

Application News

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Water Analysis / LCMS-9030 Q-TOF

Development of Screening Analysis Method for Per- and poly-fluoroalkyl Substances (PFAS) in Surface Water on LC-Q-TOF

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are man-made fluorinated compounds persistent in the environment including water, soils, food and living organisms. Standard methods such as EPA 537 were established for monitoring PFAS in drinking water and surface water^[1]. MRM based methods are used for targeted screening of up to about 30 PFASs. However, PFAS is a large collective of compounds and many of them are not determined by the existing analytical methods^[1,2]. As such, PFAS risk potential presents in the environment may be underestimated. This study aims to establish a new approach for screening both known and undiscovered PFASs in water bodies based on high mass accuracy, characteristic MS/MS pattern and the specific mass defect features of PFASs on Q-TOF LC-MS

2. Experimental

Thirty-four PFAS standards were purchased from wellington Laboratories. Stock solutions of the 34 PFAS standards were mixed and diluted with Milli-Q® water. Mixed standards was injected for targeted screening method establishment. Water samples (underground water and rivers) were obtained, filtered and concentrated for 50 times under a stream of nitrogen. The sample was then directly injected to the Q-TOF LCMS for analysis. A LCMS™-9030, Q-TOF liquid chromatograph mass spectrometer system with heated ESI, was used in this study. A GIST-C18 (100 mm x 2.1 mm; 2 µm) column was used for separation of PFASs with a gradient elution of 45 minutes (Table 1).

Table 1. Analytical conditions for PFASs on LCMS-9030 Q-TOF system

Column	Shim-pack™ GIST C18 (100 mm x 2.1 mm; 2 µm)
Flow rate	0.4 mL/min
Mobile phase	A : 5mM NH4Ac in water B : Acetonitrile
Oven Temp.	40°C
Inj. vol	10 µL
Gradient Elution (B)	10% (0 to 2 min) → 95% (35 to 40 min) → 10% (40.1 to 45 min)

Interface	ESI Heated
MS Mode	Negative MS & DDA
CID gas	Argon, 270 kPa
Block Temperature	300°C
DL Temperature	250°C
Interface Temp.	400°C
Nebulizing Gas Flow	Nitrogen, 3 L/min
Drying Gas Flow	Nitrogen, 10 L/min
Heating Gas Flow	Zero Air, 10 L/min

Table 2. PFAS standards and mass accuracy obtained on Q-TOF 9030

No.	Compound Name	Chemical Formula	CAS No	RT/min	Theoretical m/z	Measured m/z	Mass Accuracy / ppm
1	PFBA	C4HF7O2	375-22-4	4.85	212.9792	212.9787	2.2
2	PFFA	C5HF9O2	2706-90-3	8.78	262.9760	262.9755	1.8
3	PFFxA	C6HO2F11	307-24-4	11.58	312.9728	312.9725	1.2
4	PFFpA	C7HF13O2	375-85-9	13.78	362.9696	362.9693	0.8
5	PFOA	C8HF15O2	335-67-1	15.65	412.9664	412.9662	0.6
6	PFNA	C9HF17O2	375-95-1	17.32	462.9632	462.9631	0.3
7	PFDA	C10HF19O2	335-76-2	18.90	512.9600	512.9598	0.4
8	PFUdA	C11HF21O2	2058-94-8	20.41	562.9568	562.9571	0.5
9	PFDoA	C12HF23O2	307-55-1	21.88	612.9537	612.9537	0.1
10	PFTDA	C13HO2F25	72629-94-8	23.32	662.9505	662.9506	0.2
11	PFTeDA	C14HO2F27	376-06-7	24.71	712.9473	712.9471	0.3
12	PFFxDA	C16HF31O2	67905-19-5	27.40	812.9409	812.9409	0.0
13	PFODA	C18HF35O2	16517-11-6	29.87	912.9345	912.9355	1.1
14	PF-3,7-DMOA	C10HF19O2	172155-07-6	17.97	468.9702	468.9704	0.5
15	HPFFpA	C7H2F12O2	1546-95-8	11.94	344.9790	344.9789	0.3
16	L-PFBS	C4F9SO3H	29420-49-3	11.63	298.9430	298.9436	2.1
17	L-PFHxS	C6F13HO3S	82382-12-5	16.26	398.9366	398.9371	1.2
18	LPFFpS	C7HF15O3S	21934-50-9	18.07	448.9334	448.9343	1.9
19	L-PFOS	C8F17O3HS	4021-47-0	19.74	498.9302	498.9308	1.2
20	L-PFDS	C10HF21SO3	2806-15-7	22.82	598.9238	598.9243	0.7
21	H4PFuNA	C11HF21O2	2058-94-8	20.41	562.9568	562.9570	0.3
22	FOSA-I	C8H2F17NO2S	754-91-6	21.9	497.9462	497.9460	0.5
23	N-MeFOSAA	C9H4F17NO2S	2355-31-9	18.78	511.9619	511.9608	2.0
24	N-EtFOSAA	C10H6F17NO2S	2991-50-6	19.39	525.9775	525.9774	0.2
25	N-MeEFOFOSAA	C11H6F17NO4S	2355-31-9	18.78	569.9673	569.9674	0.2
26	N-EtFOSAA	C12H8F17NO4S	2991-50-6	19.4	583.9830	583.9830	0.0
27	FHEA	C8H3F13O2	53826-12-3	13.03	376.9853	376.9850	0.7
28	FOEA	C10H3F17O2	27854-31-5	16.45	476.9789	476.9783	1.3
29	6:2 FTS	C8H5F13SO3	27619-94-9	14.89	426.9674	426.9679	1.3
30	8:2 FTS	C10H5F17SO3	27619-96-1	18.13	526.9610	526.9615	1.1
31	HFPO-DA	C6HF11O3	13252-13-6	12.45	328.9677	328.9672	1.8
32	9Cl-PF3ONS	C8F16O4SClK	73606-19-6 (F-53B)	20.88	530.8950	530.8961	2.1
33	11Cl-PF3OUdS	C10F20O4SClK	83329-89-9	23.88	630.8886	630.8896	1.5
34	ADONA	C7H2F12O4	958445-44-8	14.51	376.9689	376.9689	0.0

3. Results and Discussion

3.1 Targeted screening and HRMS library for 34 PFASs

A mixed standard of 34 PFASs was used to set up targeted screening method on Q-TOF. All the PFASs are ionised in negative ESI mode efficiently to form $[M-H]^-$ ions, except for PF-3,7 DMOA which ionised to form $[M-HCOO]^-$. Mass accuracy of the PFASs are within 3 ppm with external mass calibration method (without lock-mass or internal standard for

post-run mass correction) (Table 2). Additionally, MS/MS spectra for all 34 PFASs were acquired by data-dependent acquisition (DDA) function. A library containing precursors, MS/MS spectra and retention times was established based on the 34 PFAS in LabSolutions™.

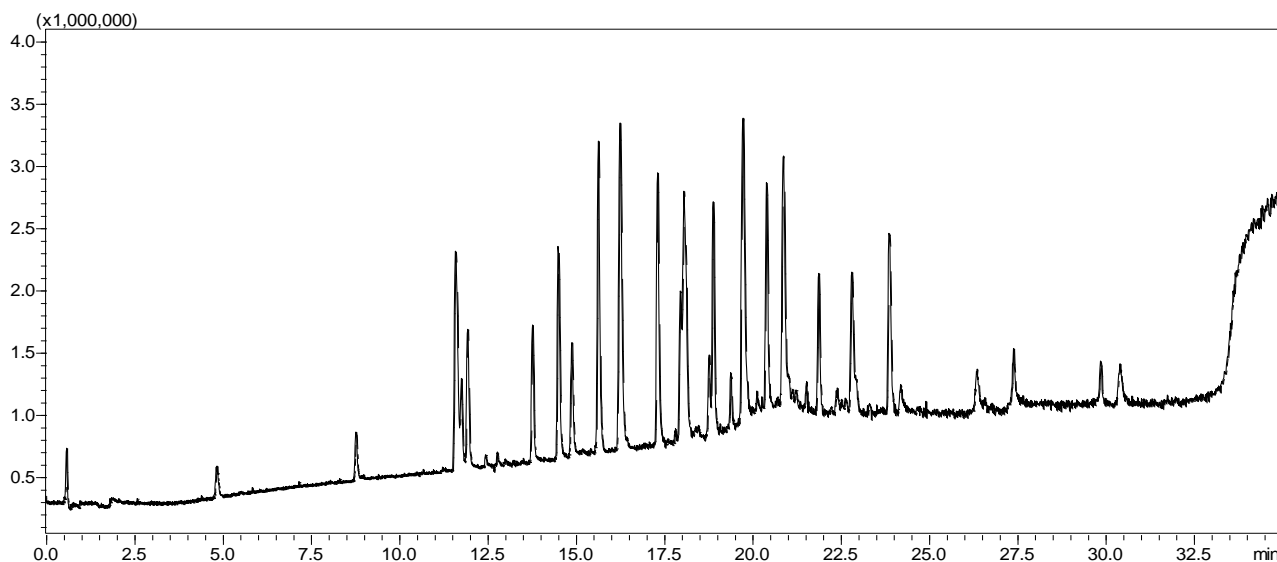


Figure 1: TIC in full mass range mode of a mixed standard of 34 PFASs with each at 100 ng/mL in Milli-Q® water. Peak ID, RT and mass accuracy are shown in Table 2.

3.2 Workflow for screening of undiscovered PFAS

In general, untargeted screening aiming to find undiscovered PFAS is challenging due to lack of information^[2,3]. Hence, it is important to design first a workflow to extract unique information enabling to find PFAS candidates from HRMS data easily before further identification via structural analysis.

Mass defect filtering: Specifically for PFAS, we can employ mass defect filtering technique to screen and detect PFAS candidates. Mass defect is the difference between the nominal mass and the exact mass of a molecule. PFASs comprise different classes of compounds with one common feature: F atoms (18.9984 Da) replace all the H atoms (1.0078 Da) on the C skeleton. As a result, all the PFASs have negative mass defects. For instance, the mass defects for the 34 PFASs are from -14.7 mDa (FHEA, (-) m/z 376.9850) to -114.4 (11Cl-PF3OUdS, (-) m/z 630.8886). It is therefore possible to use mass defect filtering (MDF) method as an easy tool to find PFAS candidates from the detectable ions in an unknown sample. A tentative mass defect filtering range was set from -10 mDa to -120 mDa in the current work.

Diagnostic fragment ions: PFAS produces unique fluorinated fragments such as $C_2F_5^-$ (m/z 118.9926) and $C_3F_7^-$

(m/z168.9893). Some functional groups have characteristic fragmentation patterns. For example, the sulfonic acid functional group (-SO₃H) can produce fragments SO₃⁻ ions (m/z79.9574), FSO₃⁻ ions (m/z98.9558) under high CE conditions. These highly specific fragments can be used as diagnostic ions to search against the DDA spectra for PFAS candidates. Accordingly, a workflow for screening of undiscovered PFAS is set up as shown in Figure 2.

3.3 Analysis of water sample using targeted and untargeted screening method

Water samples obtained from collaborators were analysed with MS and DDA modes. The raw samples were filtered and concentrated for 50 times without further clean-up. An exclusion list of ions from solvent and mobile phases was utilised, which could exclude their interference in precursor selection during DDA run. Data analysis was performed first for targeted PFAS (34) registered in the in-house library. Upon library searching of a selected peak, a matching list against the registered PFASs might be generated based on retention time, accurate mass and MS/MS patterns. For example, both PFOA and PFOS were found in one of the

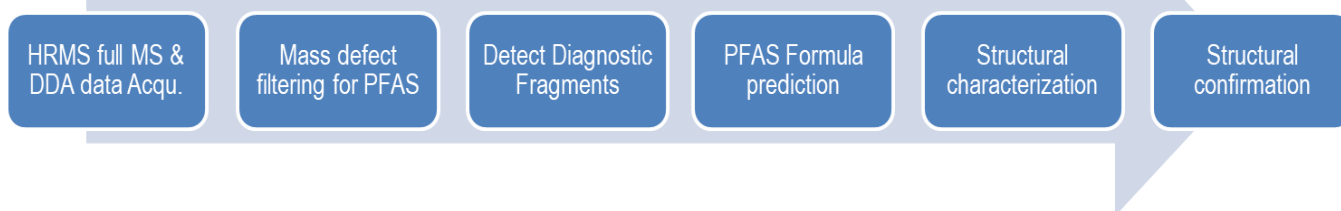


Figure 2: Workflow for screening and structural analysis of undiscovered PFASs by Q-TOF LCMS-9030

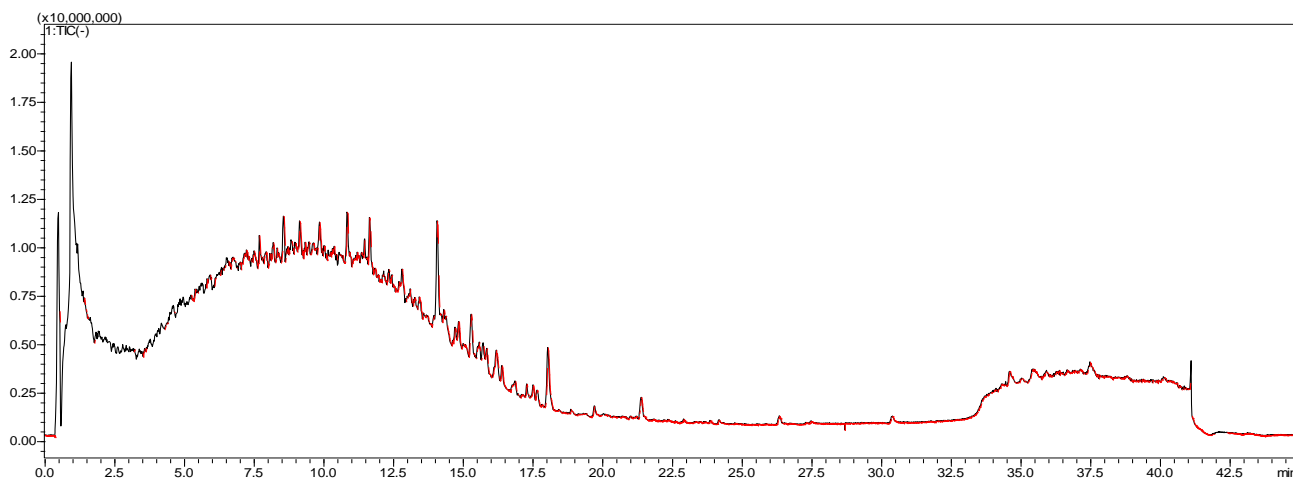
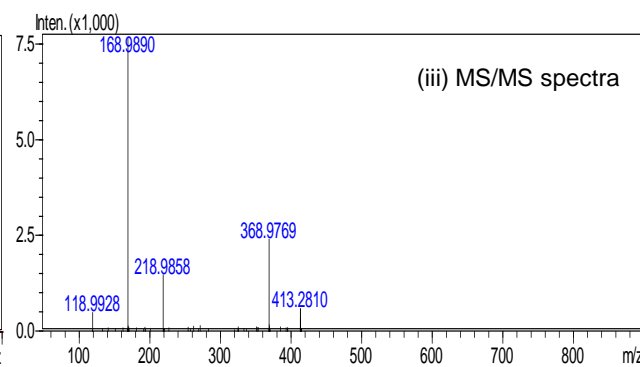
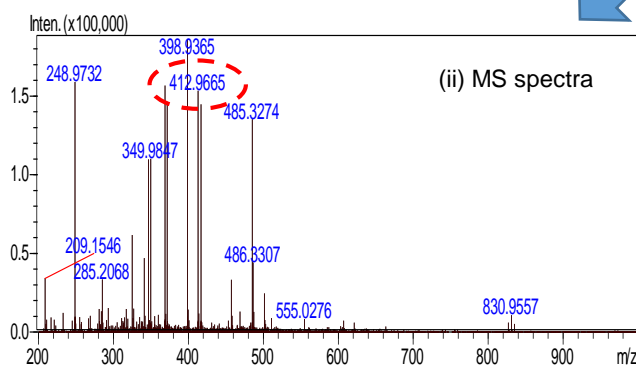
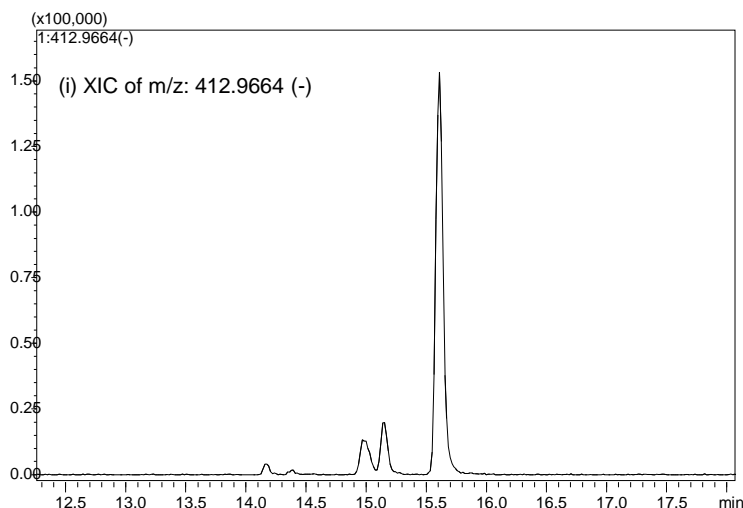


Figure 3: Chromatogram in full mass range of an unknown water sample W26 on LCMS-9030

water samples W26 (Figure 3). Figure 4 illustrates the identification process of PFOA in sample W26 through library research via matching RT, MS, MS/MS spectra. The similarities of MS and MS/MS spectra for PFOA are 96% and 92%, respectively. The mass error measured of PFOA is 0.18 ppm.

The same water sample W26 was subjected to further investigation to find any undiscovered PFAS candidate using the mass defect filtering method with a threshold from [-10 mDa] to [-80 mDa] as described above. In addition, the specific PFAS diagnostic fragment ions of $C_2F_5^-$ and $C_3F_7^-$ were also used. Formula Predictor software and Structural Analytics software in LabSolutions™ are used in the subsequent structural elucidation and identification. A precursor ion of (-)m/z382.94179 was found via mass defect filtering to be a PFAS candidate due to: (i) a matched mass defect (-58.2 mDa), (ii) both $C_2F_5^-$, $C_3F_7^-$ were found. Moreover, using the formula predictor s/w, a most-like formula of $C_6F_{13}SO_2H$ with a mass error of +0.26 ppm was obtained. The fragments found

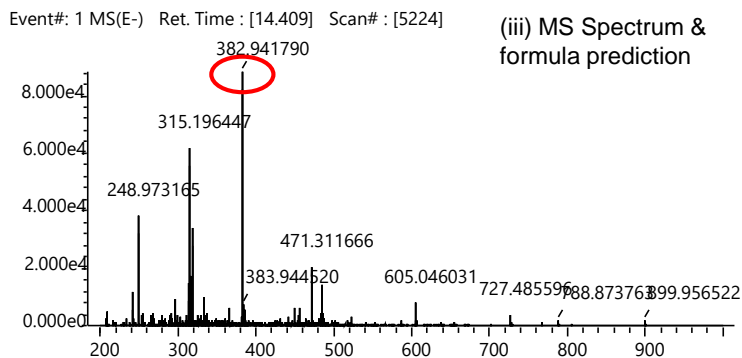
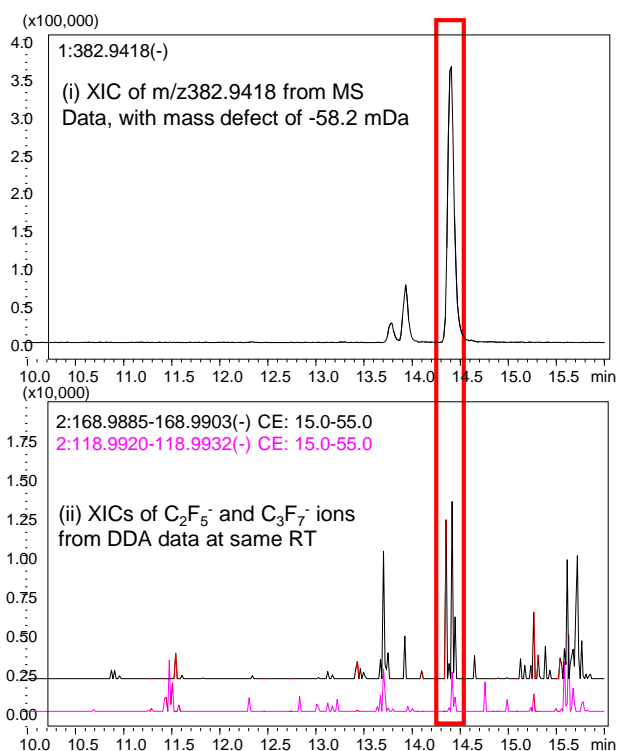


Hit#	Similarity	Report	CAS#	Compound Name	Theory MW	Formula
1	96	<input checked="" type="checkbox"/>	335-67-1	PFOA	413.9737	C8F15O2H
2	96	<input type="checkbox"/>	0-0-0	MPFOA	0.0000	13C4C4F15O

Hit#	Similarity	Report	CAS#	Compound Name	Theory MW	Formula
1	92	<input checked="" type="checkbox"/>	335-67-1	PFOA	413.9737	C8F15O2H

Figure 4: Identification of PFOA in a water sample. (i) Extracted chromatogram of FOAS; (ii) MS and library search result; (iii) MS/MS spectra and library search result

In the MS/MS spectrum match $C_6F_{13}^-$ and FSO_2^- , which correspond to PFAS skeletal structure and a head group, respectively. The PFAS found in sample W26 is likely to be perfluorohexyl sulfonic acid or its analogue. The above data analysis procedure is illustrated in details in Figure 5.



#	Score	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)
1	97.83	382.941690	382.941790	0.100	C6 H O2 F13 S	[M-H]-	0.2609
2	71.55	382.941414	382.941790	-0.376	C8 H2 N2 O7 F6 S	[M-H]-	0.9816
3	67.71	382.942557	382.941790	-0.767	C5 H3 N2 O8 F7 S	[M-H]-	-2.0027
4	55.10	382.940958	382.941790	0.832	C13 H5 O5 F4 S Cl	[M-H]-	2.1718
5	54.83	382.941015	382.941790	0.775	C7 H4 O8 F7 Cl	[M-H]-	2.0226

Event#: 2 MS/MS(E-) Precursor: 382.9418 CE:15.0-55.0 Ret. Time: [14.417]
Scan#: [5225] 3.83e3

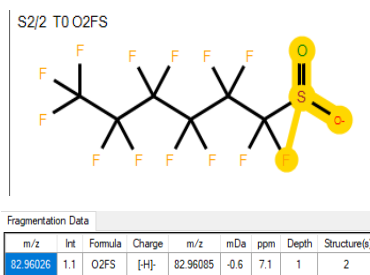
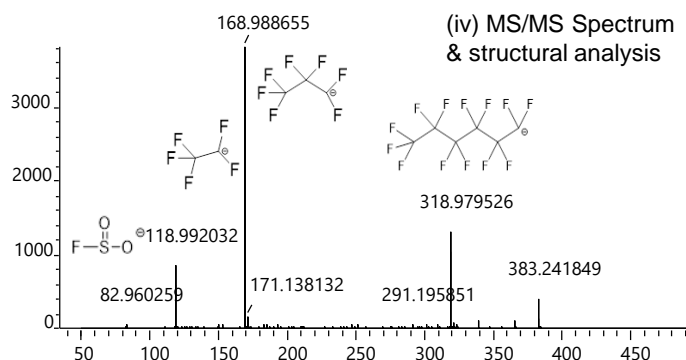


Figure 5: Illustration of finding an undiscovered PFAS: (i) XIC of (-)m/z 382.9417 from MS data via mass defect filtering; (ii) XIC of $C_2F_5^-$ and $C_3F_7^-$ ions from DDA data, (iii) MS spectrum and formula prediction, (iv) MS/MS spectrum and structural analysis; (v) assignment of fragment ion using Structural Analysis s/w.

4. Conclusions

A comprehensive screening method for targeted and undiscovered PFASs on Q-TOF mass spectrometer has been established and used in analysis of water samples. With DDA mode, MS and MS/MS data were acquired concurrently. An in-house library consisting of 34 PFASs with RT, MS and MS/MS spectra were used in targeted screening. A workflow was proposed and used to find and identify undiscovered PFAS based on mass defect filtering and specific diagnostic ions of PFASs. PFOA and PFOS were found in sample W26 via library search directly. While, a new PFAS, perfluorohexyl sulfonic acid ($C_6F_{13}SO_2H$) or its analogue, was identified successfully in the same sample using the workflow established.

References

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