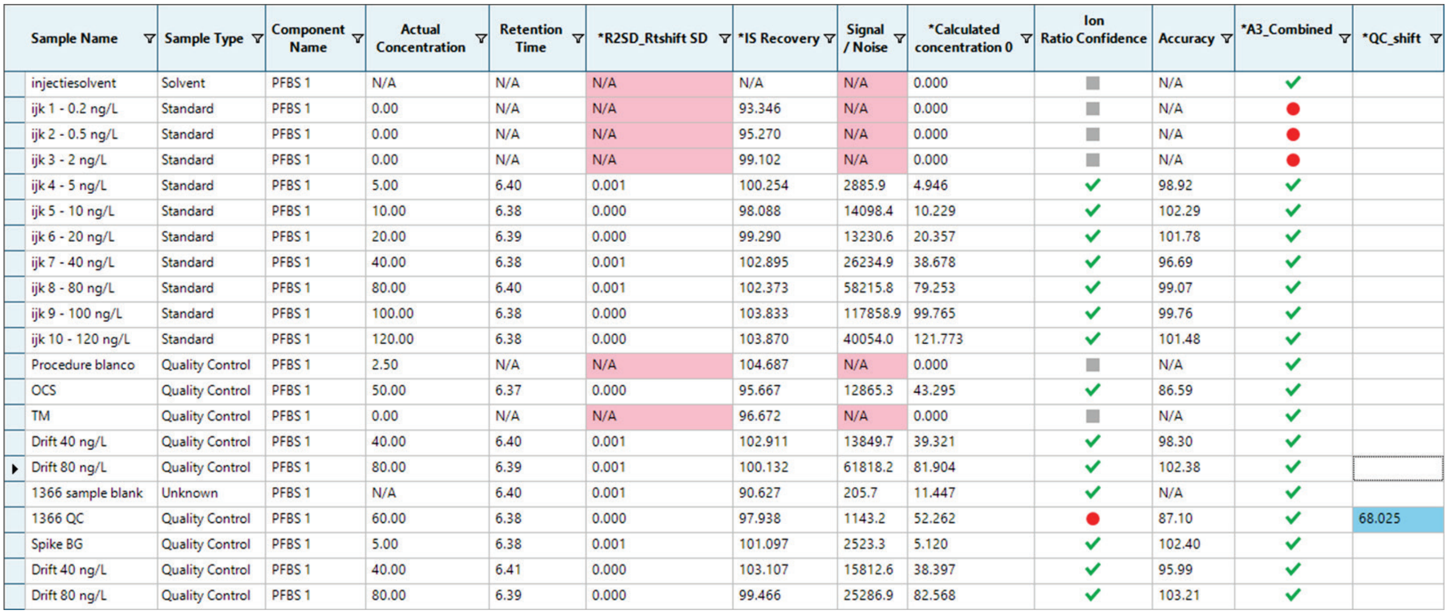
To ensure consistent data quality during routine analysis, several quality control (QC) checks were implemented based on WAC/VI/A/003 and VMM guidelines.[7] The SCIEX OS software allows for user-defined calculated columns and flagging rules to be added to a Results Table,[8,9] eliminating the need for external software, reducing transcription errors and compliance risks. These features were setup within the processing method, enabling automatic flagging as soon as the samples were acquired. The custom flagging rules highlighted outliers in blue or red for a simple and clear review of the data quality and analysis performance (example shown in **Figure 3**). This approach reduced data analysis and review time by 50%. The custom calculations and flagging rules can be downloaded by contacting sciexnow@SCIEX.com.

The specific QC checks were:

- **Calibration curve:** The calibration standards were run with each batch, and the QC criteria specified that the calibration line must have an r^2 value ≥ 0.99. The requirements for a linear calibration curve were: (1) at least 4 calibration points, (2) the lowest point must not be below the LOQ_w, (3) only one point can be removed from the curve, and (4) each calibration point must have an accuracy within 20%, or 25% at the LOQ_w. For a quadratic calibration curve: (1) at least 5 calibration points, (2) the lowest point must not be lower than the LOQ_w, (3) only one point can be removed, and (4) each calibration standard must be within 10%, or 15% at LOQ_w. In addition, the reported concentration range is restricted if the lowest or highest calibration points are omitted.
- **Procedural blank:** Each analytical batch series must include a procedural blank consisting of the injection solvent spiked with internal standard. The PFAS concentration in the procedural blank must not exceed LOD_w. The procedural blank concentration trends were tracked in a Shewhart control chart within the laboratory information management system (LIMS).
- **LOQ_w in the injection solvent:** At the end of each batch, the instrument sensitivity was confirmed using the LOQ_w standard prepared in the injection solvent. Similar to the criteria for the calibration curve, the accuracy must be 25% for PFAS compounds having a linear curve and 15% for those having a quadratic curve.
- **Spiked sample QC:** Every 20th sample was spiked with a known analyte concentration. All compound recoveries must be between 70% and 130%. Recoveries for EtPFOSAA, PFBA, PFBS, linear PFHxS, linear PFOA and linear PFOS were recorded on a Shewhart control chart in LIMS (see **Figure 1** for the PFBS chart).
- **Drift:** Calibration drift was evaluated every fifteen samples and at the end of each batch. The measured concentration may deviate up to 20% from the actual value. For a linear calibration line, one calibration standard sample was injected; for a quadratic line, two calibration standards were injected, with the additional one in the upper third of the curve.

Table 2: Validation parameters for the analysis of PFAS in surface water. Abbreviations: CV = intra-reproducibility variation coefficient %, LOD$_{\text{instr}}$ = instrument detection limit, LOD$_{\text{w}}$ = limit of detection for surface water, LOQ$_{\text{w}}$ = limit of quantification for surface water, U = measurement uncertainty.[5, 6]

Compound ID	LOD$_{\text{instr}}$ (ng/L)	Regression	LOD$_{\text{w}}$ (ng/L)	LOQ$_{\text{w}}$ (ng/L)	%CV$_{\text{concentration}}$	U (%)	Shelf life (weeks)
10:2 FTS	2.0	Linear	5	10	6.1	14.7	4
4:2 FTS	2.0	Linear	5	10	7.9	16.7	4
6:2 diPAP	0.100	Linear	5	10	6.2	27.4	4
6:2 FTS	0.005	Quadratic	5	10	9.6	26.4	4
6:2/8:2 diPAP	0.200	Linear	5	10	7.5	41.9	4
8:2 FTS	0.100	Quadratic	1	2	5.7	25.3	4
DONA	0.050	Linear	2.5	5	7.9	23.1	4
EtPFOSA	0.100	Linear	0.5	1	6.0	27.4	4
EtPFOSA$_{\text{sum}}$	0.100	Linear	5	10	6.7	30.5	4
EtPFOSAA	0.100	Quadratic	0.5	1	4.7	11.6	4
HFPO-DA	0.500	Linear	0.5	1	6.9	23.0	4
MePFBSA	0.500	Linear	5	10	7.6	18.8	4
MePFBSAA	0.050	Linear	0.5	1	4.0	13.4	4
MePFOSA	0.050	Linear	2.5	5	10	29.2	4
MePFOSA$_{\text{sum}}$	0.200	Linear	5	10	9.4	29.6	4
MePFOSAA	0.100	Linear	0.5	1	4.5	12.6	4
PFBA	0.100	Quadratic	2.5	5	10	42.2	4
PFBS	0.050	Linear	2.5	5	4.0	24.5	4
PFBSA	0.050	Linear	2.5	5	7.5	36.3	4
PFDA	0.500	Linear	5	10	8.2	27.2	4
PFDoDA	0.200	Linear	1	2	11	49.7	4
PFDoDS	0.200	Linear	5	10	5.5	15.2	4
PFDS	0.050	Quadratic	0.5	1	5.7	17.3	4
PFECHS	0.050	Quadratic	0.5	1	9.0	26.6	4
PFHpA	0.005	Linear	2.5	5	13	29.2	4
PFHpS	0.100	Linear	2.5	5	8.2	22.0	4
PFHxA	0.100	Linear	2.5	5	12	42.4	4
PFHxDA	0.500	Linear	5	10	9.1	40.6	4
PFHxS	0.050	Quadratic	2.5	5	4.8	23.1	4
PFHxS$_{\text{sum}}$	0.050	Quadratic	5	10	4.1	30.9	4
PFHxSA	0.100	Quadratic	0.5	1	4.5	9.6	4
PFNA	0.500	Linear	5	10	6.5	26.5	4
PFNS	0.100	Quadratic	0.5	1	5.8	15.7	4
PFOA	0.200	Quadratic	5	10	10	26.3	4
PFOA$_{\text{sum}}$	1.00	Quadratic	5	10	13	33.4	4
PFOS	0.005	Linear	2.5	5	13	37.5	4
PFOS$_{\text{sum}}$	0.200	Linear	5	10	14	42.5	4
PFOSA	0.050	Linear	0.5	1	4.9	12.0	4
PFOSA$_{\text{sum}}$	0.050	Linear	5	10	15	32.7	4
PFPeA	5.0	Linear	5	10	4.6	41.2	4
PFPeS	0.100	Linear	0.5	1	4.3	17.7	4
PFTeDA	0.500	Linear	5	10	9.2	33.9	4
PFTrDA	0.200	Linear	5	10	11	24.5	4
PFTrDS	0.100	Linear	5	10	6.2	29.5	4
PFUnDA	0.100	Linear	2.5	5	7.4	2.4	4
PFUnDS	0.1	Linear	5	10	6.5	15.5	4



Sample Name	Sample Type	Component Name	Actual Concentration	Retention Time	*R2SD_Rtshift SD	*IS Recovery	Signal / Noise	*Calculated concentration 0	Ion Ratio Confidence	Accuracy	*A3_Combined	*QC_shift
injectiesolvent	Solvent	PFBS 1	N/A	N/A	N/A	N/A	N/A	0.000	■	N/A	✓	
ijk 1 - 0.2 ng/L	Standard	PFBS 1	0.00	N/A	N/A	93.346	N/A	0.000	■	N/A	●	
ijk 2 - 0.5 ng/L	Standard	PFBS 1	0.00	N/A	N/A	95.270	N/A	0.000	■	N/A	●	
ijk 3 - 2 ng/L	Standard	PFBS 1	0.00	N/A	N/A	99.102	N/A	0.000	■	N/A	●	
ijk 4 - 5 ng/L	Standard	PFBS 1	5.00	6.40	0.001	100.254	2885.9	4.946	✓	98.92	✓	
ijk 5 - 10 ng/L	Standard	PFBS 1	10.00	6.38	0.000	98.088	14098.4	10.229	✓	102.29	✓	
ijk 6 - 20 ng/L	Standard	PFBS 1	20.00	6.39	0.000	99.290	13230.6	20.357	✓	101.78	✓	
ijk 7 - 40 ng/L	Standard	PFBS 1	40.00	6.38	0.001	102.895	26234.9	38.678	✓	96.69	✓	
ijk 8 - 80 ng/L	Standard	PFBS 1	80.00	6.40	0.001	102.373	58215.8	79.253	✓	99.07	✓	
ijk 9 - 100 ng/L	Standard	PFBS 1	100.00	6.38	0.000	103.833	117858.9	99.765	✓	99.76	✓	
ijk 10 - 120 ng/L	Standard	PFBS 1	120.00	6.38	0.000	103.870	40054.0	121.773	✓	101.48	✓	
Procedure blanco	Quality Control	PFBS 1	2.50	N/A	N/A	104.687	N/A	0.000	■	N/A	✓	
OCS	Quality Control	PFBS 1	50.00	6.37	0.000	95.667	12865.3	43.295	✓	86.59	✓	
TM	Quality Control	PFBS 1	0.00	N/A	N/A	96.672	N/A	0.000	■	N/A	✓	
Drift 40 ng/L	Quality Control	PFBS 1	40.00	6.40	0.001	102.911	13849.7	39.321	✓	98.30	✓	
Drift 80 ng/L	Quality Control	PFBS 1	80.00	6.39	0.001	100.132	61818.2	81.904	✓	102.38	✓	
1366 sample blank	Unknown	PFBS 1	N/A	6.40	0.001	90.627	205.7	11.447	✓	N/A	✓	
1366 QC	Quality Control	PFBS 1	60.00	6.38	0.000	97.938	1143.2	52.262	●	87.10	✓	68.025
Spike BG	Quality Control	PFBS 1	5.00	6.38	0.001	101.097	2523.3	5.120	✓	102.40	✓	
Drift 40 ng/L	Quality Control	PFBS 1	40.00	6.41	0.000	103.107	15812.6	38.397	✓	95.99	✓	
Drift 80 ng/L	Quality Control	PFBS 1	80.00	6.39	0.000	99.466	25286.9	82.568	✓	103.21	✓	

Figure 3: SCIEX OS Results Table for the PFBS quantifier transition. The data was constructed to demonstrate flagging rules. Calibration point 5 ('Ijk 5 – 20 ng/L') was manipulated, flagging the 'ion ratio' and 'A3_combined'. Similarly, for the first drift ('drift 40 ng/L – ijk 6') and the spiked sample ('QC'), flagging 'ion ratio', 'Accuracy', and 'QC_shift'. Abbreviations: A0 and A3 = part of accuracy formulas for the calculated columns of the calibration curve, ICS = independent control standard, Ijk = calibration point, IS = internal standard, LOQ$_w$ in inj solv = Spiked injection solvent with the limit of quantitation from surface water, N/A = not applicable, PFBS = perfluorobutanesulfonic acid, QC = spiked sample with known standard concentration, Rt = retention time, SD = standard, TM = technical mixture.

- **Independent control standard (ICS):** The ICS was purchased from a different supplier than the calibration standards and used to confirm their accuracy. The ICS samples must contain at least one-third of the PFAS analytes in the original calibration mix and the recovery must range from 80% to 120%.
- **Chromatographic separation of PFOS isomers:** Chromatographic separation of the branched (PFOS$_{branched}$) and linear (PFOS$_{linear}$) isomers was confirmed by injecting a sample of technical grade PFOS prepared in injection solvent. The LC column performance is acceptable if the valley height between the branched and linear peaks was less than 70% of the lowest peak (PFOS$_{branched}$). An example of satisfactory PFOS isomer separation is shown in **Figure 4**
- **Internal Standard (IS) recovery:** The IS recovery in the samples was calculated relative to the average IS response in the calibration standards. The recovery internal standards must be between 30% and 200%.

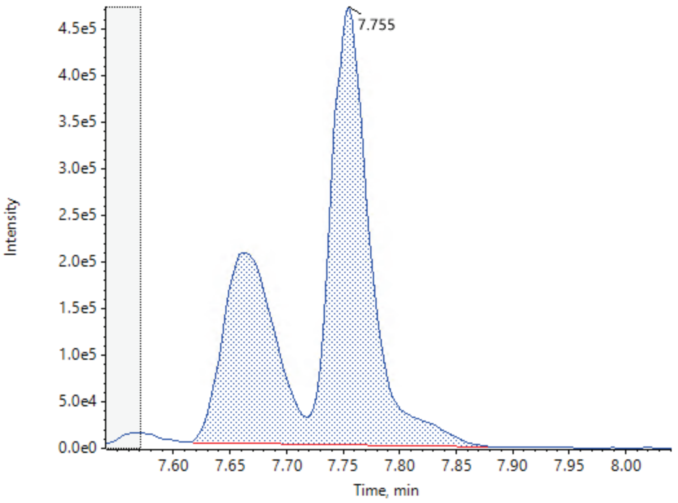


Figure 4. Separation of branched and linear isomers in a technical grade PFOS standard.

- **Retention time shift:** The retention time shift was performed via the most recent batch for the first eluting PFAS compound, PFBA. The maximum retention time deviation must not exceed 2.5% for the last calibration standard plus the observed shift for the corresponding internal standard. If a different isotope-labelled compound is used as the internal standard, the deviation may be up to 5%. Custom calculations are presented in **Table 3**.

Table 3: Custom calculations used to evaluate the shift in retention time for PFBA.

Formula name	Formula
R1SD_Rtshift SD	*ABS([Retention Time] - MEAN([Retention Time]])*
R2IS_Rtshift IS	ABS([IS Retention Time]-MEAN([IS Retention Time]])
R2_Rt HLOQ	MEAN([Retention Time])
R2_Rt HLOQ PFBA	GET([R2_Rt HLOQ];'PFBA 1')
R2SD_Rtshift SD	ABS([R1SD_Rtshift SD] - [R2IS_Rtshift IS]) / [R2_Rt HLOQ PFBA]

- **Ion ratio:** For positive identification, the ion ratio in the sample must be similar to the ion ratio from the calibration standards. The ion ratio is calculated as the peak area of the qualifier transition divided by the quantifier transition, multiplied by 100. The ion ratio deviation is calculated as the difference between the calibration standard ion ratio and the sample ion ratio, divided by the calibration standard ion ratio, multiplied by 100%. Maximum deviations are 30% for ion ratios above 10% and 50% for ion ratios below 10%.

Conclusions

- A sensitive and robust method was developed for the analysis of 40 PFAS compounds in surface water using the SCIEX 7500 system
- The method was based on EU directive 2020/2184 and WAC/IV/A/025, and has achieved accreditation with a flexible scope under ISO 17025
- The calculated columns and custom flagging rules within the SCIEX OS were used to enhance data processing and review resulting in a 50%-time savings

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