A Low Femtogram Target Screen Method for Perfluorinated Compounds in Food Matrices and Potable Water Using the Agilent 6460 Triple Quadrupole LC/MS System Equipped with Agilent Jet Stream Technology

Application Note

Environmental, Food Safety

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Abstract

In this application note, we outline a viable method for reliably detecting low-femtogram levels of perfluorinated carboxylates (PFCA) and sulphonates (PFSA) without background component interference, with an inline contaminant trap LC modification. Good chromatographic resolution of all perfluorinated compounds was observed. A representative suite of PFCAs and PFSAs were analyzed herein and were all detected at on-column levels lower than 75 fg in drinking water matrices (S/N >3.) The most sensitive analytes PFHxS, PFDS and PFBS were detected at 2.6, 3.2 and 5 fg levels, respectively. Method detection limits for spiked pork liver matrix extract samples were below 600 fg on-column for the entire analyte suite. No detectable background contamination was observed in blank injections for any analyte in this study. Linearity for up to five orders of magnitude with R^2 values above 0.996 for the entire suite were recorded.

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Introduction

Exposure, bioaccumulation and potential toxicity continue to be issues in environmental biota and their food webs from emerging contaminants such as perfluoronated byproducts (PFC) from industrial processing. Since background levels of such analytes are significant and stable in the atmosphere already, it is difficult to obtain reliable and accurate low-level on-column measurements.

In this application note, we present a case in which a suite of perfluorinated carboxylates (PFCA) and sulfonates (PFSA) were screened at low fg on-column levels in a potable water matrix and in spiked (pork) liver samples with zero background interference using dynamic multiple reaction monitoring (dynamic MRM) [1]. This approach allowed us to gain reliable positive identifications and extremely low limits of detection. By utilizing an inline contaminant trap configuration we assured cleanliness of the HPLC system and allowed use of inline membrane degassing without compromising system dead-volume or analysis speed.

A comprehensive evaluation of a suite of PFCAs & PFSAs including isotopically labeled ISTDs was undertaken which examined sensitivity and linearity of each component. Appropriate dynamic MRM transitions were identified using automatic instrument optimizations of fragmentor (frag) and collision energy (CE) voltages and applied to the chromatographic method dynamically to maximize analyte signal quality at lower concentrations. Optimal settings for the Agilent Jet Stream Technology [2] were determined for the complete PFCA & PFSA suite effectively increasing the sensitivity to around 14x that of normal electrospray ionization (ESI) conditions.

Table 1. Compounds Analyzed for this Study

Target compounds	
perfluoro-1-butanesulfonate	(PFBS)
perfluoro-n-hexanoic acid	(PFHxA)
perfluoro-n-heptanoic acid	(PFHpA)
perfluoro-1-hexanesulfonate	(PFHxS)
perfluoro-n-octanoic acid	(PFOA)
perfluoro-n-nonanoic acid	(PFNA)
perfluoro-1-octanesulfonate	(PFOS)
perfluoro-n-undecanoic acid	(PFUA)
perfluoro-1-decanesulfonate	(PFDS)
perfluoro-n-dodecanoic acid	(PFDoA)
perfluoro-n-tridecanoic acid	(PFTriA)
perfluoro-n-tetradecanoic acid	(PFTA)

Experimental

This analysis was performed using an Agilent 6460A triple quadrupole LC/MS with an Agilent 1200SL Series LC system. The LC system consisted of a binary pump (G1312B), vacuum degasser (G1379B), a low carryover automatic liquid sampler (G1367D), thermostatted column compartment (G1316B) and MassHunter data system.

Sample Handling

Sample handling is a critical element in the measurement of trace amounts of perfluorinated carboxylates and sulphonates, since background levels can be prevalent and derived from laboratory consumables and protective lab-wear. The series of analyses outlined in this application note considered this and precautions were taken to eliminate any such cross-contamination. Silanized glass vials were used with aqueous diluents that had been passed through a solid-phase extraction. Nitrile rubber vial caps and non PTFE-containing pipette tips were used. Only nitrile rubber derived protective laboratory gloves were worn.

Instrumentation

Rapid Resolution HPLC Conditions and Configuration

Configuration:

Agilent 1200 Series Binary Pump SL:	(G1312B)
High Performance WP Sampler SL Plus:	(G1367D)
Sampler Thermostat:	(G1330B)
Thermostatted Column Compartment SL	(G1316B)

Method Conditions:

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 50 mm, 1.8 µm	
Column temperature:	55 °C	
Injection volume:	1 µL	
Autosampler temp:	4 °C	
Needle wash:	Flushport (100% methanol), 5 seconds	
Mobile phase:	A = 2 mM NH_4 acetate in water	
	B = 2 mM NH_4 acetate in methanol	
Gradient flow rate:	0.5 mL/min	
Gradient:	Time (min)	%B
	0	6
	0.5	6
	6	95
	8	95
Total run time:	9.0 min (including 1 min equilibration time)	

Mass Spectrometer Dynamic MRM Conditions and Configuration

Configuration:

Agilent 6460 Triple Quadrupole Mass Spectrometer equipped with Agilent Jet Stream Technology

Ion Source Conditions:

Ion mode:	ESI/Agilent Jet Stream, Negative
Capillary voltage:	3750 V
Nozzle voltage:	0 V
Drying gas (nitrogen):	4 L/min
Drying gas temperature:	320 °C
Nebulizer gas (nitrogen):	60 psi
Sheath gas temperature:	350 °C
Sheath gas flow:	12 L/min

Dynamic MRM acquisition:

Cycle time:	250 ms
Total dynamic MRMs:	29
Maximum concurrent MRMs:	12
Retention time window:	30 sec
Minimum/maximum dwell:	17.33/246.50 ms
Q1 and Q2 resolution:	0.7 amu [unit]
Delta EMV:	0 V

Dynamic MRM triple quad MS parameters are listed in Table 2. All fragmentor voltage (frag) settings, respective collision energies (CE), and most abundant MS/MS product ions per analyte were determined automatically using the Agilent MassHunter Optimizer software.

Ion Source Optimization

In order to achieve the optimal and most sensitive Agilent 6460 ESI MS source conditions for the complete suite of analytes, each dynamic MRM method transition was measured using a single mixed standard repetitively. In addition, each subsequent sample injection was also measured using a systematic and single source parameter change. This was to obtain the best and most sensitive method conditions for an optimized method, but only had to be undertaken once.

In reality, a single set of source parameter conditions are not necessarily the optimum settings for all analytes in a suite (or assay) so a compromise set of conditions were determined for the suite of perfluorinated analytes. A subsequent

Table 2. Dynamic MRM PFSA/PFCA Settings

Compound name	Precursor ion mass	Q1-resolution	Product ion mass	Q2-resolution	Fragmentor voltage	Collision energy (eV)	Retention time (min)	RT delta (min)	Ion polarity
PFBS	298.9	unit	80	unit	133	45	3.623	1	Negative
PFBS (Q)	298.9	unit	98.9	unit	133	29	3.623	1	Negative
PFDA	512.9	unit	469	unit	102	5	5.543	1	Negative
PFDA (C13)2	514.9	unit	469.9	unit	102	5	5.542	1	Negative
PFDoA	612.9	unit	569	unit	97	5	5.961	1	Negative
PFDoA (C13)2	614.9	unit	570	unit	97	5	5.961	1	Negative
PFDoA (Q)	612.9	unit	169	unit	97	25	5.961	1	Negative
PFDS	598.9	unit	80	unit	205	94	5.752	1	Negative
PFHpA	362.9	unit	319	unit	66	5	4.626	1	Negative
PFHpA (Q)	362.9	unit	169	unit	66	13	4.626	1	Negative
PFHxA	312.9	unit	268.9	unit	66	5	4.143	1	Negative
PFHxA (C13)2	314.9	unit	269.9	unit	66	5	4.141	1	Negative
PFHxS	398.9	unit	80	unit	174	49	4.671	1	Negative
PFHxS (O18)2	402.9	unit	83.9	unit	174	49	4.671	1	Negative
PFHxS (Q)	398.9	unit	99	unit	174	45	4.671	1	Negative
PFNA	462.9	unit	418.9	unit	66	5	5.296	1	Negative
PFNA (C13)5	467.9	unit	423	unit	66	5	5.296	1	Negative
PFNA (Q)	462.9	unit	169	unit	66	17	5.296	1	Negative
PFOA	412.9	unit	368.9	unit	86	5	5.003	1	Negative
PFOA (C13)4	416.9	unit	371.9	unit	86	5	5.001	1	Negative
PFOA (Q)	412.9	unit	169	unit	86	13	5.003	1	Negative
PFOS	498.9	unit	80	unit	210	50	5.302	1	Negative
PFOS (C13)4	502.9	unit	80	unit	210	50	5.301	1	Negative
PFOS (Q)	498.9	unit	99	unit	210	50	5.302	1	Negative
PFTA	712.9	unit	669	unit	112	9	6.255	1	Negative
PFTriA	662.9	unit	619	unit	102	9	6.117	1	Negative
PFUA (C13)2	564.9	unit	519.9	unit	92	5	5.764	1	Negative
PFUA	562.9	unit	519	unit	92	5	5.762	1	Negative
PFUA (Q)	562.9	unit	169	unit	92	21	5.762	1	Negative

technical note that details the complete source optimization of this suite of compounds will soon be published.

Results and Discussion

Inline Contaminant Trapping

For highly sensitive measurements of PFCAs and PFSAs at low femtogram on-column levels, it was necessary to ensure the removal of background PFC contamination derived from sample work-up, mobile phase impurities or instrument components. PFCAs and PFSAs are typically hard to break down naturally. Their precursors are widely released into the atmosphere which are degraded to terminal PFCAs and PFSAs.

One approach is to stop PFCAs and PFSAs from entering the high-pressure HPLC flow system by effectively trapping them using a small inline reverse phase column or cartridge immediately after the respective pump head, prior to the point at which the gradient mix is achieved. Figure 1 schematically shows this configuration with a low dead-volume binary pump setup.

The positioning of the inline contaminant trap [Agilent ZORBAX Eclipse Plus C18 (4.6 mm × 30 mm, 3.5 µm, p/n-959936-902)] was prior to the mixing point of the gradient pump and on the aqueous pump channel (in this case Pump A.) It was exposed to a 100% isocratic aqueous mobile phase and effectively trapped all PFCA and PFSA contaminants from entering the HPLC flow path. Further, since the inline trap was before the gradient mix point, it had zero dead-volume implications to the HPLC separations. *It must be noted that this is a nonstandard configuration and as such may not be supported by Agilent Technologies.*

Moreover, extreme care regarding sample handling techniques was observed so that PFCAs and PFSAs were not introduced artificially during the preparation process. For example, careful choice of silanized glass vials with rubber septa were a necessity as were the use of non PTFE-containing pipette tips and protective clothing (nitrile rubber gloves). Sample diluent was also isocratically pumped through a C18 flash column to remove background PFCAs and PFSAs prior to use.

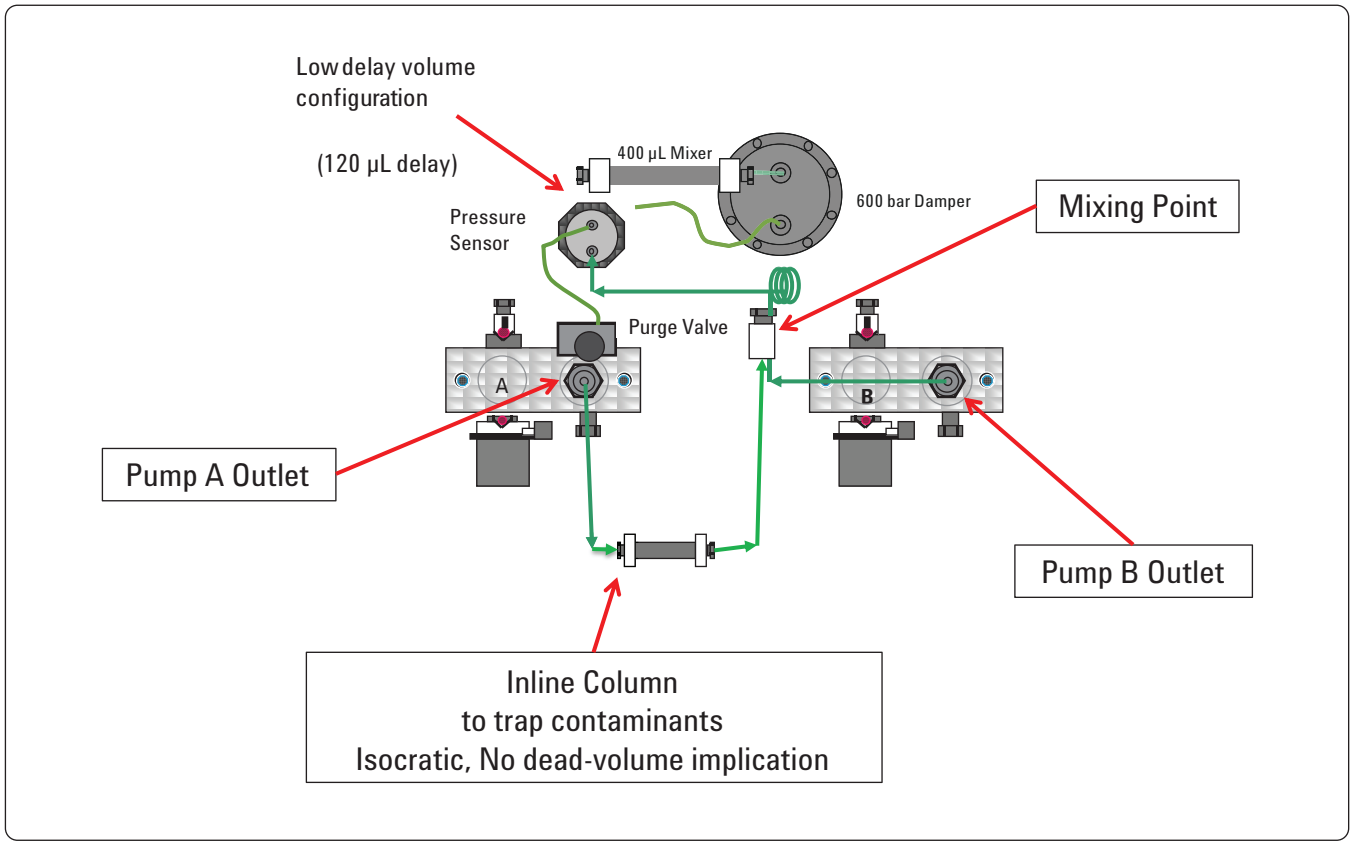

Figure 1. Inline contaminant trapping schematic.

A typical low-level injection (10 fg on-column) featuring a PFHxS (transition 398.9→80 *m/z*) is illustrated in Figure 2 in triplicate, together with a blank injection prior to these analyses. The blank sample baseline was completely clear of residual PFHxS, as a result of the cleanliness of the HPLC system from the inline contaminant trap. This was also true for all other analytes in this study; due to space restrictions, only PFHxS is shown here. The complete set of data will be published in a future application note.

Figure 2 also indicates the outstanding high level of sensitivity of the Agilent 6460 triple quad MS for the negative polarity analysis of such perfluorinated analytes spiked into potable water matrix. In this study, LODs for PFHxS were the lowest for the suite, giving an average of 2.6 fg on-column over triplicate injections and defined as having a signal-to-noise (S/N) ratio of greater than 3. All other analytes in the PFC suite exhibited LODs of less than 75 fg on-column. Figure 3 illustrates an overlaid chromatogram for all PFC analytes at a level of 100 fg on-column.

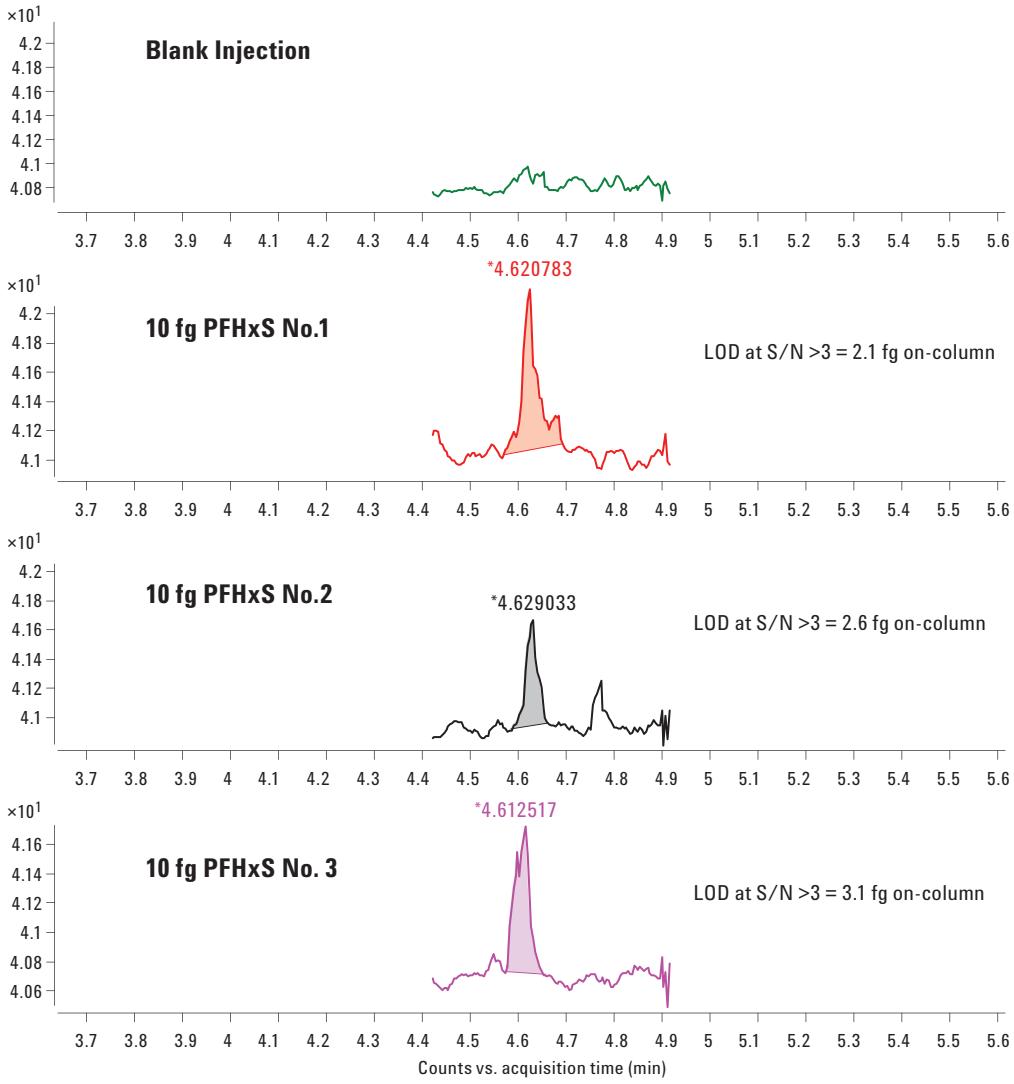

Figure 2. Blank injection with 3x replicates of PFHxS Standard, 10 fg on-column, spiked potable water.

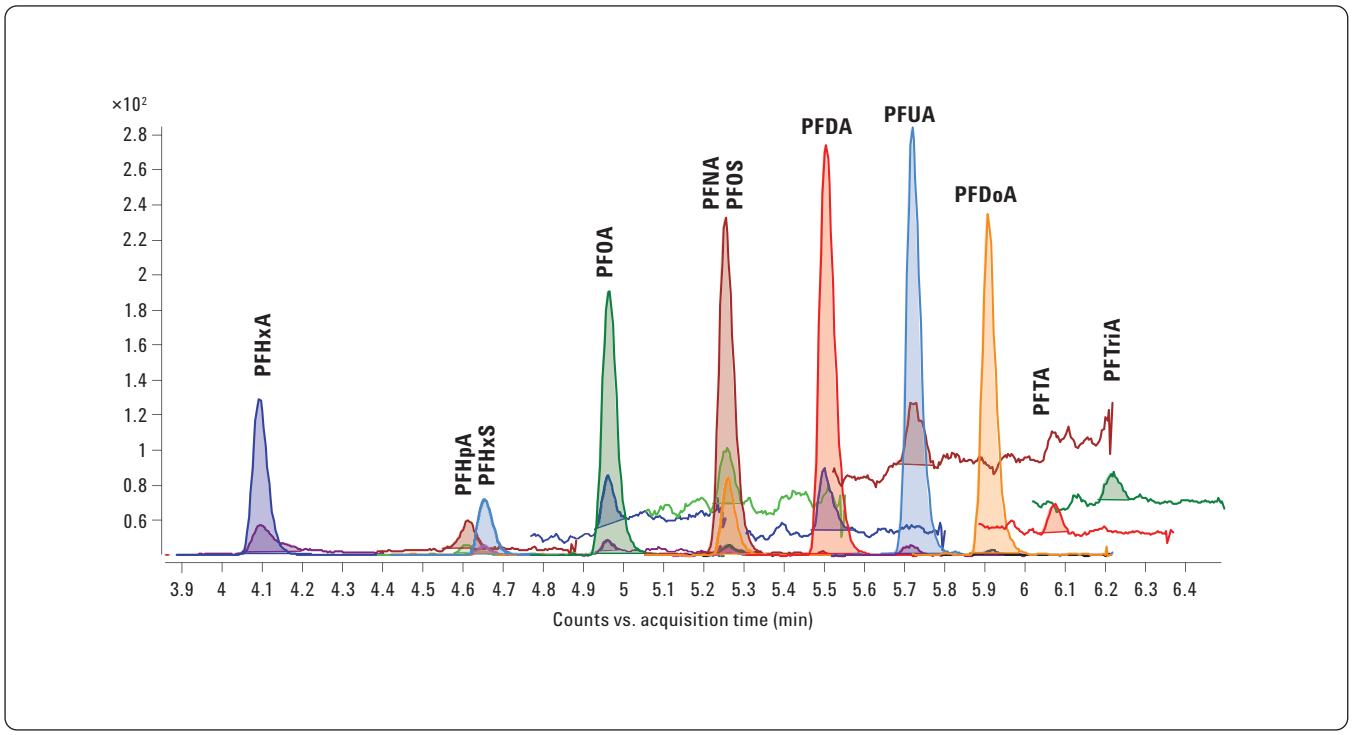

Figure 3. PFCA/PFSA Suite dynamic MRM chromatogram (overlaid) at 100 fg with quantifier and qualifier ions (spiked potable water).

Limits Of Detection (Potable Water Spiked Samples)

Table 3 outlines the limit of detection (LOD) values observed for the suite of PFCs undertaken in this study and spiked into untreated potable water matrix. All PFCA/PFSA LODs in this evaluation were below a value of 75 fg on-column. They were achieved with no background carryover at extremely high sensitivity by careful optimization of fragmentation and collision energy parameters and careful fine-tuning of Agilent Jet Stream and ion source parameters.

A typical ISTD-corrected calibration curve for one of the analytes in the suite (PFOS) is outlined in Figure 4. The linearity R^2 value was found to be 0.99957820 for triplicate injections for more than five orders of magnitude.

Table 3. LOD Results for Spiked Potable Water Samples

Compounds	LOD (fg on column, S/N >3)
PFBS	5
PFHxA	8.4
PFHpA	12.2
PFHxS	2.6
PFOA	43.7
PFNA	75
PFOS	5.7
PFDA	36.3
PFUA	44
PFDS	3.2
PFDoA	55.9
PFTriA	74.2
PFTA	21.7

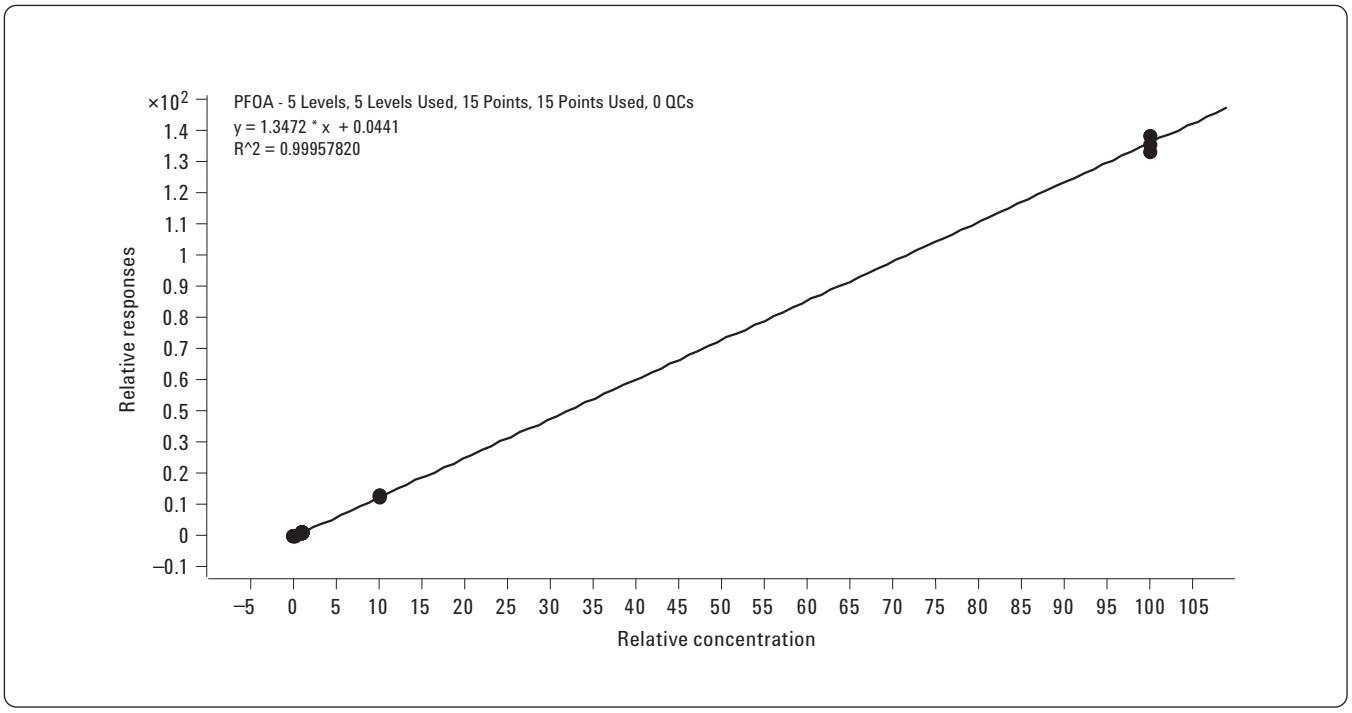

Figure 4. PFOA linearity over five orders of magnitude in potable water (10 fg – 100 pg on-column. [N = 3]).

Method Detection Limits (Spiked Pork Liver Samples)

Table 4 summarizes the method detection limits (MDL) which were observed for each individual PFCA or PFSA analyte when applied to spiked liver extracts.

More than half of the compounds showed a precision value significantly less than 10% RSD (based on peak area) at this challenging MDL concentration.

MDL values in spiked pork liver extracts ranged between 600 fg and 45 fg on-column for this reported methodology.

Table 4. PFCA/PFSA Method Detection Limits

Compounds (spiked pork liver extract)	Method detection limit (fg on column, S/N >10)
PFBS	97.7
PFHxA	110.5
PFHpA	249
PFHxS	44.62
PFOA	291.5
PFNA	421.3
PFOS	58.3
PFDA	275.3
PFUA	303.9
PFDS	54.9
PFDoA	594.5
PFTriA	494.5
PFTA	503.2

Conclusions

A highly sensitive low-femtogram dynamic MRM Agilent 6460 triple quad LC/MS method has been presented for the analysis of a suite of PFCAs and PFSAs analytes that illustrates excellent precision at low-femtogram on-column levels in a complex food matrix and potable water.

Background PFCA and PFCS interferences normally associated with low-level analyses of such perfluorinated suites were eliminated by careful preparation of samples, sample handling and an inline flow contaminant trapping cartridge set-up within the HPLC flow path.

Complete ion source optimization was undertaken for each of the analytes in the suite. This effectively increased the analytical sensitivity by at least a factor of 14x compared with standard ESI source settings.

References

1. "New Dynamic MRM Mode Improves Data Quality and Triple Quad Quantification in Complex Analyses," Agilent Technologies publication 5990-3595EN.
2. "Agilent Jet Stream Thermal Gradient Focussing Technology," Agilent Technologies publication 5990-3494EN.

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