Application Note: 51936

Sensitive and Accurate Quantitation of Perfluorinated Compounds in Human Breast Milk using Selected Reaction Monitoring Assays by LC/MS/MS

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Key Words

- PFC-free Accela
- TSQ Vantage
- High Resolution MS
- H-SRM
- Perfluorinated Compounds

Overview

Perfluorinated compounds (PFCs) are ubiquitous and persistent pollutants that bioaccumulate in animals and humans. The potential toxicity of these chemicals has fueled efforts to develop robust analytical techniques for measuring low levels of PFCs in human matrices. Quantitative selected reaction monitoring (SRM) assays were developed for six PFCs using the Thermo Scientific TSQ Vantage triple-stage quadrupole mass spectrometer (MS) coupled to a PFC-free Thermo Scientific Accela LC system. Using this method, PFCs were accurately and reproducibly detected at ppt concentrations in neat solution and in human milk matrix. Exceptionally sensitive and accurate, this integrated LC-MS platform is ideally suited for robust ultra-trace analysis of PFCs in a wide range of matrices.

Introduction

The unique water-, oil-, grease-, stain- and heat-resistant properties of perfluorinated compounds (PFCs) have led to their widespread use in diverse industrial applications and multiple consumer products for over fifty years. Resistant to degradation, many of these synthetic compounds have become persistent and ubiquitous environmental pollutants. Bioaccumulation of PFCs in wildlife and in humans as well as studies linking some of these chemicals to developmental, reproductive and systemic toxicity in laboratory animals have led to efforts to regulate these compounds and have prompted the need for PFC monitoring and risk assessment in humans.[1,2] PFCs are detectable in human serum and breast milk and have even been found to be present in the blood of newborns, possibly through lactational transfer from mothers.[3] Determination of exposure pathways and health outcomes requires sensitive and accurate methods for trace-level analysis of PFCs in a range of human and environmental matrices.

PFCs encompass neutral and ionic species that contain the perfluorinated alkyl moiety. While gas chromatography-mass spectrometry (GC/MS) methods have been used to analyze volatile neutral PFCs and derivatives of ionic PFCs, many of these chemicals are more amenable to other analytical techniques. Liquid chromatography-tandem mass spectrometry (LC/MS/MS) is the method of choice for the analysis of ionic PFCs in a variety of matrices, but accurate quantification has proven to be difficult using this technique due to background PFC contamination and matrix interferences. These analytical challenges underscore the need for a high performance LC-MS platform capable of exceptional sensitivity, selectivity and accuracy.

The TSQ Vantage™ triple-stage quadrupole MS coupled to the Accela™ high speed LC system enables rapid, accurate and robust LC/MS/MS analysis of small molecules and biomolecules. Delivering up to a 10-fold improvement in signal-to-noise ratio compared to existing triple-quadrupole MS systems, the TSQ Vantage mass spectrometer facilitates high-sensitivity quantitation in matrix-rich samples and enhances analytical accuracy and precision. The instrument is capable of high resolution (0.2 Da. FWHM) selection of precursor ions, enabling highly selective reaction monitoring (H-SRM) for greater analytical selectivity and accuracy. The Accela system, together with 1.9 µm particle columns, enables fast and efficient chromatographic separations over an expansive range of flow rates and pressures.

In this note, we demonstrate highly sensitive, accurate and reproducible analysis and quantitation of six PFCs in neat solution and in human breast milk matrix using selected reaction monitoring (SRM) and H-SRM on the integrated UHPLC Accela-TSQ Vantage LC-MS platform. Elimination of PFC contamination from the analytical system was achieved by using a PFC-free Accela pump with a pre-cleaned PFC-free degasser and replacing Teflon® tubing with PEEK tubing. The excellent sensitivity and selectivity afforded by SRM on the TSQ Vantage system obviated the need for any further modifications of the LC configuration, a distinct advantage over other commercial platforms that require the use of in-line contaminant traps or column-switching methods for PFC analysis.

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Materials and Methods

Sample Preparation

PFC Standard Solutions

Standards for perfluoro-1-butanesulfonate (PFBS), perfluoro-1-hexanesulfonate (PFHxS), perfluoro-n-heptanoic acid (PFHpA), perfuoro-1-decanesulfonate (PFDS), perfluoro-n-undecanoic acid (PFUnA), and perfluoro-n-dodecanoic acid (PFDoA) were obtained from a proprietary source. A stock solution of a mixture of these six PFCs was prepared at a concentration of 1 mg/L. Calibration solutions, with concentrations of 0.04-2.5 ng/mL (ppb), were prepared by serial dilution of the stock solution in 60:40 (v/v) methanol/water. Two internal standards, m-PFUnA and m-PFHxS, were added into each calibration solution and sample at 2 ng/mL (ppb) concentration.

Milk Matrix A

A 2 g human breast milk sample, obtained from a proprietary source, was diluted in acetonitrile to precipitate proteins. Weak anion exchange solid-phase extraction was performed and the resulting PFC extract was eluted using 2% ammonium hydroxide in methanol, evaporated to dryness and reconstituted in 60% methanol/water (0.6 mL).

Milk Matrix B

The six PFCs were spiked into Matrix A at concentrations of 0.1 ng/mL to generate Matrix B.

Milk Matrix C

To generate Matrix C, six PFCs were spiked into Matrix A at concentrations of 0.3 ng/mL.

Milk Matrix D

Matrix D was prepared by spiking the six PFCs into Matrix A at concentrations of 1.0 ng/mL.

LC/MS Analysis

Instrumentation

LC/MS analysis was performed on a PFC-free Accela 600 LC system and PAL autosampler coupled to a TSQ Vantage triple-stage quadrupole mass spectrometer. The PFC-free Accela pump was equipped with a pre-cleaned PFC-free degasser and all Teflon tubing was replaced with PEEK tubing.

LC Parameters

Column:	Thermo Fisher Scientific Hypersil GOLD PFP column (100 x 3 mm, 1.9 µm particle size)			
Mobile Phase:	A: 5 mM ammonium acetate and 10% methanol/water B: 2 mM ammonium acetate/99% methanol			
Flow Rate:	see gradient			
Column Temperature:	ambient			
Sample Injection Volume:	10 µL			
Gradient:	Time (min)	A%	B%	Flow rate (µL/min)
	0.0	70	30	400
	0.5	70	30	400
	1.0	54	46	400
	4.0	30	70	400
	9.0	12	88	400
	9.4	12	88	400
	9.6	0	100	400
	9.7	0	100	500
	11.0	0	100	500
	11.1	70	30	500
	14.5	70	30	500
	15.0	70	30	400

MS Parameters

Negative Ion Mode Ionization with HESI Probe	
Heated Electrospray Ionization Source Conditions:	
Spray Voltage:	3500 V
Capillary Temperature:	300 °C
Sheath Gas:	60 au
Auxiliary Gas:	15 au
Vaporizer Temperature:	400 °C
Resolution for SRM Setup:	Q1, Q3 = Unit [0.7 Da. FWHM]
Resolution for H-SRM Setup:	Q1 = 0.2 Da. FWHM; Q3 = 0.7 Da. FWHM

#	Parent	Product	Collision Energy	RT Start	RT End	S-Lens	Name
1	299.0	80.2	43	3.15	4.15	115	PFBS
2	299.0	99.2	34	3.15	4.15	115	PFBS
3	299.0	169.0	23	3.15	4.15	115	PFBS
4	399.0	80.2	45	4.7	5.7	89	PFHxS
5	399.0	99.2	35	4.7	5.7	89	PFHxS
6	399.0	169.1	29	4.7	5.7	89	PFHxS
7	403.0	84.2	43	4.7	5.7	89	m-PFHxS
8	403.0	103.2	37	4.7	5.7	89	m-PFHxS
9	363.0	169.0	10	5.0	6.0	51	PFHpA
10	363.0	319.0	17	5.0	6.0	51	PFHpA
11	598.9	99.1	47	7.1	8.1	128	PFDS
12	598.9	230.1	50	7.1	8.1	128	PFDS
13	598.9	80.3	47	7.1	8.1	128	PFDS
14	562.9	269.0	18	7.75	8.75	62	PFUnA
15	562.9	519.0	12	7.75	8.75	62	PFUnA
16	564.9	520.0	18	7.75	8.75	64	m-PFUnA
17	612.9	169.0	25	8.4	9.4	78	PFDoA
18	612.9	569.0	12	8.4	9.4	78	PFDoA

Table 1: SRM transitions monitored for the detection of PFBS, PFHxS, PFHpA, PFDS, PFUnA and PFDoA.

Results and Discussion

Separation of PFC Standards

A total of fifteen unique SRM transitions were monitored for PFBS, PFHxS, PFHpA, PFDS, PFUnA and PFDoA, and three were monitored for the internal standards m-PFHxS and m-PFUnA (Table 1). Using the modified PFC-free LC-MS platform, a mixture of the six PFC standards was separated and detected under 10 minutes (Figure 1). All of the compounds were baseline resolved with the elution order of PFBS, PFHxS (m-PFHxS), PFHpA, PFDS, PFUnA (m-PFUnA) and PFDoA. As the majority of interferences from matrices elute early at void volume, elution of the first compound at 3.64 min ensured a robust quantitation method.

Linearity and Sensitivity

Excellent linearity in detector response was observed over the range of 0.04-2.5 ppb, with correlation coefficients greater than 0.999 for all transitions. Representative calibration curves for PFBS and PFUnA, obtained using the internal standard method, are shown in Figure 2, with coefficients of 0.9997 and 0.9996 respectively.

The sensitivity of the method is dependent on the levels of interferences that are present in the blank and in the solvents used. Limits of detection (LODs) and limits of quantitation (LODs), defined as S/N ratio of 3 and 10, respectively, are shown in Table 2. LODs ranged from 2–174 ppt, and LOQs ranged from 5–756 ppt. PFBS and PFDS were detectable at 2 ppt and quantifiable at 5 ppt. Figure 3 shows the separation and detection of 10 ppt PFBS and 10 ppt PFDS at different SRM transitions, and the corresponding blanks as comparisons. The higher LOD and LOQ values observed for PFHpA, PFUnA and PFDoA may be attributed to interferences present in the blank and mobile phases.

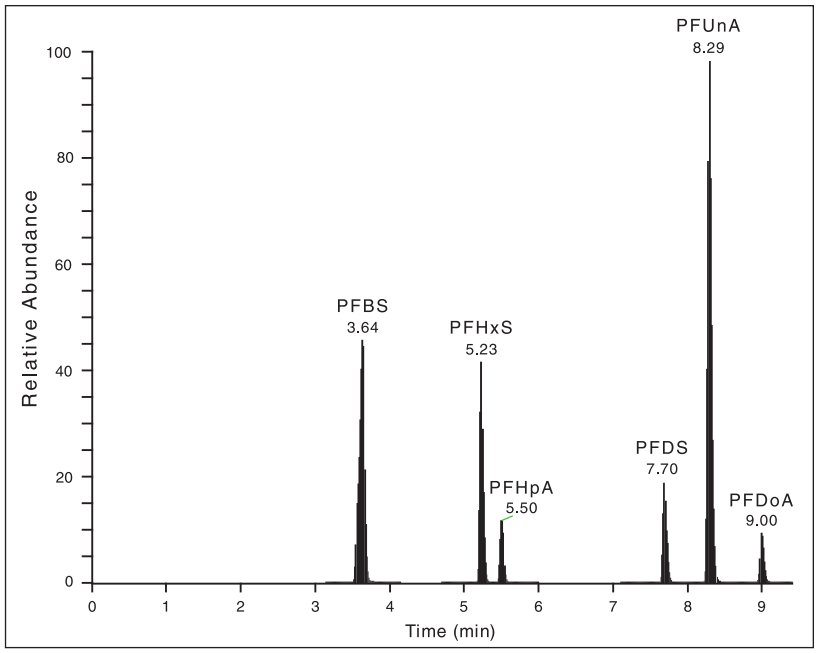

Figure 1: Separation and detection of six PFC standards at 2.5 ppb concentrations.

Compounds	SRM	LOD (ppt)	LOQ (ppt)		
PFBS	298.9 > 80.2	2	5	SRM	
	298.9 > 99.2	5	12	SRM	
PFHxS	398.9 > 80.2	21	83	SRM	
	398.9 > 99.2	12	66	SRM	
PFHpA	362.9 > 169.0	174	756	SRM	Blank Contamination
	362.9 > 319.0	120	457	SRM	Blank Contamination
PFDS	598.9 > 80.2	2	7	SRM	
	598.9 > 99.2	3	9	SRM	
PFUnA	562.9 > 269.0	35	156	SRM	
	562.9 > 519.0	52	235	SRM	
PFDoA	612.9 > 169.0	59	296	SRM	
	612.9 > 569.0	64	295	H-SRM	

Table 2: LODs and LOQs of the PFC standards. LOQs were estimated from triplicate injections (CV < 15%) of standard solutions at concentration levels corresponding to a signal-to-noise ratio of 10.

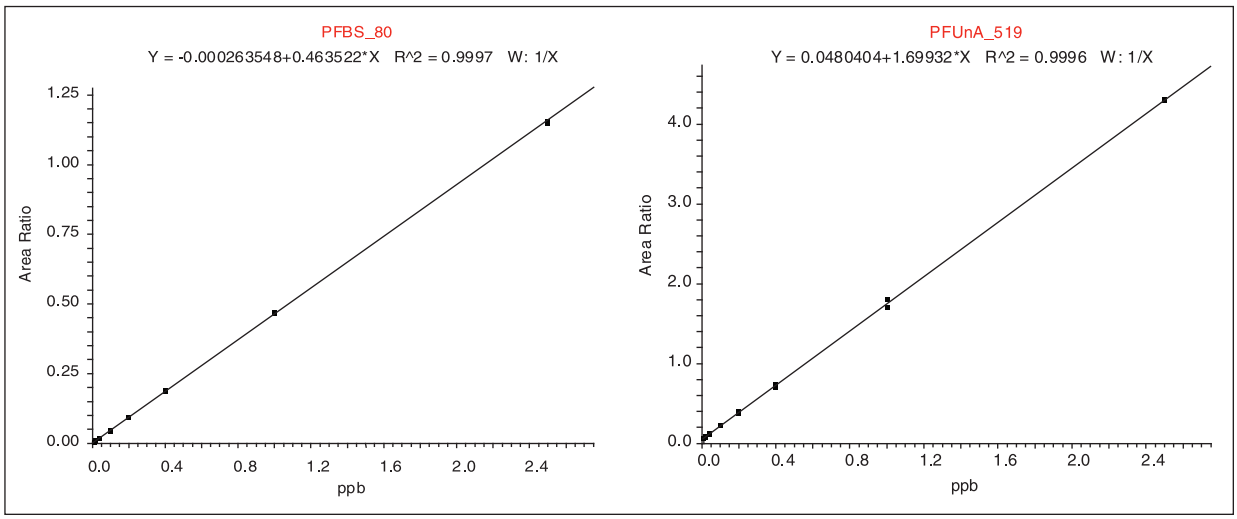

Figure 2: Representative calibration curves of PFBS and PFUnA standards.

Significant background interference was observed for the SRM transition 613 > 569 of PFDoA at Q1 resolution of 0.7 Da. FWHM, therefore H-SRM was employed. As shown in Figure 4, using the higher Q1 resolution of 0.2 Da. FWHM removed the matrix interference without compromising sensitivity. Moreover, sensitive and unambiguous PFC detection was achieved without the use of in-line trapping or column switching.

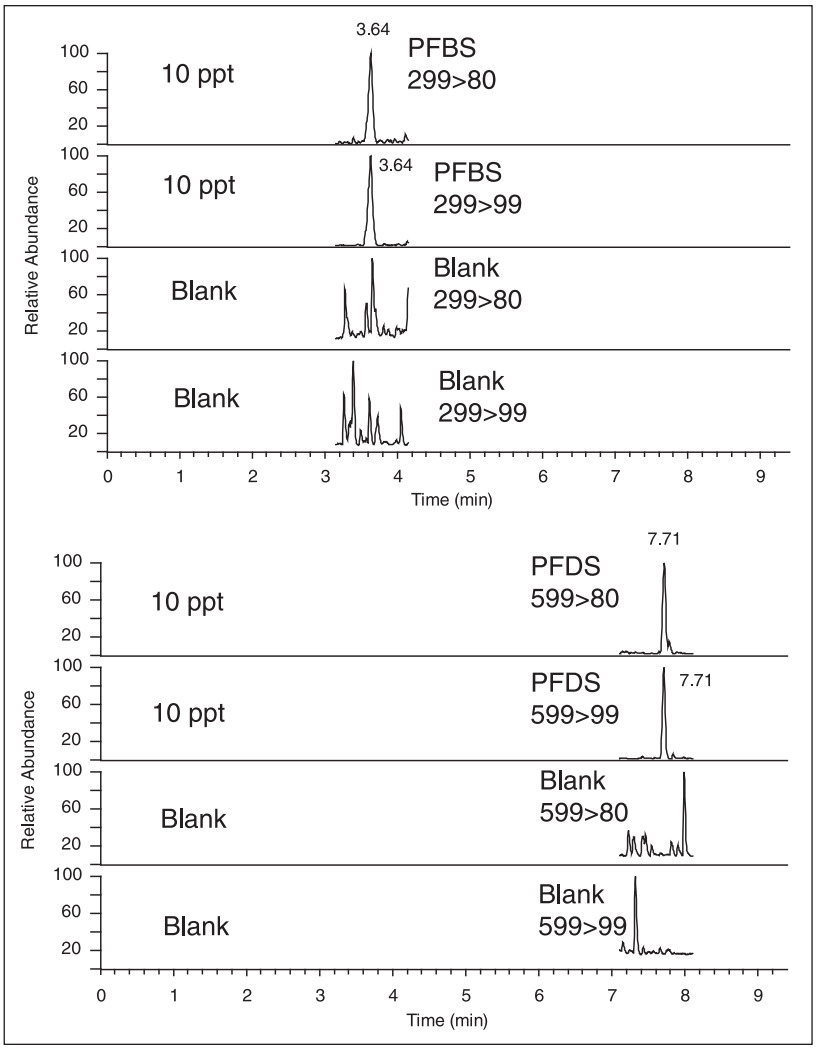

Figure 3. Separation and detection of 10 ppt of PFBA and PFDS.

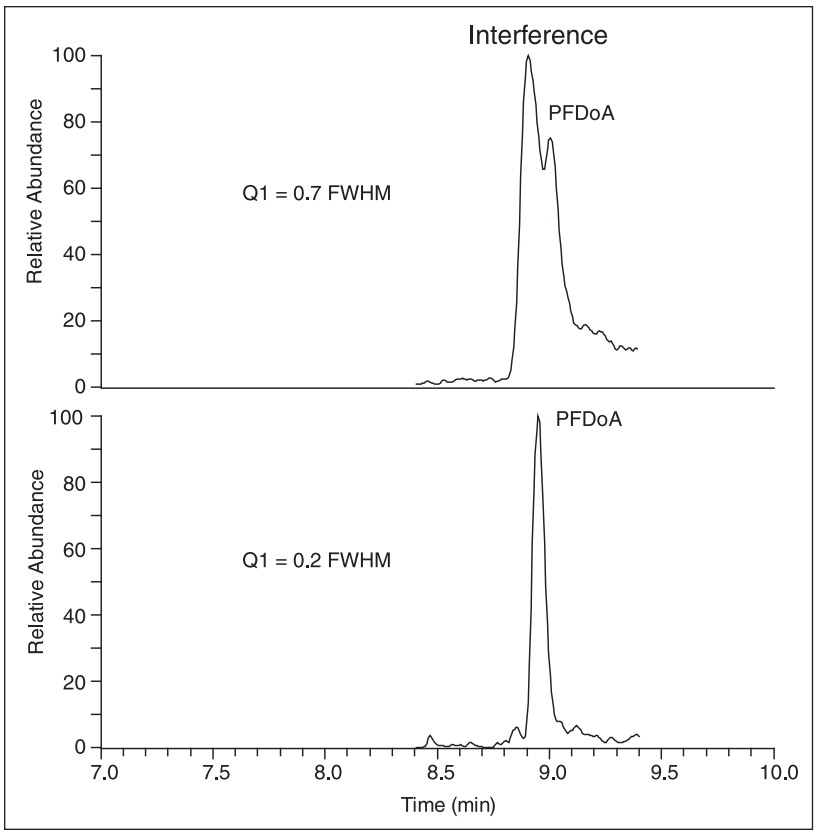

Figure 4: H-SRM eliminates interference peaks without any change in signal intensity.

Analysis of PFCs in Human Milk Matrix

To evaluate the applicability of this technique to complex matrices, the SRM assays were used to analyze and quantitate PFCs in human breast milk. UHPLC separation of the six PFC analytes in a spiked milk matrix was achieved within 9 minutes (Figure 5). All analytes were baseline resolved using the optimized LC method.

Reproducibility was investigated by analyzing fifteen replicate injections of a spiked matrix (Table 3). Peak area RSDs for compounds and internal standards were 10.8% and 11.0% respectively, the response ratio RSD was 1.29%, and retention time RSD was 0.29%, indicating excellent method and system reproducibility, particularly of the LC pump.

File Name	Peak Area	ISTD Area	Response Ratio	RT (Min)
Mark D_0 17	149 369	8 268 9	1.806	8.29
Mark D_0 18	147 075	8 081 9	1.820	8.27
Mark D_0 19	145 882	8 127 6	1.795	8.29
Mark D_0 20	146 012	7 990 7	1.827	8.29
Mark D_0 21	143 987	8 071 2	1.784	8.27
Mark D_0 22	143 095	8 011 6	1.786	8.25
Mark D_0 23	140 298	7 802 3	1.798	8.25
Mark D_0 67	121 597	6 929 2	1.755	8.25
Mark D_0 68	119 763	6 776 4	1.767	8.29
Mark D_0 69	119 149	6 654 3	1.791	8.27
Mark D_0 70	121 775	6 647 6	1.832	8.32
Mark D_0 71	113 885	6 376 6	1.786	8.27
Mark D_0 72	115 138	6 271 2	1.836	8.31
Mark D_0 73	116 884	6 561 6	1.781	8.24
Mark D_0 74	114 601	6 358 6	1.802	8.31
RSD%	**11**	**10.8**	**1.29**	**0.29**

Table 3: Reproducibility (RSD) of instrument performance for fifteen replicate injections of Matrix D. Peak area is the LC peak area response for fifteen injections. Peak area was used for quantitation, both for the internal standard method and external standard method. ISTD area = peak area of the internal standard. Response ratio is the peak area of the compounds over the peak area of the internal standard, and was used for quantitation with the internal standard method. RT = retention time.

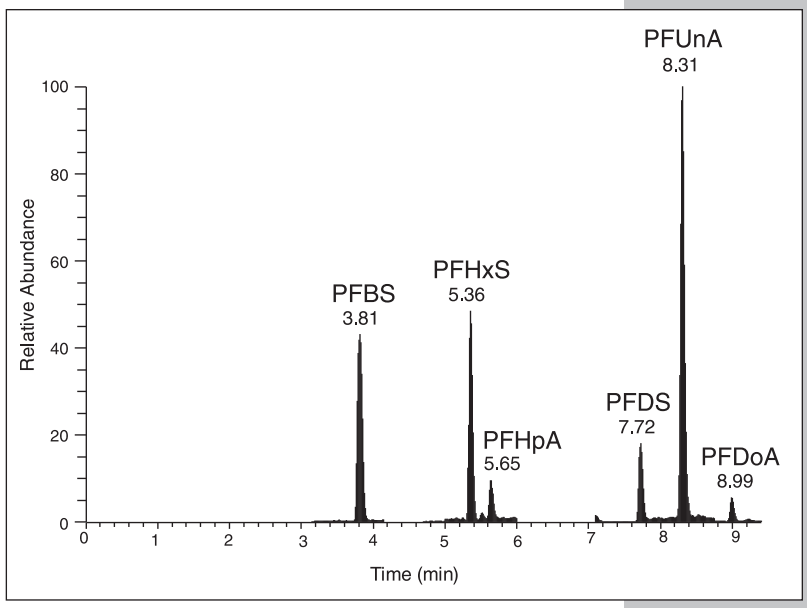

Figure 5: The separation and detection of the PFCs in human milk matries C

Table 4 summarizes the concentrations of the PFCs detected in a human milk sample (Matrix A). PFBS, PFHxS, PFHpA, PFDS, and PFUnA were detected at concentrations of less than 60 ppt, while PFDoA was not found to be present in the sample. Assay accuracy was investigated using spiked milk matrices B, C, and D and internal and external standards (Table 3). For PFHxS and PFUnA, the two PFCs for which internal standards were available, using the internal standard method was significantly more accurate (98–110%) than the external standard method (81–144%) in the concentration range 0.1–1.0 ng/mL. While internal standards eliminate the matrix effect to facilitate greater quantitative accuracy, they are expensive and may be difficult to obtain. Using the external standard method, the accuracy of all PFC analytes was 81–144% in the concentration range 0.1–1.0 ng/mL.

		PFBS	PFHxS	PFHpA	PFDS	PFUnA	PFDoA
Matrix A (unknown)	Measured value with IS (ppt)		48.0			12.0	
	Measured value with ES (ppt)	10.0	40.0	50.0	50.0	35.0	0.0
Matrix B (Matrix A + spiked 100 ppt)	Measured value with IS (ppt)		152			115	
	Measured value with ES (ppt)	110	145	185	150	195	130
	Method Accuracy with IS (%)		103			103	
	Method Accuracy with ES (%)	100	104	123	100	144	130
Matrix C (Matrix A + spiked 300 ppt)	Measured value with IS (ppt)		382			340	
	Measured value with ES (ppt)	260	290	365	285	420	280
	Method Accuracy with IS (%)		110			109	
	Method Accuracy with ES (%)	84	85	104	81	125	93
Matrix D (Matrix A + spiked 1000 ppt)	Measured value with IS (ppt)		1023			1042	
	Measured value with ES (ppt)	930	945	1255	935	1495	985
	Method Accuracy with IS (%)		98			103	
	Method Accuracy with ES (%)	92	91	120	89	144	99

Table 4: PFC concentrations (ppt) in human milk matrix A and spiked milk matrices B, C, and D. Note: The method accuracy was calculated with the formula of 100 x measure value/(measure value of Matrix A + spiked value).

Conclusion

A highly sensitive, accurate and robust SRM-based approach for PFC analysis was developed on a PFC-free Accela-TSQ Vantage LC-MS platform. PFCs were accurately and reproducibly detected at ppt levels in neat solution and in human milk. The unique H-SRM capability of the TSQ Vantage instrument removed interference peaks and significantly improved selectivity. Furthermore, unlike other approaches, this platform does not require trapping or column switching techniques to ensure exceptional sensitivity in high chemical backgrounds.

References

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