Analysis of Perfluoroalkyl Acids Specified Under the UCMR3 Using the QTRAP® 6500 LC/MS/MS System

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Overview

This application note highlights the sensitivity and precision of the QTRAP® 6500 LC/MS/MS system for the analysis of perfluoroalkyl acids (PFAAs) in drinking water. The PFAAs analyzed are a subset of EPA Method 537 (Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry [LC/MS/MS])[1], comprising the PFAAs outlined in the Unregulated Contaminant Monitoring Rule 3 Assessment Monitoring list (UCMR3).[2] Statistically validated method detection limits range from 1.4 – 35.9 ng/L.

Introduction

PFAAs are ubiquitous chemicals that are used in a variety of industrial and consumer products including carpets, cookware, paints, shampoos, food packaging, etc.[3] PFAAs have high thermal and chemical stability and are highly resistant to degradation in aquatic environments. Typical concentrations of PFAAs found in various water sources range from pg/L to µg/L levels.

Within the scope of EPA 537 there are 14 PFAAs (Table 1). Of these 14, six are specified in the UCMR3 Assessment Monitoring list: PFBS, PFHpA, PFHxS, PFOA, PFOS and PFNA.

This paper describes the performance of the QTRAP® 6500 system for the evaluation of the PFAAs in the UCMR3 using the guidelines laid out in EPA 537.

Table 1. PFAAs in EPA Method 537. Those compounds in bold type face are included in the UCMR3 Assessment Monitoring list.

Compound	Abbreviation	CASRN	UCMR3 MRL (ng/L)
Perfluorohexanoic acid	PFHxA	307-24-4	-
Perfluoroheptanoic acid	**PFHpA**	**375-85-9**	**10**
Perfluorooctanoic acid	**PFOA**	**335-67-1**	**20**
Perfluorononanoic acid	**PFNA**	**375-95-1**	**20**
Perfluorodecanoic acid	PFDA	335-76-2	-
Perfluoroundecanoic acid	PFUnA	2058-94-8	-
Perfluorododecanoic acid	PFDoA	307-55-1	-
Perfluorotridecanoic acid	PFTrDA	72629-94-8	-
Perfluorotetradecanoic acid	PFTA	376-06-7	-
Perfluorobutanesulfonic acid	**PFBS**	**375-73-5**	**90**
Perfluorohexanesulfonic acid	**PFHxS**	**355-46-4**	**30**
Perfluorooctanesulfonic acid	**PFOS**	**1763-23-1**	**40**
N-methyl perfluorooctane-sulfonamidoacetic acid	NMeFOSAA	-	-
N-ethyl perfluorooctane-sulfonamidoacetic acid	NEtFOSAA	-	-

Experimental

Sample preparation and data processing were carried out according to EPA Method 537 without deviation (EPA 537 sections 10, 11 and section 12), unless specifically noted. All required quality control parameters (EPA 537 section 9.3) were met or exceeded for each batch of calibrators and/or samples analyzed. Quantitation was performed using MultiQuant™ 3.0 software. All calibration curves had a 1/x concentration weighting and were forced through the intercept as specified in EPA 537 section 10.2.6. For carboxylic acids $^{13}C_2$-PFOA was used as the internal standard (ISTD), while all sulfonic acids used $^{13}C_4$-PFOS as the ISTD. The surrogates used were $^{13}C_2$-PFHxA and $^{13}C_2$-PFDA, both of which were fortified into samples at 40 ng/L.

Analyses were carried out using the SCIEX QTRAP® 6500 system coupled with an Agilent 1260 HPLC (degasser, binary pump and column oven) with an Eksigent ULC 100 HTC-xt autosampler. The mobile phase consisted of 20mM ammonium acetate with methanol. Gradient parameters are provided in Table 2. All samples were analyzed with a 5 µL injection (vs. 10 µL in EPA 537) onto an Atlantis T3 analytical column (150 x 2.1 mm, 5 µm) heated to 35˚C. An Atlantis T3 column (50 x 2.1mm, 5 µm) was also used as a delay column.

Table 2. LC gradient conditions

Time (min)	Flow Rate (µL/min)	A (%)	B (%)
0.0	450	60	40
1.0	450	60	40
6.0	450	35	65
6.1	350	35	65
14.0	350	10	90
15.0	350	10	90
15.1	350	60	40
16.0	450	60	40
18.0	450	60	40

The QTRAP® 6500 system was operated in negative polarity Electrospray Ionization (ESI) using Multiple Reaction Monitoring (MRM) and the *Scheduled* MRM™ algorithm. ESI source and MRM parameters are outlined in Tables 3 and 4.

Table 3. ESI source parameters

Parameter	Value
Polarity	negative
Curtain Gas	30 psi
Collision Gas	12 psi
IonSpray Voltage	-4500 V
Temperature	400˚C
GS1	30 psi
GS2	30 psi

Table 4. MRM transitions, retention time (RT), Declustering Potential (DP), and Collision Energy (CE) for target PFAAs, ISTDs (*) and surrogates (^)

Compound	Q1	Q3	RT	DP (V)	CE (V)
PFBS 1	298.8	79.8	6.8	-60	-68
PFBS 2	298.8	98.9	6.8	-60	-36
PFHpA 1	362.8	318.8	10.7	-5	-12
PFHpA 2	362.8	168.8	10.7	-5	-22
PFHxS 1	398.9	79.7	10.7	-70	-86
PFHxS 2	398.9	98.7	10.7	-70	-74
PFOA 1	412.8	368.9	12.1	-5	-14
PFOA 2	412.8	168.7	12.1	-5	-24
PFOS 1	498.9	79.8	13.2	-60	-122
PFOS 2	498.8	98.9	13.2	-60	-98
PFNA 1	462.9	418.9	13.3	-30	-14
PFNA 2	462.9	218.9	13.3	-30	-24
*$^{13}C_2$-PFOA**	414.9	369.8	12.1	-20	-14
*$^{13}C_4$-PFOS**	502.9	79.8	13.3	-10	-102
*$^{13}C_2$-PFHxA^*	314.8	269.8	8.9	-15	-12
*$^{13}C_2$-PFDA^*	514.9	469.9	14.3	-25	-16

Results and Discussion

EPA 537 permits deviation from the LC conditions provided in the method. To that end, the method presented here used an Atlantis T3 column (5 µm) and a gradient that was designed to increase method throughput, while still providing sufficient chromatographic resolution (Figure 1).

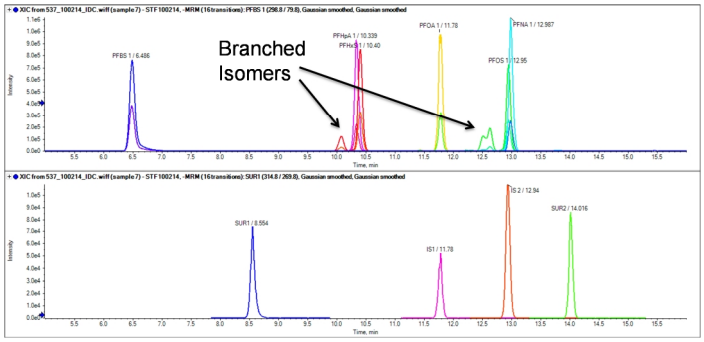

Figure 1. Final chromatography using a 20mM ammonium acetate / methanol mobile phase. Targets are shown on top with branched isomers of PFHxS and PFOS indicated. ISTDs ($^{13}C_2$-PFOA and $^{13}C_4$-PFOS) and surrogates are shown on the bottom (SUR1 = $^{13}C_2$-PFHxA and SUR2 = $^{13}C_2$-PFDA)

For PFHxS and PFOS the presence of additional small peaks points to the presence of branched isomers, which are known contaminants in the technical PFAAs suggested for purchase in EPA 537. When present, these isomers were summed into a combined value for the branched and linear isomers. This adheres to section 12.4 of EPA 537.

Initial Calibration

The Initial Calibration (EPA 537 section 10.2) was carried out using the UCRM3 Assessment Monitoring list as a guide, with the lowest calibration level for each target compound corresponding to ½ of the UCMR3 reporting limit (Table 1). Owing to the high sensitivity of the QTRAP® 6500 system these low ng/L levels were easily obtained for all compounds, with Signal-to-noise values (S/N) of 50 to 1700 after 1-point Gaussian smoothing using a peak-to-peak algorithm (Figure 2). All calibration acceptance criteria specified in EPA 537 section 10.2 were met.

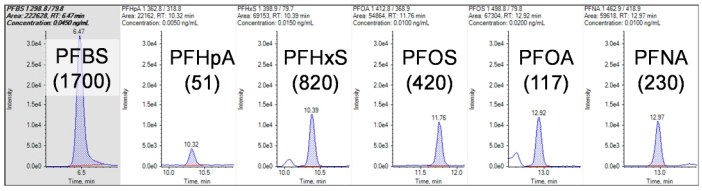

Figure 2. Signal-to-noise values (S/N) for the low calibrators. Low calibration levels for each compound are ½ of the UCMR3 reporting limits

The correlation (r) value for all calibration curves were > 0.99 (Figure 3).

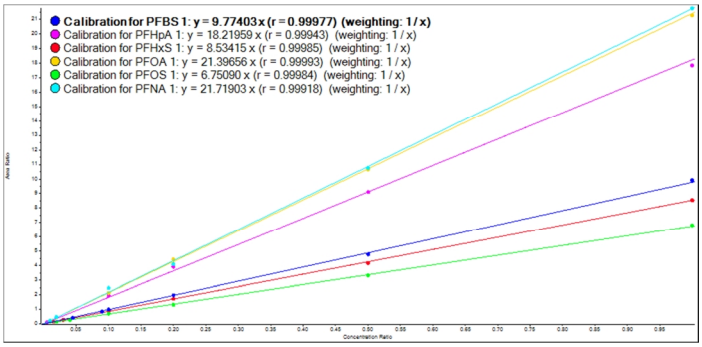

Figure 3. Calibration lines and regression equations for all six PFAAs

Initial Demonstration of Capability

To demonstrate method suitability for EPA 537 it is necessary to perform an Initial Demonstration of Capability (IDC) following the Initial Calibration. In addition to the ongoing QC criteria specified in EPA 537 section 9.3, adhering to the IDC necessitates the following:

1. Extraction of four Laboratory Fortified Blanks (LFB) to assess Accuracy (±30%) and Precision (RSD <20%). Fortification should correspond to a mid-level calibrator.
2. PFBS and $^{13}C_2$-PFHxA (surrogate) must have peaks Asymmetry Factors between 0.8 to 1.5.
3. Extraction of seven LFBs that must meet a Prediction Interval of Results (PIR) of 50 to 150% to define the Method Reporting Limits (MRL).
4. Determination of Method Detection Limits (MDL). This is an optional part of the IDC that requires seven replicates prepared over three days. In this study the MRL replicates were used.
5. All targets compounds in a Laboratory Reagent Blank (LRB) and Field Reagent Blank (FRB) after the Initial Calibration must quantify to <1/3 of MRL.
6. Evaluate method accuracy (±30%) using a Quality Control Sample (QCS) that is sourced from a vendor other than the one that provided the calibration samples.

Each of these criteria are discussed below.

Accuracy and Precision

Fortification for evaluation of Accuracy and Precision was done at 200 ng/L. This corresponded to calibration level four of six. For the four replicates extractions analyzed the relative standard deviations (RSD) ranged from 3.1 to 9.8%, while the recoveries ranged from 89 to 96% (Table 5). All of these values were within the EPA 537 specified ranges of < 20% RSD and ±30% recoveries.

Table 5. Method performance

Compound	Precision (%)	Accuracy (%)	QCS (%)		RPD (%)
			Batch 1	Batch 2	
PFBS	3.5	91	71.2	87.6	5.65
PFHpA	6.1	89	86.0	109.0	0.20
PFHxS	3.3	93	95.3	116.0	4.81
PFOA	4.7	96	96.8	101.4	3.84
PFOS	3.1	92	91.9	111.5	5.11
PFNA	9.8	91	72.8	103.6	9.21

Asymmetry Factor

To ensure acceptable chromatography of the two earliest eluting peaks in the method, the user is required to calculate the Asymmetry Factor (A_S) for every batch of samples analyzed. In the present method this corresponded to PFBS and $^{13}C_2$-PFHxA. The A_S was calculated from a mid-level calibrator of 200 ng/L. Figure 4 demonstrates that the A_S for PFBS (1.31) and $^{13}C_2$-PFHxA (1.37) meet the EPA 537 acceptance criteria of: A_S must fall in the range of 0.8 to 1.5. The A_S values were calculated automatically using MultiQuant™ software version 3.0.

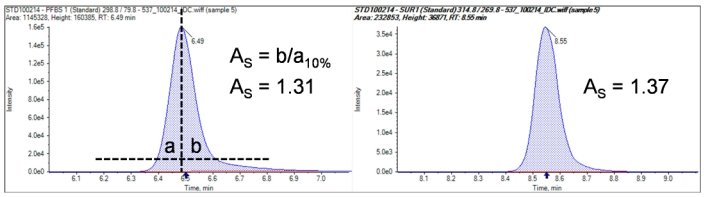

Figure 4. Asymmetry Factor for PFBS (left) and $^{13}C_2$-PFHxA (right). The example on the left demonstrates how MultiQuant™ software 3.0 calculates A_S.

Method Reporting Limits

As the current method was designed to meet the UCMR3 reporting limits, the levels used to fortify the seven extractions required for the calculation of the Method Reporting Limit (MRL) correspond to the UCMR3 reporting limits. To be a valid MRL the results of the seven replicate extractions must meet a set of statistical criteria, which are outlined in detail in section 9.2.5 of EPA 537. Briefly, the calculations are:

$$HR_{PIR} = 3.963s$$

$$\frac{Mean + HR_{PIR}}{Fortified\ Concentration} \times 100\%$$

HR_{PIR} = Half Range for the prediction interval of results
s = the standard deviation of replicate analyses
3.963 = a constant value for seven replicates

The PIR must be within 50 and 150% to be a validated MRL. Using the above equations on samples that had been fortified at the UCMR3 reporting limits yielded acceptable PIR values (Table 6). Based on these calculations and the UCMR3 reporting limits that were used as sample fortification guidelines, all compounds in the current method were validated.

Table 6. MRL and MDL determination and statistical verification

Compound	Fortification Level (ng/L)	Lower PIR (%)	Upper PIR (%)	MDL (ng/L)
PFBS	90	81	99	8.3
PFHpA	10	75	114	1.4
PFHxS	30	86	99	1.6
PFOA	20	77	109	3.1
PFOS	40	56	144	35.9
PFNA	20	75	98	7.0

Method Detection Limits

The Method Detection Limit (MDL) was calculated using the following equation:

$$MDL = s \times t_{(n-1,1-\alpha=0.99)}$$

s = the standard deviation of replicate analyses
$t_{(n-1,1-\alpha=0.99)}$ = Student's t value for the 99% confidence level with $n-1$ degrees of freedom
n = number of replicates

Using the MRL extracts, the calculated MDLs ranged from 1.4 to 35.9 ng/L. It is conceivable that the QTRAP® 6500 could detect lower concentrations based on the S/N for the low calibrators (Figure 2).

Laboratory Reagent Blank

A Laboratory Reagent Blank (LRB) is a system blank that has been taken through the entire extraction procedure to assess for background contamination. Following the Initial Calibration a LRB was assessed. Once MRLs were established, the LRB was evaluated with regards to the background levels relative to the calculated MRLs (Figure 5).

In the present method, all target compounds were observed well under 1/3 of their respective MRLs.

Quality Control Sample and Ongoing QC Results

The Quality Control Sample (QCS) was evaluated at 200 ng/L for all compounds to verify the validity of the Initial Calibration. All compounds met the ±30% accuracy criterium for the QCS samples (Table 5).

Three components of the ongoing QC requirements specified in EPA 537, the LRB, Asymmetry Factor and QCS, have already been discussed as they are also specified components of the IDC. In addition, the following ongoing QC criteria were required:

1. Laboratory fortified blank (LFB) should be analyzed with each batch. Acceptance criteria will depend on the fortified concentration, which should change from batch-to-batch.
2. Internal standard (ISTD) responses should not deviate more than 50% from the average ISTD response in the initial calibration and the ISTD in all samples should be 70-140% of the response in the latest continuing calibration check (CCC).
3. Surrogate recovery should be ±30% of the expected value.
4. Laboratory fortified sample matrix (LFSM) and a duplicate (LFSMD) should yield accuracies within ±30% of expected values and the relative percent difference (RPD) between the LFSM and LFSMD must be < 50%.
5. A field reagent blank (FRB) should not contain residue levels > 1/3 of the calculated MRLs.

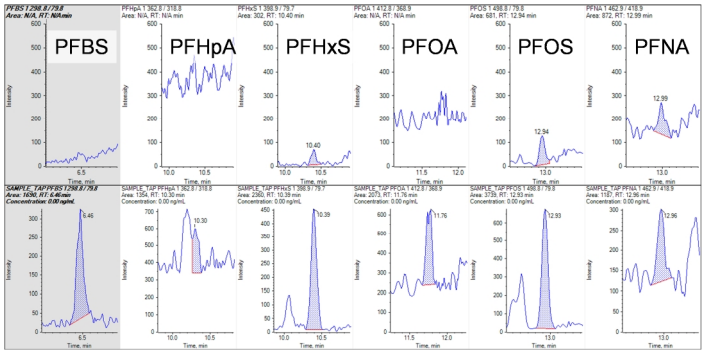

Figure 5. LRB (top) and FRB (bottom) results. Both LRB and FRB results showed background levels that were all < 1/3 of the calculated MRLs. The FRB matrix was finished tap water.

Table 6. LRB and FRB background levels in comparison to the MRL

(ng/L)	PFBS	PFHpA	PFHxS	PFOA	PFOS	PFNA
1/3 MRL	30	3.3	10	6.7	13.3	6.7
LRB	-	-	0.06	-	0.2	0.2
FRB	0.3	0.3	0.4	0.8	0.3	0.2

The first four of these criteria were all met or exceeded in all samples discussed herein. The RPD results ranged from 0.2 to 9.2, well within the ±30% RPD permitted in EPA 537 (Table 5). The FRB matrix in this study was finished tap water. Figure 5 demonstrates that all compounds were < 1/3 of the calculated MRLs, which meets EPA 537 criteria and further validates the RPD results since there was negligible background PFAA contamination in the sample matrix.

There is also criteria for CCCs (low CCC accuracy 50-150%; mid/high CCC accuracy 70-130%; surrogate accuracy 70-130%) that were met for all samples analyzed.

Conclusion

The QTRAP® 6500 LC/MS/MS system is a sensitive and robust platform for the analysis of PFAAs in drinking water. The demonstrated MRLs easily meet the UCMR3 reporting limits.

References

1 EPA Method 537 'Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography / Tandem Mass Spectrometry LC/MS/MS)' version 1.1 (2009) http://www.epa.gov/microbes/documents/Method%20537_FINAL_rev1.1.pdf

2 Unregulated Contaminant Monitoring Rule 3 (UCMR3) http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/

3 M.F. Rahman et al.: 'Behavior and Fate of Perfluoroalkyl substances (PFAs) in Drinking Water Treatment: A Review.' Water Research 50 (2014) 318-340

Abbreviations

A_s – asymmetry factor
CASRN – chemical abstracts registration number
CCC – continuing calibration check
CE – collision energy
DP – declustering potential
EPA – environmental protection agency
ESI – electrospray ionization
FRB – field reagent blank
HR_{PIR} – half range prediction interval of results
IDC – initial demonstration of capability
ISTD – internal standard
LFB – laboratory fortified blank
LFSM – laboratory fortified sample matrix
LFSMD – laboratory fortified sample matrix duplicate
LRB – laboratory reagent blank
MDL – method detection limit
MRL – method reporting limit
MRM – multiple reaction monitoring
PFAAs – perfluoroalkyl acids
PIR – prediction interval of results
QCS – quality control sample
RPD – relative percent difference
RSD – relative standard deviation
RT – retention time
S/N – signal-to-noise
UCMR3 – unregulated contaminant monitoring rule 3 assessment monitoring list

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