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Application News

Untargeted Screening of Per- and Polyfluoroalkyl Substances by HRAM-DIA Method on LCMS-9030

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User Benefits

- A sensitive untargeted screening method was established based on HRAM-DIA data acquisition on LCMS-9030. The method was successfully verified with 14 PFAS standards at 1 ng/mL in water.
- Data analysis was performed using LabSolutions Insight Explore™ – Analyze. PFAS-like species could be extracted by specific elemental settings which functions as mass defect filtering for PFAS. This procedure was used for an unknown sample, and 16 PFAS-like species were discovered and characterized.

■ Introduction

Contamination of per- and polyfluoroalkyl substances (PFAS) are found everywhere in water, soils, sediments, fish, foods, textiles and human blood etc. Targeted screening and quantitation of PFAS in drinking water by LC-MS/MS are established in reference to the US EPA Method 537 and ISO 21675: 2019, ASTM D7979 etc. Such MRM-based methods are widely adopted in the analysis of up to 29 PFAS or more [1]. However, PFAS represents a large collective of compounds [2] and many of them are not determined by the existing methods. This study aims to establish a method of untargeted screening for both known and undiscovered PFAS in water samples. The method was established based on LC-Q-TOF data, i.e., HRAM spectrum (MS) and DIA deconvolution spectrum (MS/MS), relying on the specific mass defect feature of PFAS [3, 4] using LabSolutions Insight Explore – Analyze program. Fourteen PFAS including PFOA and PFOS were used as standards for verifying the performance of this HRAM-DIA method in terms of detection sensitivity and identification. The established approach was applied to real water sample analysis and the found PFAS-like species were further characterized via database and library searches, and structural elucidation using LabSolutions Insight Explore – Assign program.

■ Experimental

Reagents, PFAS standards and samples

Acetonitrile (LCMS grade) and methanol (LCMS grade) were obtained from commercial suppliers. Ammonium acetate (>99%) of LCMS grade was used as additives in the mobile phase prepared from Milli-Q water. Sixteen PFAS standards (Table 2) were purchased from Wellington Laboratories and Apollo scientific. M-PFOS (with $^{13}C_4$) and M-PFOA (with $^{13}C_4$) were used as internal standards in method development. Water samples were collected and subjected to analysis for the discovery of unknown PFAS in the study.

LC-Q-TOF analytical conditions

Details of the analytical conditions on LCMS-9030, Q-TOF system (Shimadzu Corporation, Japan) are shown in Table 1. LabSolutions v5.114 and LabSolutions Insight Explore v3.8 SP4 were used for data acquisition in MS and DIA mode and data processing of HRAM spectra and DIA MS/MS spectra for efficient detection and identification of targeted and untargeted PFAS.

Table 1 Analytical conditions of PFAS on LCMS-9030

LC Conditions	
Column	Shim-pack™ Velox, C18 (2.1x100 mm, 2.7 µm)
Flow Rate	0.4 mL/min
Mobile Phase	A: 5 mM Ammonium acetate in water B: Acetonitrile
LC gradient	B: 10% (0-0.5 min) → 85% (8.5 min-9 min) → 10% (9.1 min-12 min) → stop
Delay column	Shim-pack Velox, C18 (2.1x50 mm, 2.7 µm)
Oven Temp.	40°C
Injection Vol.	50 µL
Interface Conditions and MS mode	
Interface	ESI Heated
Interface Temp.	300°C
DL Temp.	250°C
Heat Block Temp.	400°C
Nebulizing Gas	3 L/min (N2)
Heating Gas Flow	10 L/min (Air)
Drying Gas Flow	10 L/min (N2)
MS mode	MS (-), m/z 100~1000 DIA (-), m/z 50~1000; with CE 25V and Spread (+/-) 20V Loop time: 1.01 sec

Untargeted screening method

As shown in Table 1, MS and DIA events were set up for data acquisition. Data analysis and processing were performed with the LabSolutions Insight Explore suite, which include Analyze and Assign etc. The Analyze is for the deconvolution of DIA data to generate a precursor list and provide various functions of in-depth data analysis such as deconvoluted MS/MS spectrum, formula prediction and library search etc. The Assign is for identification and structural elucidation, which links to database searches such as ChemSpider and PubChem. Both Analyze and Assign were highly efficient and flexible in data processing and result display. Two PFAS libraries were installed and used in this study: (1) an in-house PFAS HRMS MS/MS library including spectra of 34 PFAS standards [4] and (2) MS-DIAL PFAS_Neg library [5].

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■ Results and Discussion

1. Detection of PFAS by HRAM

Table 2 shows the results of detection of 14 PFAS and 2 ISTD in pure water from HRAM data (event 1) acquired on LCMS-9030 using the method as described above. The XIC chromatograms of 1 ng/mL sample is displayed in Figure 1a and the spectrum of the first XIC peak (PFPA) is shown in Figure 1b. The lowest detectable concentrations by HRAM are 0.01 ng/mL for 11 PFAS, 0.02 ng/mL for PFDA, 0.05 ng/mL for PFPA and 0.1 ng/mL for PFTrA and PFTeA. The mass accuracy is better than (+/-) 3.3 ppm, with most compounds less than (+/-) 2 ppm. Linear calibration curves were established from the lowest concentrations to 5 ng/mL for all the PFAS with R^2 between 0.94 and 0.99. PFOA, PFOS and their isotope labelled standards (M-PFOA, M-PFOS) could also be detected at 0.01 ng/mL level. These results indicate that a highly-sensitive screening and quantitation method could be established based on HRAM on LCMS-9030.

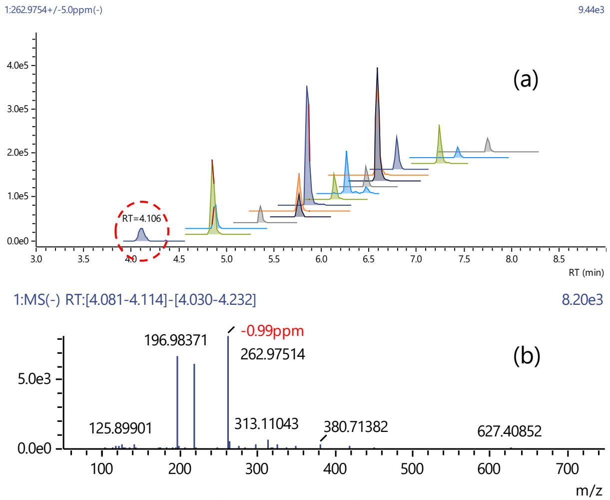


Figure. 1 (a) XICs of 14 PFAS and 2 ISTD (1 ng/mL each); (b) MS spectrum of the 1st peak (PFPA).

Table 2 Sixteen PFAS information, accurate mass, measured error, RT and calibration curve range on LCMS-9030

PFAS (Abbr.)	Formula	CAS No.	[M-H]- (Meas.)	[M-H]- (Calc.)	Error (ppm)	RT (min)	Range (ng/mL)	R^2
PFPA	$C_5HF_9O_2$	2706-90-3	262.9751	262.9760	-3.27	4.12	0.05 ~ 5	0.989
PFBS	$C_4F_9SO_3H$*	29420-49-3	298.9421	298.9430	-3.01	4.86	0.01 ~ 5	0.941
PFHxA	$C_6HO_2F_{11}$*	307-24-4	312.9718	312.9728	-2.91	4.88	0.01 ~ 5	0.981
PFHpA	$C_7HF_{13}O_2$*	375-85-9	362.9687	362.9696	-2.20	5.36	0.01 ~ 5	0.982
PFOA	$C_8HF_{15}O_2$*	335-67-1	412.9655	412.9664	-1.99	5.77	0.01 ~ 5	0.982
M-PFOA	$^{13}C_4C_4HF_{15}O_2$	N.A.	416.9791	398.9366	-1.73	5.77	0.01 ~ 5	0.982
PFHxS	$C_6F_{13}HO_3S$*	82382-12-5	398.9358	462.9632	-1.75	5.86	0.01 ~ 5	0.950
PFNA	$C_9HF_{17}O_2$*	375-95-1	462.9624	512.9600	-1.60	6.13	0.01 ~ 5	0.983
PF-3,7-DMOA	$C_{10}HF_{19}O_2$	172155-07-6	468.9692	512.9600	-1.83	6.27	0.01 ~ 5	0.985
PFDA	$C_{10}HF_{19}O_2$*	335-76-2	512.9592	498.9302	-1.58	6.47	0.02 ~ 5	0.988
PFOS	$C_8F_{17}O_3HS$*	4021-47-0	498.9296	502.9436	-1.22	6.59	0.01 ~ 5	0.955
M-PFOS	$^{13}C_4C_4F_{17}O_3HS$	N.A.	502.9429	562.9568	-1.31	6.59	0.01 ~ 5	0.969
PFUnA	$C_{11}HF_{21}O_2$*	2058-94-8	562.9562	598.9238	-1.12	6.80	0.02 ~ 5	0.998
PFDS	$C_{10}HF_{21}SO_3$	2806-15-7	598.9232	662.9505	-1.09	7.24	0.01 ~ 5	0.987
PFTrA	$C_{13}HO_2F_{25}$*	72629-94-8	662.9497	712.9473	-1.27	7.43	0.1 ~ 5	0.991
PFTeA	$C_{14}HO_2F_{27}$*	376-06-7	712.9463	262.9760	-1.42	7.75	0.1 ~ 5	0.983

** Targeted PFAS by EPA 537 method*

2. Detection of PFAS by DIA

The DIA data of the same data set was processed with the LabSolutions Insight Explore - Analyze to generate a long list of precursors via deconvolution, followed by applying predicting formula. To look for PFAS-like compounds and species, the key settings of elements include F: 6~50, H: 1~5, O: 1~5, C: 1~50, S: 0~1 and N: 0~1. These settings restrict the elemental composition of candidate with a negative mass defect and could be used to find PFAS-like species [4]. Figure 2 shows an example of this approach. A precursor peak (*m/z*262.9751) generated from DIA data (Figure 2a) appeared at the same RT as PFPA in MS XIC (Figure 1a). The mass defect measured is -24.9 mDa, which is very close to the calculated mass defect of PFPA (-24.0 mDa). The corresponding deconvoluted MS/MS spectrum as shown in Figure 2b matches perfectly to the PFPA MS/MS spectrum in the PFAS library (Figure 2c). These results confirm that PFPA of 1 ng/mL spiked in water can be detected firmly via the DIA analysis approach.

The detection and identification results of the 14 spiked PFAS in water are compiled into Table 3. As can be seen from the Table, all the 14 PFAS were detected and identified via the above approach using the Analyze.

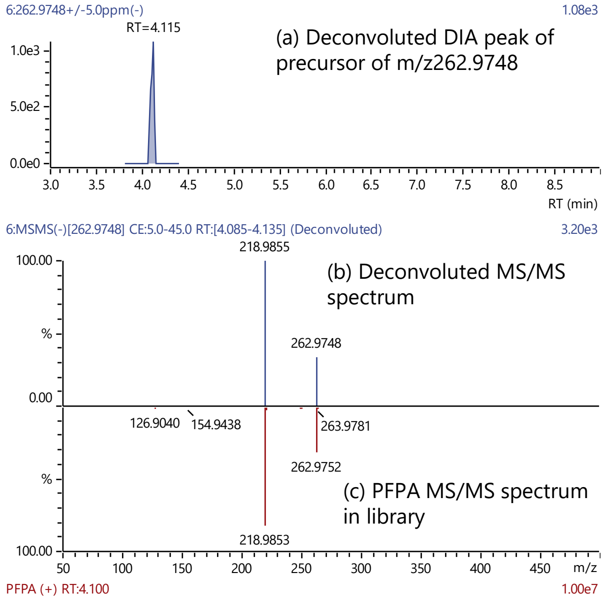


Figure 2 Detection of PFPA (1 ng/mL in water) by DIA peak (a) and deconvoluted spectrum (b), which matches to the library spectrum of PFPA (c)

Table 3 Detection and identification of 14 PFAS (1 ng/mL) from DIA data using Analyze program

PFAS (Abbr.)	PFAS Formula	Precursor generated from DIA data	Precursor RT (min)	Precursor ion formula obtained	Deconvoluted spectrum from DIA data	PFAS Lib Search
PFPA	$C_5HF_9O_2$	262.9748	4.12	$[C_5HO_2F_9\text{-}H]^-$	262.9748, 218.9855	Confirmed
PFBS	$C_4F_9SO_3H$	298.9421	4.86	$[C_4HO_3F_9S\text{-}H]^-$	298.9421, 98.9552, 79.9565	Confirmed
PFHxA	$C_6HO_2F_{11}$	312.9721	4.88	$[C_6HO_2F_{11}\text{-}H]^-$	312.9721, 268.9819, 118.9918	Confirmed
PFHpA	$C_7HF_{13}O_2$	362.9695	5.35	$[C_7HO_2F_{13}\text{-}H]^-$	362.9695, 318.9791, 168.9888	Confirmed
PFOA	$C_8HF_{15}O_2$	412.9656	5.77	$[C_8HO_2F_{15}\text{-}H]^-$	412.9656, 368.9757, 218.9857, 168.9884	Confirmed
PFHxS	$C_6F_{13}HO_3S$	398.9357	5.85	$[C_6HO_3F_{13}S\text{-}H]^-$	398.9357, 118.9918, 98.9548, 79.9567	Confirmed
PFNA	$C_9HF_{17}O_2$	462.9625	6.14	$[C_9HO_2F_{17}\text{-}H]^-$	462.9625, 418.9725, 218.9856, 168.9885	Confirmed
PF-3,7-DMOA	$C_{10}HF_{19}O_2$	468.9693	6.26	$[C_9HF_{19}\text{-}H]^{-*}$	468.9693, 446.9687, 268.9822	Confirmed
PFDA	$C_{10}HF_{19}O_2$	512.9592	6.46	$[C_{10}HO_2F_{19}\text{-}H]^-$	512.9592, 468.9694, 268.9823, 218.9855, 168.9884	Confirmed
PFOS	$C_8F_{17}O_3HS$	498.9294	6.58	$[C_8HO_3F_{17}S\text{-}H]^-$	498.9294, 168.9883, 118.9921, 98.9551, 79.9565	Confirmed
PFUnA	$C_{11}HF_{21}O_2$	562.9565	6.80	$[C_{11}HO_2F_{21}\text{-}H]^-$	562.9565, 518.9660, 318.9789, 268.9818, 218.9848, 168.9882	Confirmed
PFDS	$C_{10}HF_{21}SO_3$	598.9232	7.24	$[C_{10}HO_3F_{21}S\text{-}H]^-$	598.9232	Confirmed
PFTrA	$C_{13}HO_2F_{25}$	662.9486	7.43	$[C_{13}HO_2F_{25}\text{-}H]^-$	662.9486, 618.9597	Confirmed
PFTeA	$C_{14}HO_2F_{27}$	712.9465	7.75	$[C_{14}HO_2F_{27}\text{-}H]^-$	712.9465, 668.9566, 168.9880	Confirmed

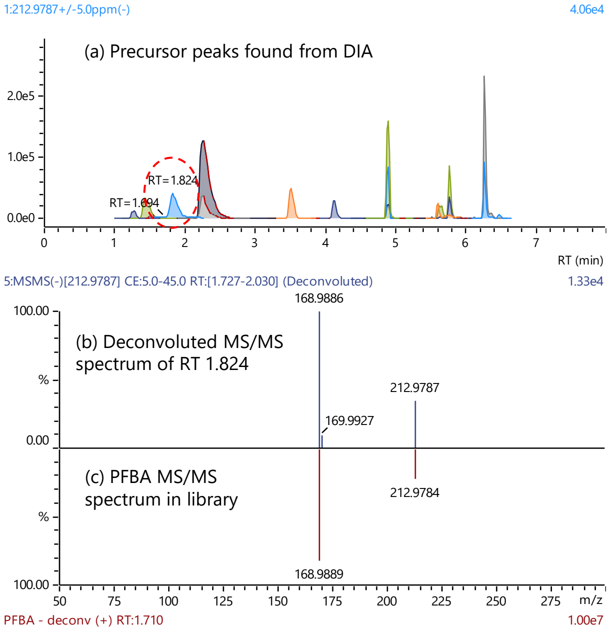
** PF-3,7-DMOA is ionized to form $[M\text{-}HCOO]^- = [C_9HF_{19}\text{-}H]^-$*

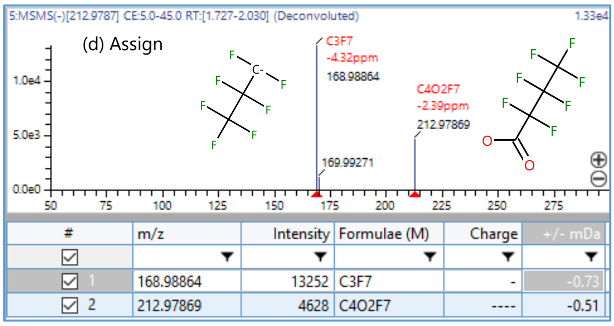
The results indicate that PFAS can be detected from DIA data, which is the basis to use the HRMS-DIA method for untargeted screening of PFAS in water samples.

3. Untargeted screening by HRAM-DIA method

The above data processing using Analyze was adopted for untargeted screening of PFAS in unknown samples. The obtained DIA data of a water sample was processed with Analyze and a long list of precursors (>800) was produced. By applying formula prediction with specific elemental settings as described above to all precursors, 16 PFAS-like precursors were generated (Table 4), which feature with the characteristic negative mass defects. Upon applying library search, five candidates were found in the PFAS libraries: PFBA (SI=94%), PFCA-unsaturated (SI=65%), 6:2 fluorotelomer sulfonic acid (SI=55%), PFOA (SI=61%) and PF-3,7-DMOA (SI=76%). In addition, PFCA-diether H_substituted was found to match *m/z*626.9530 spectrum with a very low SI (23%). For such poor library matched and the remaining totally un-matched species, their identities could rely only on the characterization through structural elucidation analysis using the Assign program.

The 16 PFAS-like precursor peaks extracted from DIA data via Analyze are displayed in Figure 3a. Taking the peak at 1.824 min (*m/z*212.9787) as an example, the deconvoluted MS/MS spectrum and library search are shown in Figure 3b and 3c. The results confirm the presence of PFBA in the sample. If the deconvoluted MS/MS spectrum was sent to Assign program which links to database search (ChemSpider), heptafluorobutyric acid (CAS No.: 375-22-4) was found as a matched structure and the precursor as well as a fragment were annotated (Figure 3d). It is actually same as perfluorobutanoic acid (PFBA). Another representative example is candidate #15, which was detected at 6.26 min with a precursor ion of





#	m/z	Intensity	Formulae (M)	Charge	+/- mDa
☑	▼	▼	▼	▼	▼
☑ 1	168.98864	13252	C3F7	-	-0.73
☑ 2	212.97869	4628	C4O2F7	----	-0.51

Figure 3 (a) Detection of unknown PFAS-like compounds from DIA, (b) deconvoluted spectrum of peak at RT 1.824, (c) matches to library spectrum of PFBA; (d) Assign to Heptafluorobutyric acid structure and fragment (same as PFBA).

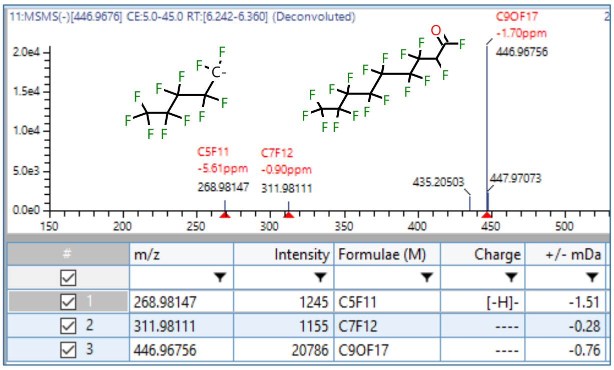
Table 4 Untargeted screening for detection and identification of PFAS from a water sample by HRAM-DIA on LCMS-9030

Candi-date #	Precursor from DIA	RT (min)	Precursor ion Formula	Error (ppm)	Deconvoluted DIA MS/MS spectrum	PFAS Library search	ID by Assign (ChemSpider)
1	402.9979	1.26	$[C_{13}H_4OF_{12}\text{-}H]^-$	-4.6	402.9979	Not found	(2E)-2,3,4,4,5,5,6,6,7,7,7-Undecafluoro-1-(4-fluorophenyl)-2-hepten-1-one
2	404.0146	1.43	$[C_{15}H_5NOF_{10}\text{-}H]^-$	1.9	404. 0146, 376.9950	Not found	N-[2,3,5,6-Tetrafluoro-4-(trifluoromethyl) phenyl]-2-(trifluoromethyl) benzamide
3	212.9787	1.82	$[C_4HO_2F_7\text{-}H]^-$	-2.4	212.9787, 168.9886	Perfluorobutanoic acid (PFBA)	Heptafluorobutyric acid
4	220.9864	2.23	$[C_8H_2O_3F_4\text{-}H]^-$	-1.6	220.9864, 138.9956, 79.9567	Not found	2,3,5,6-Tetrafluoro-4-formylbenzoic acid
5	219.9835	2.27	$[C_5HNO_2F_6\text{-}H]^-$	-1.5	219.9835, 81.9525	Not found	4-Cyano-2,2,3,3,4,4-hexafluorobutanoic acid
6	247.9786	3.51	$[C_6HNO_3F_6\text{-}H]^-$	-0.8	247.9786, 219.9838, 79.9566	Not found	bis(trifluoromethyl)-1,2-oxazole-4-carboxylic acid
7	196.9836	4.12	$[C_4HOF_7\text{-}H]^-$	-3.6	196.9836, 130.9923, 80.9951, 68.9951	Not found	butanal, heptafluoro-
8	246.9805	4.88	$[C_5HOF_9\text{-}H]^-$	-2.4	246.9805, 180.9887, 130.9918, 118.9919	Not found	2,2,3,3,4,4,5,5,5-Nonafluoropentanal
9	626.9530	4.88	$[C_{12}H_2O_4F_{22}\text{-}H]^-$	0.1	626. 9530, 354.9605, 312.9722, 268.9822	PFCA-diether H_substituted	No result
10	224.9786	4.89	$[C_5HO_2F_7\text{-}H]^-$	-2.8	224.9786, 174.9816	PFCA-unsaturated	1,1,1,3,5,5,5-Heptafluoro-2,4-pentanedione
11	374.9738	4.89	$[C_{10}H_5O_3F_9S\text{-}H]^-$	-1.4	374.9738, 312.9720, 268.9821	Not found	Phenyl 1,1,2,2,3,3,4,4,4-nonafluoro-1-butanesulfonate
12	426.9681	5.60	$[C_8H_5O_3F_{13}S\text{-}H]^-$	0.4	426.9677	6:2 Fluorotelomer sulfonic acid	1H,1H,2H,2H-PERFLUOROOCTANESULFONIC ACID
13	346.9742	5.77	$[C_7HOF_{13}\text{-}H]^-$	-1.6	346.9742, 280.9816, 96.9595	Not found	2,2,3,3,4,4,5,5,6,6,7,7-Dodecafluoroheptanoyl fluoride
14	412.9654	5.77	$[C_8HO_2F_{15}\text{-}H]^-$	-2.5	412.9654, 368.9753, 218.9856, 168.9887	Perfluorooctanoic acid (PFOA)	Perfluorooctanoic Acid (PFOA)
15	446.9676	6.26	$[C_9HOF_{17}\text{-}H]^-$	-1.7	446. 9676, 311.9811, 268.9815	Not found	2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Hexadecafluorononanoyl fluoride
16	468.9697	6.26	$[C_9HF_{19}\text{-}H]^-$	-3.8	468.9697, 446.9678, 268.9822, 218.9854, 168.9886	PF-3,7-DMOA	2,2,3,4,4,5,5,6,6,7,8,8,8-tridecafluoro-3,7-bis(trifluoromethyl)octanoic acid, C10HF19O2

*m/z*446.9676. PFAS Library search did not generate any result. The deconvoluted MS/MS spectrum was sent to Assign with inputting a formula of C_9HOF_{17} for searching in ChemSpider database. The Assign program generated a list of candidates for the formula and spectrum. One of most-likely candidate is 2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Hexadecafluorononanoyl fluoride. As can be seen in Figure 4, the precursor ion and two fragments match the structure essentially. However, it is worth to note that, although the identification results using Assign program and ChemSpider database may provide reference structures and information, the results are not considered as conclusions and further structural analysis is required.

■ Conclusion

In this study, an untargeted screening method based on HRAM-DIA data acquisition was established on LCMS-9030. The DIA data obtained was deconvoluted using the LabSolutions Insight Explore – Analyze to generate a list of precursors. Then, PFAS-like species were extracted by applying formula prediction with specific elemental settings. This approach was successfully verified with 14 PFAS standards at 1 ng/mL in water and applied to analyze an unknown water sample. Sixteen PFAS-like species were found. PFAS library search and structural elucidation using the Assign program were conducted for these PFAS-like species.



#	m/z	Intensity	Formulae (M)	Charge	+/- mDa
☑					
☑ 1	268.98147	1245	C5F11	[-H]-	-1.51
☑ 2	311.98111	1155	C7F12	----	-0.28
☑ 3	446.96756	20786	C9OF17	----	-0.76

Figure 4 Deconvoluted MS/MS spectrum of precursor peak at 6.26 min (*m/z*446.9676); Assign: 2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Hexadecafluorononanoyl fluoride, C_9HOF_{17}, from ChemSpider.

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04-AD-0280-EN First Edition: Oct. 2022