GenX and legacy PFAS analysis in water and sediment with high selectivity and sensitivity

Novel PFAS compounds detected using the X500 QTOF series system

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GenX is a replacement chemical for PFOA that is used industrially as a polymerization aid during fluoropolymer manufacturing. Recent studies have shown high levels of GenX contamination in the environment near manufacturing plants in North Carolina and the Netherlands (for example, in surface, ground and drinking waters, sediments, vegetation).[1, 2] In addition, the use of high resolution accurate mass spectrometry (HRMS) has detected many other PFECAs and GenX-related PFAS compounds.[3] As such, monitoring lists have expanded to capture these "novel" PFAS compounds.

Method detection limits are often limited in environmental samples because of high background levels. HRMS is well suited for analyzing compounds in complex matrices (such as sediments) due to monitoring high resolution accurate mass fragments (Figure 1). This allows for greater selectivity as compared to nominal mass spectrometry instruments.

In this application note, the analysis of perfluoroalkyl ether carboxylic acids (PFECAs), including GenX (HFPO-DA) and shorter-chain analogues, in water and sediment using targeted MRM^{HR} acquisition with the SCIEX X500 QTOF series system is described. In addition, two legacy PFAS compounds – PFOS and PFOA – were included for comparison. MRM^{HR} acquisition allowed for low to mid parts-per-trillion detection limits with enhanced selectivity.

Key features of the X500 QTOF series system

- The X500 QTOF series system is easy to use, data processing is performed with the intuitive but powerful SCIEX OS software
- MRM^{HR} acquisition uses compound-specific parameters (DP, CE) resulting in high sensitivity needed to achieve low LOQs
- High resolution fragment ions result in greater selectivity, reducing false negatives in complex environmental matrices

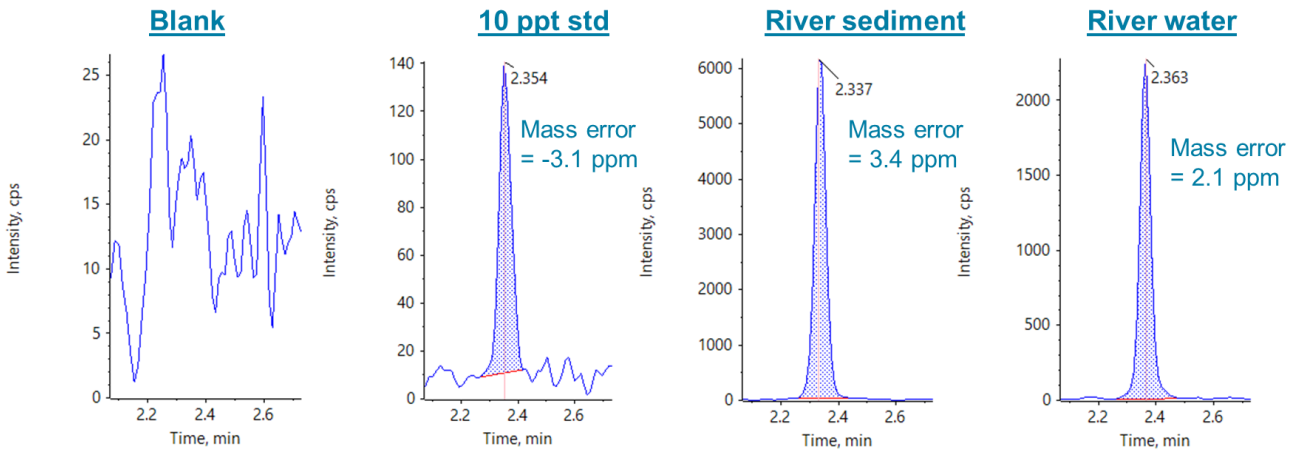

Figure 1. High resolution extracted ion chromatograms (XICs) of GenX PFAS. MRM^{HR} XIC and fragment mass error for *HFPO-DA* (m/z 329.0>284.9779) in blank, 10 ppt (pg/mL) standard, river sediment and river water.

Methods

Sample preparation: River water and sediment samples were collected from the Cape Fear River in Wilmington, NC using methanol-cleaned HDPE bottles and bags. Sample preparation methods followed published methods.[4] River water samples were filtered and extracted using Oasis WAX Plus SPE cartridges and the final eluate was reduced to 1 mL. Sediment samples were dried at 40 ºC and extracted with 20:80 MilliQ water:methanol three times. Extracts were cleaned with ENVI-CARB SPE cartridges and the eluant was reduced to 0.5 mL.

Analytical standards: Target analytes were purchased from commercial sources and included 4 PFECAs (PFMOAA, PFMoPrA, PFMOBA, HFPO-DA), as well as PFOS and PFOA (Figure 2).

Chromatography: Chromatography was performed using a SCIEX ExionLC system. Analytes were separated using a Phenomenex Luna Omega PS C18 column (100 Å, 50 x 2.1 mm, 1.6 µm particle size) using a flow rate of 0.4 mL/min. A delay column was used to separate the analyte peaks from PFAS contamination originating from the LC system. The mobile phases were water (A) and methanol (B), both modified with 10 mM ammonium acetate. The column oven was at 40°C and the injection volume was 10 µL. Initial conditions were 10% B, immediately ramped to 55% and then ramped to 70% B over 2.9 min. The gradient was then ramped to 99% B over 0.1 min, held for 0.9 min and returned to initial conditions for a total run time was 6.5 min.

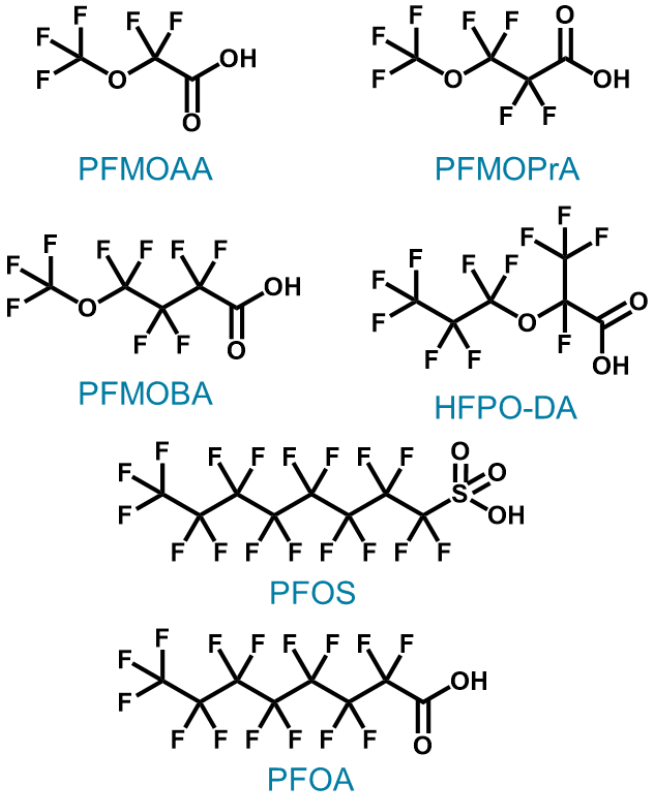

Figure 2. Chemical structures of PFAS analytes monitored.

Table 1. Compound information. Precursor and fragment m/z, fragment formula and optimized DP and CE values for PFAS analytes were monitored during the study. Note PFMOAA, PFMOPrA and PFMOBA showed only 1 stable fragment.

Analyte	Precursor m/z (Da)	Fragment m/z (Da)	Fragment formula	DP	CE
PFMOAA	*179.0*	*84.9907*	$[CF_3O]^-$	*-30*	*-12*
PFMOPrA	*229.0*	*84.9907*	$[CF_3O]^-$	*-25*	*-15*
PFMOBA	*279.0*	*84.9907*	$[CF_3O]^-$	*-55*	*-12*
HFPO-DA_1	*329.0*	*168.9894*	$[CF_3CF_2CF_2]^-$	*-30*	*-13*
HFPO-DA_2	*329.0*	*284.9779*	$[CF_3CF_2CF_2OCFCF_3]^-$	*-30*	*-6*
HFPO-DA_3	*329.0*	*184.9843*	$[CF_3CF_2CF_2O]^-$	*-30*	*-26*
PFOA_1	*413.0*	*368.9766*	$[CF_2(CF_2)_6]^-$	*-50*	*-13*
PFOA_2	*413.0*	*168.9894*	$[CF_3CF_2CF_2]^-$	*-50*	*-21*
PFOS_1	*498.9*	*79.9574*	$[SO_3]^-$	*-180*	*-65*
PFOS_2	*498.9*	*98.9558*	$[FSO_3]^-$	*-180*	*-52*

Mass spectrometry: Analysis was performed on the SCIEX X500 QTOF series system with the Turbo V ion source using electrospray ionization (ESI) in negative ion mode. Data were collected using MRM^{HR} acquisition with compound-specific optimized DP and CE parameters (Table 1). The TOF MS mass range used was 100 to 1000 Da, with the general compound dependent parameters of DP=-40 V and CE=-5 V. The source and gas conditions were GS1= 60, GS2= 60, CUR= 30, CAD= 10, TEM= 550°C, ISV = -2500V. The fragment ion formulas and exact masses are also shown in Table 1.

Data processing: Samples were processed with SCIEX OS software 2.1 using the Analytics module.

Advantages of using MRM^{HR} for quantitative PFAS analysis

The X500 QTOF series system utilizes a time-of-flight mass analyzer to detect the precursor and fragment ions with high resolution and high mass accuracy as compared to a quadrupole mass analyzer in traditional nominal mass instruments. As such, the QTOF collects mass data with high resolution (30-40K resolution) and high accuracy (<5 ppm accuracy) which ultimately results in greater analyte specificity. This provides two key advantages for quantitative PFAS analysis. First, chromatograms show lower background noise which generally results in higher S/N and thus lower detection limits. This is particularly beneficial with complex matrices such as sediment and serum. Second, increased selectively can mass resolve endogenous interferences that would otherwise result in false positives and poor data quality. For example, the separation of PFOS and PFHxS interferences in human serum and plasma.[5]

Chromatography results

All analytes showed good retention and peak shape using the Phenomenex Luna Omega PS C18 column (Figure 3). The PS C18 stationary phase is a unique mixed-mode phase and the particle surface contains a positive charge that has been shown to improve retention of acidic compounds. As shown in Figure 3, the PS C18 column showed good retention of the highly polar shorter-chain compounds (e.g. PFMOAA and PFMOPrA).

Sensitivity, precision, accuracy and linear dynamic range

Low parts-per-trillion (ppt, pg/mL) sensitivity was shown for the novel PFECAs and legacy PFAS (Table 2). This demonstrates excellent sensitivity, particularly if the samples have been concentrated using common SPE sample preparation methods for PFAS. MRM^{HR} XICs for the LLOQ standards are shown in Figure 4. Overall, the TOF MS and MRM^{HR} XICs showed similar LLOQ values although the MRM^{HR} XIC generally had lower background.

Analyte precision (n=3) was very good, generally <20% CV% for the LLOQ standard. Interestingly, the MRM^{HR} XIC precision was typically better than the TOF MS XIC. Accuracy of the LLOQ standard was excellent, typically ranging from 90-110%. Overall, these trends show very good sensitivity as well as quantitative data quality at low ppt (pg/mL) concentrations.

Finally, approximately 3 orders of linear dynamic range was shown with r^2 values typically greater than 0.998.

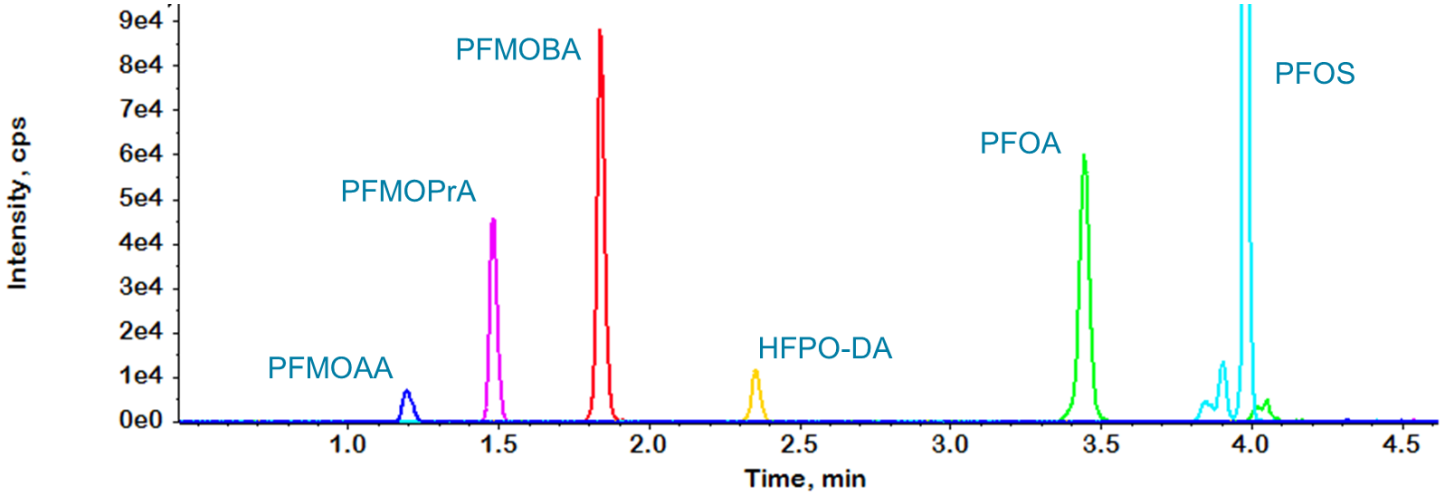

Figure 3. Good separation using mixed mode chromatography. TOF MS XIC of 1 ng/mL standard. Novel PFAS compounds showed excellent peak shape and retention using Phenomenex Luna Omega PS C18 column. Note y-axis is truncated to better show lower responsive analysis and thus the PFOS peak is cut off.

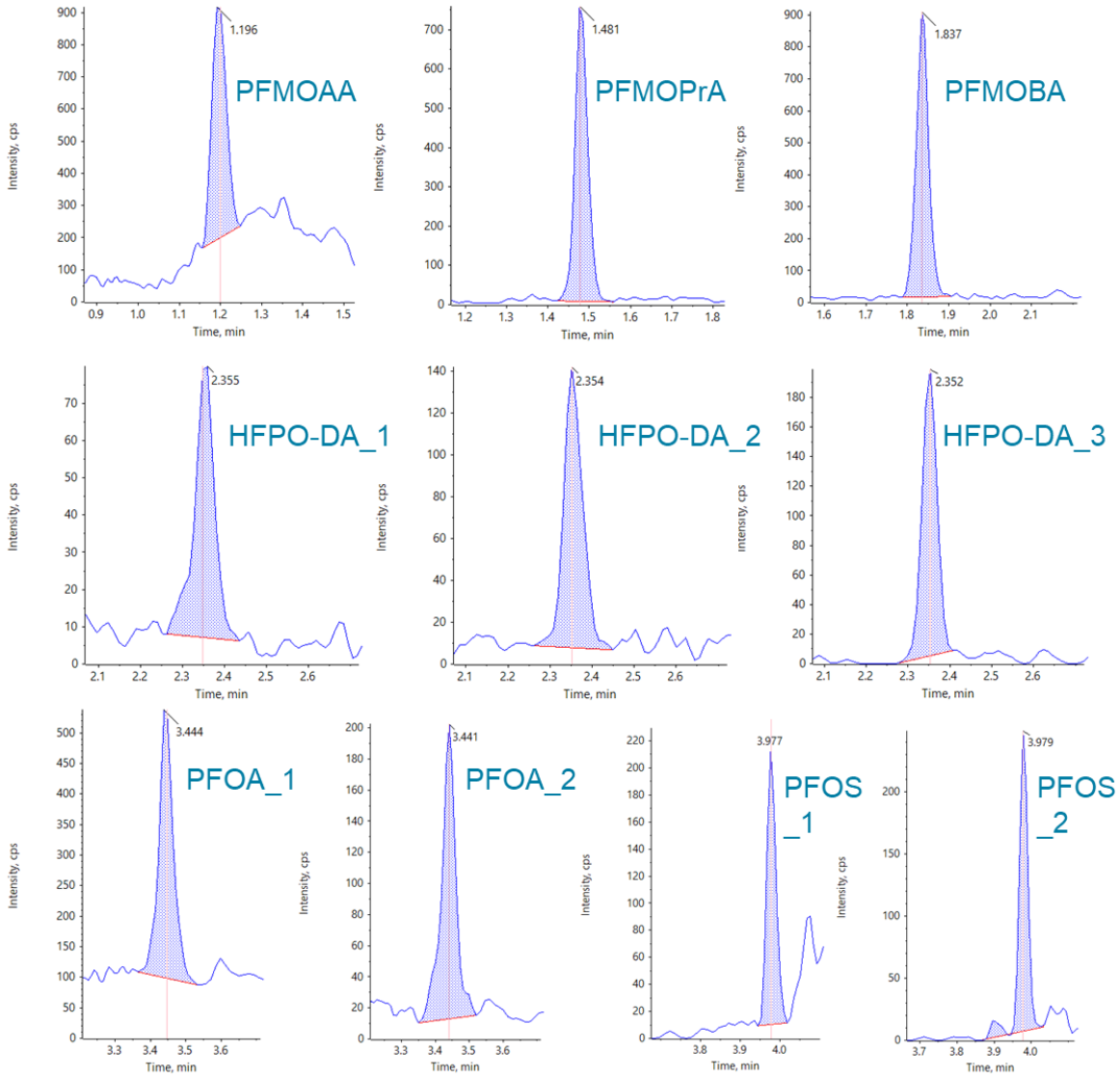

Figure 4. Lower limits of quantification for the GenX compounds. MRM^{HR} XICs of the LLOQ standard for PFMOAA (41 pg/mL), PFMOPrA (21 pg/mL), PFMOBA (22 pg/mL, HFPO-DA_1 (10 pg/mL), HFPO-DA_2 (10 pg/mL), HFPO-DA_3 (50 pg/mL), PFOA_1 (7.5 pg/mL), PFOA_2 (19 pg/mL), PFOS_1 (10 pg/mL) and PFOS_2 (21 pg/mL).

Cape Fear river water and sediment

River water and sediment samples were analyzed from the Cape Fear river basin to demonstrate method performance. Results for HFPO-DA (GenX) are shown in Figure 1 and demonstrate the ability of the X500 QTOF series system to measure this novel PFAS in environmental samples. Both the river water and sediment showed very low background, partially due to monitoring the accurate mass fragment during MRM^{HR} acquisition. An additional benefit of using HRMS for quantification is the ability to measure the analyte mass accuracy thus increasing detection confidence. The X500 QTOF series system is capable of 5 ppm mass accuracy which is 2 mDa accuracy for a 400 Da compound. As shown in Figure 1, the HFPO-DA mass accuracy in the river water and sediment was <5 ppm.

In addition to HFPO-DA, the other 3 novel PFECAs as well as the legacy PFAS, PFOA and PFOS were detected in the river water and sediment samples (Figure 5). Interestingly, the PFMOPrA XIC showed 2 distinct peaks due to the presence of the structural isomers, PFMOPrA and PMPA[3]. However, the standard only contained PFMOPrA. Overall, these results show the Cape Fear river basin is contaminated with novel ether-based PFAS in addition to the well-known HFPO-DA. Further, the X500 QTOF series system can confidently identify and quantitative environmental levels of these novel PFAS.

Table 2. Comparing sensitivity between the TOF MS and MS/MS data. LLOQ (pg/mL) concentration, calibration range (pg/mL) and r^2 for the TOF MS and MRM^{HR} scans. CV (%) and accuracy (%) given at the LLOQ value. N=3 replicates.

	Scan Type	LLOQ (pg/mL)	CV (%)	Accuracy (%)	Calibration range (pg/mL)	r^2
PFMOAA	*TOF MS*	*41*	*23*	*113*	*21-8240*	*0.998*
	MRM^{HR} 1	*41*	*8.7*	*97*	*21-8240*	*0.997*
PFMOPrA	*TOF MS*	*21*	*21*	*102*	*8.5-8500*	*0.998*
	MRM^{HR} 1	*21*	*5.9*	*92*	*8.5-8500*	*0.993*
PFMOBA	*TOF MS*	*9*	*20*	*79*	*9-8550*	*0.998*
	MRM^{HR} 1	*22*	*2*	*93*	*9-8550*	*0.994*
HFPO-DA	*TOF MS*	*50*	*8.8*	*73*	*50-10,000*	*0.993*
	MRM^{HR} 1	*10*	*3.5*	*79*	*10-10,000*	*0.997*
	MRM^{HR} 2	*10*	*19*	*95*	*10-10,000*	*0.997*
	MRM^{HR} 3	*50*	*15*	*86*	*50-10,000*	*0.996*
PFOA	*TOF MS*	*7.5*	*14*	*86*	*7.5-7500*	*0.999*
	MRM^{HR} 1	*7.5*	*5.2*	*102*	*7.5-7500*	*0.998*
	MRM^{HR} 2	*19*	*15*	*97*	*15-7500*	*0.999*
PFOS	*TOF MS*	*21*	*16*	*89*	*21-8275*	*0.999*
	MRM^{HR} 1	*10*	*5.9*	*123*	*10-8275*	*0.999*
	MRM^{HR} 2	*21*	*16*	*85*	*21-8275*	*0.999*

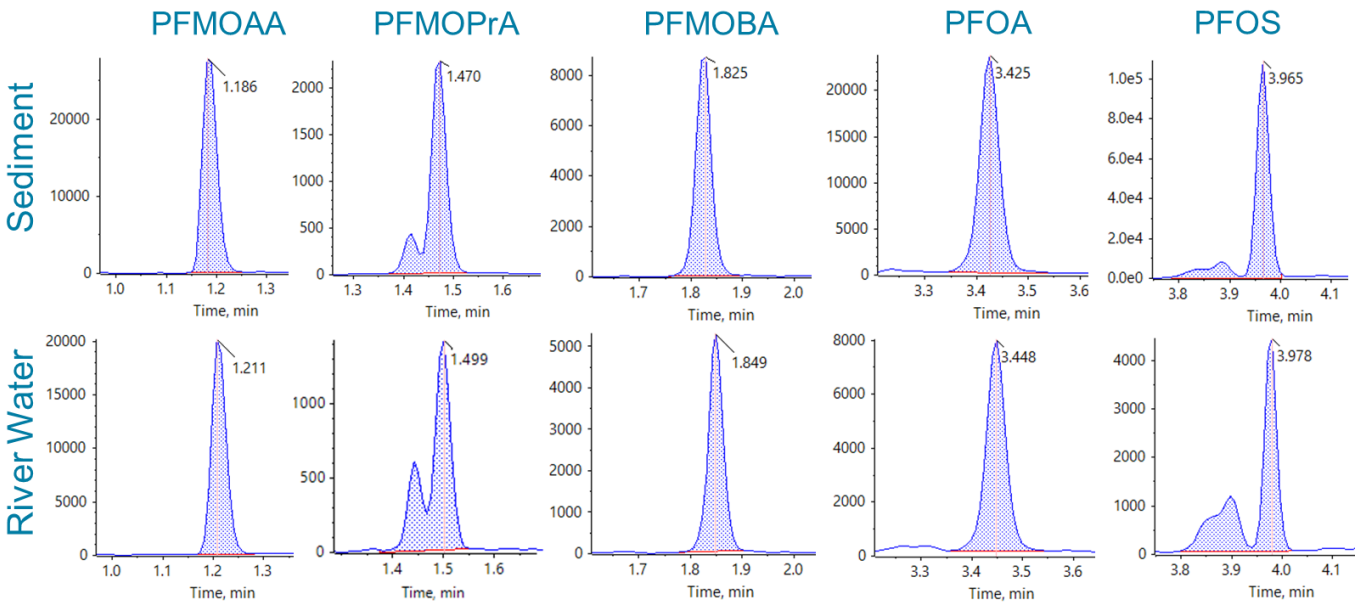

Figure 5. Detection in river and sediment samples. MRM^{HR} XICs of PFMOAA, PFMOPrA, PFMOBA, PFOA and PFOS in sediment and river water from the Cape Fear river basin near Wilmington, NC.

Conclusions

MRM^{HR} method was developed using the X500R QTOF series system to analyze novel perfluoroalkyl ether carboxylic acids (PFECAs), including GenX (HFPO-DA) and legacy PFAS.

- Increased specificity obtained by monitoring accurate mass fragmentions is important when analyzing complex matrices such as sediment
- Ability to obtain accurate mass error information improves confidence in analyte detection
- Very good sensitivity was obtained for all compounds in low ppt (pg/mL) concentrations.

References

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