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Application News

PFAS non-targeted screening / LCMS-9030

MDF-Based Workflow for Non-Targeted Screening for Per- and Polyfluoroalkyl Substances

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User Benefits

- A data analysis workflow based on mass defect filtering (MDF) was developed for non-targeted screening for PFAS from DDA data acquired on LCMS-9030.
- The workflow was verified with 29 PFAS in a mixed sample. All the 29 PFAS were picked up easily. In addition, 42 unknown PFAS were found in the same sample.
- Restricted settings for PFAS were adopted to obtain PFAS formula and Assign-MOL database search was used to provide tentative identification for unknown PFAS

■ Introduction

It is estimated that over 4000 per- & polyfluoroalkyl substances (PFAS) have been manufactured and used since 1940s. In recent years, analysis of PFAS in various samples (environmental, consumable products and human blood etc.) has become important due to the concerns of public health [1,2]. LC-MS/MS method has played key roles in targeted screening & quantitation of PFAS specified by the US EPA and other authorities. Due to large number, great structural diversity and limited resources in MS/MS library, non-targeted screening and identification of PFAS are highly challenging. LC-HRMS offers the possibility to unknown discovery without priori hypotheses or authentic standards. Recently, many research papers in non-targeted screening for PFAS by high resolution mass spectrometry were published [2-6]. This study is a continuous work to further develop and test a non-targeted screening approach established [5, 6] using a mixed sample of 29 PFAS. First, mass defect filtering (MDF) method was used to screen for all PFAS from DDA data acquired on LCMS-9030. Then, identification of the found PFAS was carried out using an approach described as restricted settings for PFAS to obtain PFAS formula, followed by MS/MS library search and Assig-MOL search in public database.

■ Experimental

Reagents and PFAS standards

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 ultrapure water. A ready to use mixed sample of 29 PFAS was purchased from supplier (each 2 ug/mL in methanol), which was diluted with ultrapure water to 1 ug/mL and 100 ng/mL as the testing samples. The ultrapure water used as diluent was analyzed as a blank sample. The information of the 29 PFAS standards are shown in Table 2.

LC-Q-TOF analytical conditions

Details of the analytical conditions for PFAS on LCMS-9030 (Shimadzu, Japan) are compiled into Table 1. The data acquisition method used includes MS and DDA events with optimized trigging parameters. Spread CE between 20 V and V 50 was used for all precursors.

Table 1 Analytical conditions of PFAS on LCMS-9030

LC Conditions	
Column	Shim-pack Velox™, C18 (2.1x100 mm, 2.7 µm), P/N: 227-32009-03
Flow Rate	0.4 mL/min
Mobile Phase	A: 5 mM Ammonium acetate in pure water B: Acetonitrile
LC gradient	B: 20% (0-0.5 min) → 80% (9-9.5 min) → 20% (9.6-12 min) → stop
Delay column	Shim-pack Velox, C18 (2.1x50 mm, 2.7 µm)
Oven Temp.	40°C
Injection Vol.	1 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 DDA (-), *m/z* 50~1000; with CE 35V and Spread (+/-) 15V Loop time: 0.15 sec

■ Results and Discussion

1. Mass defect filtering (MDF) for PFAS

With replacing fully or partially the H atoms on carbon skeleton with fluorine, the mass defect values of most PFAS (C: 4~20) fall in a special range, i.e., from -10 mDa to -120 mDa [5]. Therefore, MDF method can be used to pick up PFAS components from HRMS scan data easily. A data analysis workflow for non-targeted screening and identification for PFAS is illustrated in Figure 1. Specifically, a DDA data file is sent to the LabSolutions Insight Explore – Analyze. All precursors listed in the Analyze pane are sorted and copied into an Excel sheet, where the MD values of every precursor is calculated. The precursors which MD values fall in the range from -10 mDa to -120 mDa are regarded as PFAS candidates and are kept. The rest precursors are deleted due to their MD values out of the above range [Supporting Materials].

2. PFAS Formula Prediction

Before performing formula prediction, reviewing of the precursor spectrum is needed, because this allows us to know if the precursor is mono-isotope ion and if Cl or Br is present etc. Restricted element settings for PFAS in the formula predictor is critical, which can narrow down greatly the range of candidates within a maximum mass error [5]. The restricted settings for PFAS adopted are described as below:

1) Initial element settings restricted for PFAS: C, 4~20, F, 5~40, H, 1~10, O, 1~5, N, 0~5 and S, 0~1
2) Other elements: P, Cl, Br. Adding them when there is no fitting result using the initial settings
3) DBE: 0 and 1 are always the preferred choices
4) Number of H atom: 1 H or less H is selected first

The example in Figure 2 demonstrates the procedure. First, the MS spectrum pattern of a precursor (XIC 298.9424) confirm that the ion is mono-isotope without Cl or Br. The result obtaining with above settings generated one formula, $C_4HO_3F_9S$, which is correct to the compound (perfluorobutane-1-sulfonic acid, CAS: 375-73-5). More examples are shown in the [Supporting Materials].

3. Testing with mixed 29 PFAS sample

The mixed sample containing 29 PFAS was used to test and verify the effectiveness and reliability of the MDF-based workflow. This sample and the diluent (ultrapure water) as blank sample were analyzed with DDA method. To avoid contamination, the blank sample was injected five times before injection of the mixed 29 PFAS sample. Data analysis was carried out following the workflow as illustrated in Figure 1. The result shows that not only the 29 PFAS were picked up and confirmed, but additional 42 unknown PFAS components were discovered in the same sample. PFAS component was not found in the blank water sample. This result excludes any possibility that any of the 42 unknown PFAS components are from the solvent, mobile phase or column used.

The screening results of the 29 PFAS are summarized in Table 2. First, the 29 PFAS were picked up in the MDF step from the precursor list in Analyze pane. These PFAS show MD values between -17.7 mDa and -106.1 mDa, falling in the expected range of -10 ~ -120 mDa [5]. Next step was to obtain PFAS formula using Formula Predictor with the restricted settings for PFAS. Under the specific conditions, one or few candidates were generated (Figure 1). For more than one candidates, selecting the one with DBE=0 or 1 as is always the preferred choice. This is because most PFAS have DBE of 0 or 1, and fewer PFAS have DBE =/>2 [Supporting Material]. In addition, many PFAS has one or less H atoms, because all or most H on the carbon skeleton are replaced with F atoms.

Figure 3 shows an example, which precursor is *m/z* 530.8969. Formula predictor with the initial restricted settings for PFAS gave no result. In fact, the MS spectrum pattern indicates that Cl is present. After adding Cl in the element settings, a unique formula C8HO4F16SCl was generated, which matches the PFAS, potassium 9-chlorohexadecafluoro-3-oxanone-1-sulfonate. A more challenging example is shown in Figure 4. For the precursor *m/z* 988.9605, multiples candidates were generated with the initial settings. However, none of them (not showing) is correct, because of the molecule containing P. After adding P in the element settings, the correct formula, (Bis[2-(perfluorooctyl)ethyl] phosphate 8:2-diPAP, was obtained.

Library search of MS/MS spectrum may provide final confirmation when multiple candidates appear. The MS/MS library used are MSDIAL-PFAS and an in-house PFAS library [6]. As shown in Figure 4 (bottom), library search confirms the compound to be 8:2-diPAP, C20H9F34O4P.

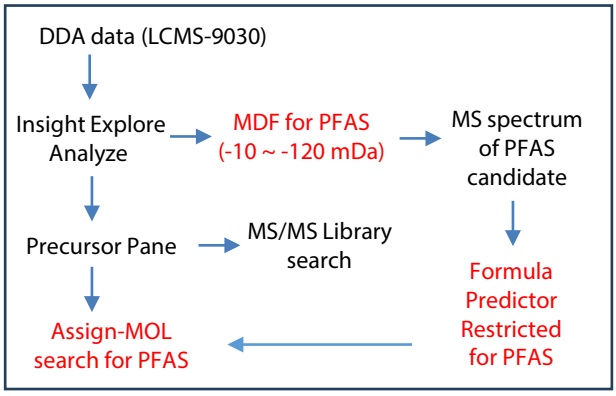


Figure. 1 Mass defect filtering (MDF) based workflow for PFAS screening in DDA data file.

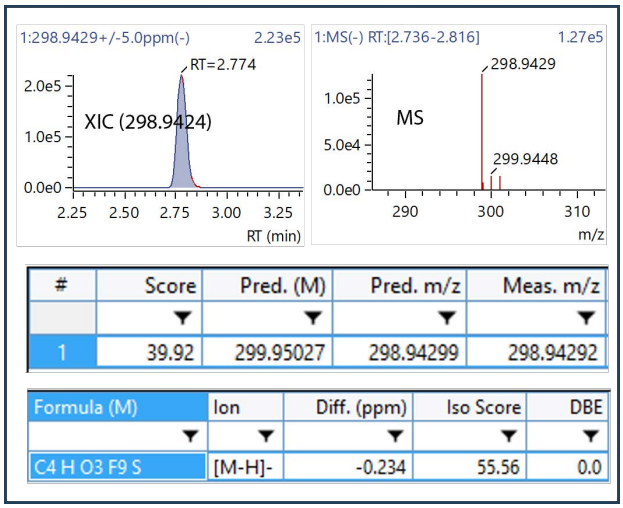


Figure. 2 Result of formula prediction for *m/z* 298.9424. Only formula C4HO3F9S is produced with allowed mass error 3 ppm.

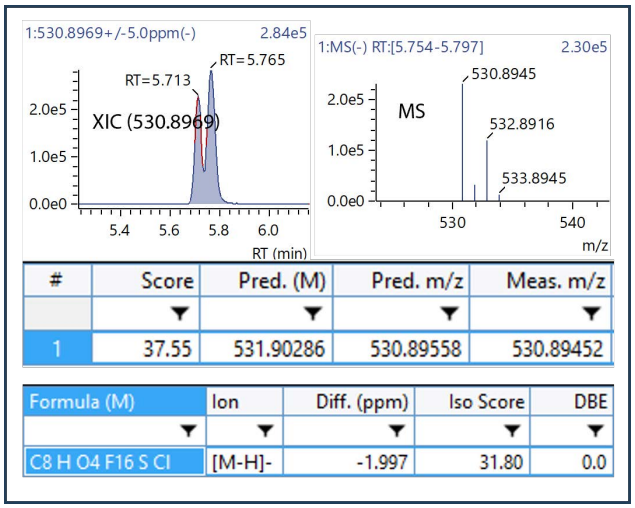


Figure. 3 Result of formula prediction for *m/z* 530.8947. A unique candidate C8HO4F16SCl obtained matches potassium 9-chlorohexadecafluoro-3-oxanone-1-sulfonate, CAS 73606-19-6.

Table 2 Detection and confirmation of 29 PFAS by MDF, Restricted formula prediction, MS/MS Library search and MOL DB search

Measured			Data Analysis MDF-based workflow								PFAS Information		
#	Precursor *m/z*	RT	Mass defect (mDa)	Formula (PFAS restriction)	Ion	Diff (ppm)	Iso Score	DBE	MS/MS lib search	Assign-MOL Search (ChemSpider or PubChem)	Abbr. Name	CAS	Formula
1	212.9783	0.857	-21.7	C4HO2F7	[M-H]-	-4.2	37.0	1	N.A.	Heptafluorobutyric acid, C4HO2F7	HFBA	375-22-4	C4HF7O2
2	262.9746	1.723	-25.4	C5HO2F9	[M-H]-	-3.8	77.2	1	N.A.	PFPeA, C5HF9O2	PFPeA	2706-90-3	C5HF9O2
3	298.9429	2.756	-57.1	C4HO3F9S	[M-H]-	-0.2	43.4	0	yes	1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulfonic acid (C4HF9O3S)	PFBS	375-73-5	C4HF9O3S
15	312.9732	2.811	-26.8	C6HO2F11	[M-H]-	-3.5	70.9	1	Yes	Perfluorohexanoic acid, C6HF11O2	PFHxA	307-24-4	C6HF11O2
20	328.9656	3.118	-34.4	C6HO3F11	[M-H]-	-3.5	69.9	1	N.A	Perfluoro-2-propoxypropanoic acid, C6HF11O3	HFPO-DA	13252-13-6	C6HF11O3
22	362.9688	3.608	-31.2	C7HO2F13	[M-H]-	-2.3	69.5	1	Yes	Perfluoroheptanoic acid, C7HF13O2	PFHpA	375-85-9	C7HF13O2
27	376.9681	3.844	-31.9	C7H2O4F12	[M-H]-	-2.3	73.5	1	yes	2,2,3-Trifluoro-3-[1,1,2,2,3,3-hexafluoro-3-(trifluoromethoxy)propoxy]propanoic acid, C7H2F12O4	NaDONA	2250081-67-3	C7H2F12O4
51	398.9357	4.246	-64.3	C6HO3F13S	[M-H]-	-2.2	71.4	0	Yes	Perfluorohexanesulfonic acid, C6HF13O3S	PFHxS	355-46-4	C6HF13O3S
42	412.9646	4.198	-35.4	C8HO2F15	[M-H]-	-2.6	71.0	1	N.A.	Perfluorooctanoic Acid, C8HF15O2	PFOA	335-67-1	C8HF15O2
32	426.9669	3.991	-33.1	C8H5O3F13S	[M-H]-	-1.9	83.3	0	Yes	3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctane-1-sulfonic acid, C8H5F13O3S	6:2 PFOS	27619-97-2	C8H5F13O3S
80	448.9328	4.856	-67.2	C7HO3F15S	[M-H]-	-1.5	66.2	0	yes	1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoroheptane-1-sulfonic acid, C7HF15O3S	PFHpS	375-92-8	C7HF15O3S
64	456.9713	4.572	-28.7	C10H2O2F16	[M-H]-	-3.0	69.2	2	yes	3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-hexadecafluorodec-2-enoic acid, C10H2F16O2	FOUEA	70887-84-2	C10H2F16O2
69	462.9628	4.721	-37.2	C9HO2F17	[M-H]-	-3.8	53.8	1	N.A.	Perfluorononanoic acid, C9HF17O2	PFNA	375-95-1	C9HF17O2
254	497.9444	7.086	-55.6	C8H2NO2F17S	[M-H]-	-1.9	41.0	0	Yes	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonamide, C8H2F17NO2S.	FOSA	754-91-6	C8F17SO2NH2
103	498.9274	5.071	-72.6	C8HO3F17S	[M-H]-	-1.5	63.4	0	Yes	Perfluorooctanesulfonic acid, C8HF17O3S	PFOS	1763-23-1	C8HF17O3S
288	511.9610	8.922	-39.0	C9HNO3F18	[M-H]-	2.6	57.9	1	Yes	N-Methylperfluorooctanesulfonamide, C9H4F17NO2S	N-MeFOSA	31506-32-8	C9H4F17NO2S
120	512.9596	5.243	-40.4	C10HO2F19	[M-H]-	-1.9	71.2	1	Yes	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluorodecanoic acid, C10HF19O2	PFDA	335-76-2	C10HF19O2
291	525.9761	9.355	-23.9	C10H3NO3F18	[M-H]-	1.8	88.1	1	Yes	3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluoro-1-decanesulfonamide, C10H6F17NO2S	N-EtFOSA	4151-50-2	C10H6F17NO2S
98	526.9618	4.997	-38.2	C10H5O3F17S	[M-H]-	-2.0	70.3	0	Yes	1H,1H,2H,2H-Perfluorodecanesulfonic acid, C10H5F17O3S	8:2 PFOS	39108-34-4	C10H5F17O3S
170	530.8939	5.7	-106.1	C8HO4F16SCl	[M-H]-	-2.0	68.3	0	Yes	2-(6-chloro-1,1,2,2,3,3,4,4,5,5,6,6-dodecafluorohexoxy)-1,1,2,2-tetrafluoroethanesulfonic acid, C8HClF16O4S	9Cl-PF3ONS	73606-19-6	C8HClF16O4S
171	562.9558	5.701	-44.2	C11HO2F21	[M-H]-	-1.6	70.3	1	Yes	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-henicosafluoroundecanoic acid, C11HF21O2	PFUnDA	2058-94-8	C11HF21O2
142	569.9667	5.389	-33.3	$C_{11}H_6NO_4F_{17}S$	[M-H]-	-1.7	69.5	1	Yes	2-[1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctylsulfonyl(methyl)amino]acetic acid, C11H6F17NO4S	N-MeFOSAA	2355-31-9	C11H6F17NO4S
167	583.9823	5.6	-17.7	$C_{12}H_8NO_4F_{17}S$	[M-H]-	-1.7	67.9	1	Yes	N-Ethylperfluorooctane sulfonamidoacetic acid, C12H8F17NO4S	N-EtPFOSAA	2991-50-6	C12H8F17NO4S
214	598.9226	6.325	-77.4	C10HO3F21S	[M-H]-	2.2	25.7	0	Yes	Perfluorodecanesulfonic acid, C10HF21O3S	PFDS	335-77-3	C10HF21O3S
202	612.9524	6.161	-47.6	C12HO2F23	[M-H]-	-1.6	61.4	1	Yes	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-tricosafluorododecanoic acid, C12HF23O2	PFDoA	307-55-1	C12HF23O2
233	662.9495	6.615	-50.5	C13HO2F25	[M-H]-	-1.4	68.8	1	Yes	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,13-pentacosafluorotridecanoic acid, C13HF25O2	PFTrA	72629-94-8	C13HF25O2
246	712.9467	7.049	-53.3	C14HO2F27	[M-H]-	-1.8	69.7	1	Yes	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,14-heptacosafluorotetradecanoic acid, C14HF27O2	PFTeA	376-06-7	C14HF27O2
276	812.9388	7.964	-61.2	C16HO2F31	[M-H]-	-2.1	65.9	1	Yes	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,15,15,16,16,16-hentriacontafluorohexadecanoic acid, C16HF31O2	PFHxDA	67905-19-5	C16HF31O2
282	988.9574	8.373	-42.6	$C_{20}H_9O_4F_{34}P$	[M-H]-	-1.8	66.7	0	Yes	bis(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl) hydrogen phosphate, C20H9F34O4P	8:2-DiPAP	678-41-1	C20H9F34O4P

Currently, ESI-based MS/MS libraries for PFAS are still limited and spectra are acquired on different types of mass spectrometers with different CID conditions. Library search often produces results with low similarity Index (SI) or even no result (Table 2).

Assign-MOL database search with fragment annotation is another approach for identification of PFAS. Compound structures are saved in MOL file format in compound database such as ChemSpider, PubChem and EPA PFAS Master List [7]. These public databases have collected huge numbers of compounds including PFAS. Hence, once a formula is obtained, it can be sent to Assign for MOL search against these database. As shown in Table 2, all the 29 PFAS were found in the Assign-MOL databases search. In addition, the fragment peaks could be annotated to the MOL structures.

4. Unknown PFAS found in sample

As mentioned in the previous session, additional 42 unknown PFAS components were found in the same sample. The screening and identification results of these unknown PFAS are shown in Table 3. The PFAS formulas listed were obtained from Formula Predictor with the restricted settings for PFAS described above. Among the 42 unknown PFAS, 30 compounds could be provided with tentative identification obtaining from the Assign-MOL search using the PFAS formula obtained. It could be concluded that these compounds are PFAS, although only tentative identification or even no identification could be provided for them.

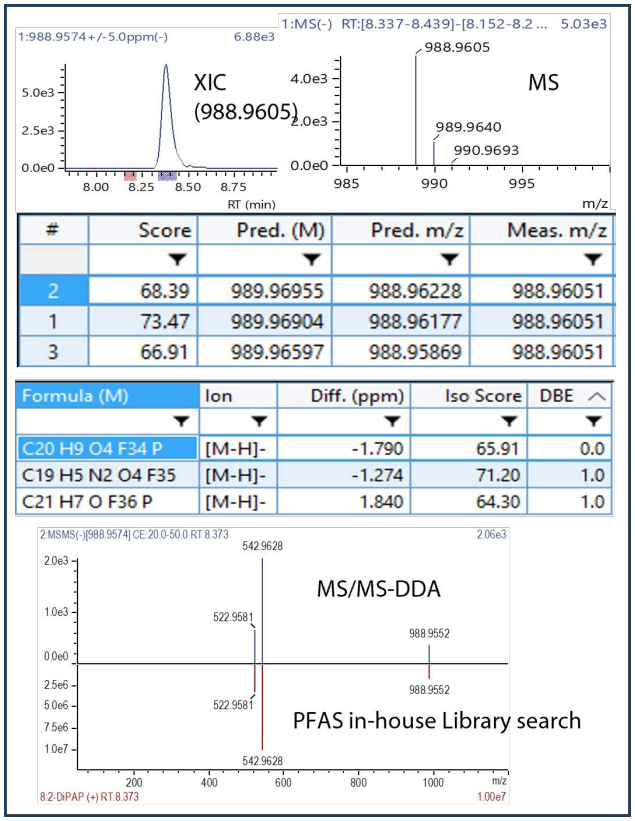


Figure. 4 (Top-middle) Formula prediction for *m/z* 988.9605, candidate C20H9O4F34P is correct (Bis[2-(perfluorooctyl)ethyl] phosphate, CAS 678-41-1). (Bottom) The PFAS is confirmed by MS/MS library search.

Table 3 Detection and identification of 42 unknown PFAS in sample by MDF-based workflow

#	Precursor *m/z*	RT	Mass defect (mDa)	Formula Predictor (PFAS restriction)	Ion	Diff (ppm)	Iso Score	DBE	MS/MS lib search	Assign-MOL Search (ChemSpider or PubChem)
17	246.9807	2.835	-19.3	C5HOF9	[M-H]-	-3.0	45.8	1	N.A.	5H-Octafluoropentanoyl fluoride, C5HF9O
28	250.9756	3.844	-24.4	C4HO2F9	[M-H]-	-2.4	84.4	0	N.A.	1-(Difluoromethoxy)-1,1,2,2-tetrafluoro-2-(trifluoromethoxy)ethane, C4HF9O2
19	268.9824	2.854	-17.7	C5H4N2O2F6S	[M-H]-	-0.8	95.9	2	N.A.	4,5-dihydroxy-4,5-bis(trifluoromethyl)imidazolidine-2-thione, Compound CID: 168355037, C5H4F6N2O2S
50	280.9822	4.236	-17.8	C6H4N2O2F6S	[M-H]-	-1.0	99.1	3	N.A.	[5-[(1,1,1,3,3,3-Hexafluoro-2-propanyl)oxy]-1,3,4-thiadiazol-2-yl]methanol, C6H4N2O2F6S
23	296.9770	3.609	-23.0	C6HOF11	[M-H]-	-2.9	76.8	1	N.A.	2,2,3-Trifluoro-3-(1,1,2,2,3,3,4,4-octafluorobutyl)oxirane, C6HOF11 or isomer
24	318.9794	3.612	-20.6	C6H4N2O2F8S	[M-H]-	-0.3	98.6	2	N.A.	No result
44	346.9739	4.219	-26.1	C7HOF13	[M-H]-	-2.4	81.3	1	N.A.	2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoroheptanal, C7HF13O or isomer
43	368.9759	4.218	-24.1	C7H4N2O2F10S	[M-H]-	-0.5	98.6	2	N.A.	No result
114	380.9746	5.226	-25.4	C5H2N2O4F12	[M-H]-	1.5	50.9	0	N.A.	No result
65	392.9754	4.573	-24.6	C6H2N2O4F12	[M-H]-	1.3	76.8	1	Not Mat	No result
71	396.9700	4.732	-30.0	C8HOF15	[M-H]-	-2.3	72.2	1	N.A.	Octanal, pentadecafluoro, C8HF15O
34	406.9609	3.994	-39.2	C8H4O3F12 S	[M-H]-	-2.7	74.2	1	N.A.	3-(1,1,2,2,3,3,4,4,5,5,6,6-dodecafluorohexyl)oxathietane 2,2-dioxide, C8H4F12O3S
70	418.9725	4.731	-27.5	C8H4N2O2F12 S	[M-H]-	-0.6	97.9	2	N.A.	2-[1,1,1,3,3,3-hexafluoro-2-[2-(1,1,1,3,3,3-hexafluoropropan-2-ylidene)hydrazinyl]propan-2-yl]sulfanylacetic acid, C8H4F12N2O2S
176	430.9720	5.711	-28.0	C6H2N2O4F14	[M-H]-	1.3	71.3	0	N.A.	No result
113	446.9668	5.222	-33.2	C9HOF17	[M-H]-	-2.1	71.9	1	Yes	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Heptadecafluorononanal, C9HF17O
112	468.9691	5.221	-30.9	C9HF19	[M-H]-	-0.3	93.1	2	Yes	2,2,3,4,4,5,5,6,6,7,8,8,8-tridecafluoro-3,7-bis(trifluoromethyl)octanoic acid, C10HF19O2
102	476.9264	5.037	-73.6	C8HO4F15S	[M-H]-	-2.3	66.5	1	N.A.	1,1,2,2,3,3,4,4,5,5,6,6,8,8,8-Pentadecafluoro-7-oxo-1-octanesulfonic acid, C8HF15O4S
204	480.9692	6.164	-30.8	C7H2N2O4F16	[M-H]-	0.7	92.3	0	N.A.	No result
159	482.9333	5.583	-66.7	C8HO2F17S	[M-H]-	-1.6	83.0	0	N.A.	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Heptadecafluoro-1-octanesulfinic acid, C8HF17O2S
172	496.9641	5.703	-35.9	C10HOF19	[M-H]-	-1.6	71.2	1	Not Mat	(E)-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,9,10,10,10-nonadecafluorodec-8-en-1-ol, C10HF19O
154	514.9025	5.426	-97.5	C8HO3F16SCl	[M-H]-	-2.6	70.2	0	Yes	8-Chloro-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-hexadecafluoro-1-octanesulfonic acid, C8HClF16O3S
174	518.9665	5.706	-33.5	C10H4N2O2F16S	[M-H]-	-0.2	95.8	2	N.A.	No result
213	524.9576	6.189	-42.4	C11HO2F19	[M-H]-	-2.0	27.3	2	Yes	(E)-2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,10,11,11,11-nonadecafluoroundec-9-enoic acid, C11HF19O2
164	525.9750	5.593	-25.0	C10H6NO2F17S	[M-H]-	-2.4	69.6	0	Yes	N-ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonamide. C10H6F17NO2S
206	546.9606	6.167	-39.4	C11HOF21	[M-H]-	-1.6	76.9	1	N.A	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-henicosafluoroundecanal, C11HF21O
203	568.9625	6.162	-37.5	C11H4N2O2F18S	[M-H]-	-0.7	96.7	2	N.A.	No result
252	580.9621	7.079	-37.9	C9H2N2O4F20	[M-H]-	0.4	95.1	0	N.A.	No result
235	596.9573	6.619	-42.7	C12HOF23	[M-H]-	-1.8	71.0	1	Not mat	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-tricosafluorododecanal, C12HF23O or isomer
234	618.9588	6.616	-41.2	C12H4N2O2F20S	[M-H]-	-0.7	98.3	2	N.A.	No result
16	626.9508	2.832	-49.2	C12H2O4F22	[M-H]-	-2.2	72.1	1	N.A.	1-[2-[2-[1,1-difluoro-2-(1,2,2-trifluoroethenoxy)ethoxy]-1,1,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoroethoxy]-1,1,2,2,3,3,4,4,4-nonafluorobutane, C12H2F22O4
130	632.9617	5.361	-38.3	C12H2O2F24	[M-H]-	2.3	24.0	0	N.A.	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12-tetracosafluorododecane-1,12-diol, C12H2F24O2
248	646.9540	7.070	-46.0	C13HOF25	[M-H]-	-1.7	69.4	1	N.A.	(E)-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,12,13,13,13-pentacosafluorotridec-11-en-1-ol, C13HF25O, or isomers
157	646.9770	5.576	-23.0	C13H4O2F24	[M-H]-	2.1	67.0	0	N.A.	(2) [1,1,2,2,3,3-hexafluoro-3-(2-methylprop-2-enoyloxy)propyl] 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoate, C15H5F21O4
5	656.9466	2.760	-53.4	C13HO4F23	[M-H]-	2.3	71.3	2	N.A.	3-[(E)-1,2-difluoro-2-(1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctoxy)ethenoxy]-2,2,3,3-tetrafluoropropanoic acid, C13HF23O4
247	668.9560	7.069	-44.0	C13H4N2O2F22S	[M-H]-	-1.0	96.3	2	N.A.	No result
274	698.9131	7.245	-86.9	C12HO3F25S	[M-H]-	-2.4	69.1	0	N.A.	Perfluorododecanesulfonic acid, C12HF25O3S
251	728.9160	7.077	-84.0	C14HO2F26Cl	[M-H]-	-2.5	65.2	1	Not Mat	14-chloro-2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14-hexacosafluorotetradecanoic acid , C14HClF26O2
277	746.9479	7.964	-52.1	C15HOF29	[M-H]-	-2.0	68.1	1	Not mat	(E)-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,14,15,15,15-nonacosafluoropentadec-13-en-1-ol , C15HF29O
280	768.9499	7.971	-50.2	C15HN2O3F27	[M-H]-	1.5	69.4	3	N.A	No result
270	774.9432	7.221	-56.8	C16 HO2F29	[M-H]-	-2.1	63.2	2	Not Mat	(E)-2,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,15,15,16,16,16-nonacosafluorohexadec-2-enoic acid, C16HF29O2
54	856.9315	4.303	-68.5	C17 H O4 F31	[M-H]-	1.2	85.1	2	No result	3-[(E)-1,2-difluoro-2-(1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-pentacosafluorododecoxy)ethenoxy]-2,2,3,3-tetrafluoropropanoic acid, C17HF31O4
76	926.9327	4.748	-67.3	C18H2O4F34	[M-H]-	-2.2	64.0	1	Not Mat	2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluorononanoic acid, C18H2F34O4

In summary, the MDF-based workflow could effectively discover PFAS components and obtain PFAS formula. Assign-MOL database search gives tentative identification for many unknown PAS. However, confirmation by MS/MS library search failed in many cases. This is likely due to (1) the PFAS are not registered in library or (2) fragmentation of PFAS did not occur sufficiently.

■ Conclusion

Based on the unique mass defect (MD) feature of PFAS, a data analysis workflow is established for non-targeted screening for PFAS. The workflow was tested and verified successfully with a mixed sample of 29 PFAS. Not only all the 29 PFAS were picked up and confirmed easily, but 42 unknown PFAS components were discovered. However, identification of unknown PFAS is challenging. Assign-MOL database search could provide tentative identification for many unknown PFAS.

■ References

1. Wang Z. et. al., "A Never-Ending Story of PFASs?", Env. Sci. & Tech., Vol. 51(5), 2017, 2508–2518.
2. Y. Liu et al., "High-resolution mass spectrometry methods for non target discovery and characterization of PFASs in environmental and human samples", Trends in Ana. Chem., Vol 121 Dec 2019, 115420.
3. Boris Bugsel et al., "Nontarget screening strategies for PFAS prioritization and identification by high resolution mass spectrometry: A review", Trends in Envi. Ana. Chem., Vol 40, Dec. 2023, e00216
4. Joseph A. Charbonnet at al., "Communicating Confidence of Per- and Polyfluoroalkyl Substance Identification via High-Resolution Mass Spectrometry', Environ. Sci. Technol. Lett. 2022, 9, 473–481.
5. Lee J. X. et. al., "Development of Screening Analysis Method for PFAS in Surface Water on LC-Q-TOF", Shimadzu Application News, AD-0200A (2020),
6. Z. Zhan et al. "Untargeted Screening of PFAS by HRAM-DIA method on LCMS-9030", Shimadzu Application News, 04-AD-0280-en (2022).
7. PFAS Master list (retired), CompTox Chemicals Dashboard (epa.gov).

[Supporting Materials]

1. Mass Defect Filtering for PFAS

- Copy and paste the Precursor List to Excel
- Calculate Mass Defect (MD) of every precursor
- Delete those precursors which MD are out of the PFAS range (-10 ~ -120 mDa)

Data: 115, DDA, Precursor (307), After MDF: ??? (MD: -10 ~ -120 mDa)

#	Event	Precursor m/z	Intensity	RT	Mass defect (mDa)	Formula Predictor (PFAS restrition)	Ion	Diff (ppm)	Iso Score	DBE	Type
1	2	212.9783	12,042	0.857	-21.7						
17	2	246.9807	41,074	2.835	-19.3						
28	3	250.9756	209,646	3.844	-24.4						
297	2	255.2315	19,325	9.939	-768.5						
298	2	255.2329	12,190	9.969	-767.1						
2	2	262.9746	26,881	1.723	-25.4						
19	2	268.9824	63,086	2.854	-17.7						
50	2	280.9822	27,178	4.236	-17.8						
302	2	283.2629	14,120	10.031	-737.1						
23	3	296.9770	88,236	3.609	-23.0						
3	2	298.9429	201,508	2.756	-57.1						
10	2	298.9894	39,952	2.771	-10.6						
4	3	299.0483	8,568	2.757	-951.7						
6	3	299.0694	6,706	2.761	-930.7						
12	2	299.1852	4,718	2.775	-814.8						
13	2	300.9385	34,249	2.777	-61.5						
158	2	311.1677	9,437	5.58	-832.3						
15	2	312.9732	8,743	2.811	-26.8						
24	2	318.9794	147,469	3.612	-20.6						
208	2	325.1830	5,539	6.173	-817.0						
20	2	328.9656	24,770	3.118	-34.4						
79	2	330.9787	40,420	4.759	-21.3						
232	2	339.1988	5,336	6.425	-801.2						
44	3	346.9739	143,333	4.219	-26.1						
22	2	362.9688	163,837	3.608	-31.2						
43	2	368.9759	337,164	4.218	-24.1						
47	2	369.0941	5,522	4.228	-905.9						
46	2	369.1164	5,536	4.225	-883.6						
27	2	376.9681	165,950	3.844	-31.9						
114	2	380.9746	57,461	5.226	-25.4						
65	2	392.9754	64,556	4.573	-24.6						

✓ Delete those MD out of the range (-10 mDa ~ -120 mDa)

Figure. S1 calculation of mass defect in Excel and delete non-PFAS precursors

Data: 115, DDA, Precursor (307), After MDF: 152 (MD: -10 ~ -120 mDa)

#	Event	Precursor m/z	Intensity	RT	Mass defect (mDa)
1	2	212.9783	12,042	0.857	-21.7
17	2	246.9807	41,074	2.835	-19.3
28	3	250.9756	209,646	3.844	-24.4
2	2	262.9746	26,881	1.723	-25.4
19	2	268.9824	63,086	2.854	-17.7
50	2	280.9822	27,178	4.236	-17.8
23	3	296.9770	88,236	3.609	-23.0
3	2	298.9429	201,508	2.756	-57.1
10	2	298.9894	39,952	2.771	-10.6
13	2	300.9385	34,249	2.777	-61.5
15	2	312.9732	8,743	2.811	-26.8
24	2	318.9794	147,469	3.612	-20.6
20	2	328.9656	24,770	3.118	-34.4
79	2	330.9787	40,420	4.759	-21.3
44	3	346.9739	143,333	4.219	-26.1
22	2	362.9688	163,837	3.608	-31.2
43	2	368.9759	337,164	4.218	-24.1
27	2	376.9681	165,950	3.844	-31.9
114	2	380.9746	57,461	5.226	-25.4
65	2	392.9754	64,556	4.573	-24.6
71	3	396.9700	52,442	4.732	-30.0
63	2	398.9349	8,719	4.415	-65.1
31	2	398.9353	5,424	3.879	-64.8

Data: 113, DDA (Milli-Q wtaer), Precursor (19), After MDF: 0 (MD: -10 ~ -120 m

#	Event	Precursor m/z	Intensity	RT	Mass defect (mDa)
8	2	255.2322	16,148	9.954	-767.8
10	2	283.2630	14,845	10.003	-737.1
17	2	405.2838	4,761	10.337	-716.2
12	2	451.2890	4,817	10.077	-711.0
3	2	465.3027	4,794	9.548	-697.3
16	2	465.3031	7,069	10.309	-696.9
13	2	465.3033	5,835	10.103	-696.7
7	2	465.3036	10,637	9.932	-696.4
19	2	465.3041	5,457	10.479	-695.9
1	2	465.3054	7,476	9.461	-694.6
5	2	465.3057	5,042	9.674	-694.3
15	2	465.3060	6,237	10.195	-694.1
11	2	465.3060	8,889	10.023	-694.0
18	2	465.3065	9,651	10.365	-693.5
6	2	465.3067	8,020	9.842	-693.3
4	2	465.3081	5,412	9.649	-691.9
14	2	465.3094	8,314	10.155	-690.6
2	3	728.5321	6,723	9.462	-467.9
9	2	742.5461	5,745	9.982	-453.9

Figure. S2 152 PFAS-like precursors found in sample (data: 115) and 0 FFAS-like precursor in diluent (Data: 113)

2. Restricted settings for PFAS in Formula Predictor

Rule	Restricted settings for PFAS	Remark
1	Initial element settings restricted for PFAS: C, 4~20, F, 5~40, H, 1~10, O, 1~5, N, 0~5 and S, 0~1	The settings cover PFAS of C4~C20; refer to ratio (F+H-N)/C = 2 for DBE=1; at least 1 O in PFAS to exclude polyfluoro alkanes and alkenes;
2	Other elements: P, Cl, Br. Adding them when there is no fitting result using the initial settings	Check the spectrum pattern if the compound contains Cl and Br; It is seldom that PFAS contains both S and P or 2 S.
3	DBE: 0 and 1 are always the preferred choices	Most PFAS have DBE=0 or 1; few with DBE=2; seldom PFAS with DBE >2 (see Table S1)
4	Number of H atom: 1 H or less H is selected first	All perfluoroalkyl carboxylic acids and perfluoroalkyl sulfonates have one H only (see Table S1)

Table S1 29 PFAS compound information, formula, BDE and H

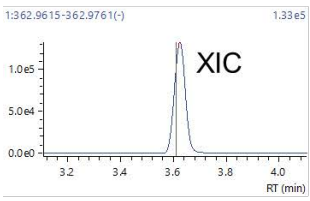
No.	Name	Abbrev.	CAS	Formula	Formula	DBE	H atom
1	Perfluoro-n-butanoic acid	HFBA	375-22-4	C4HF7O2	$CF_3(CF_2)_2COOH$	1	1
2	Perfluoro-n-pentanoic acid	PFPeA	2706-90-3	C5HF9O2	$CF_3(CF_2)_3COOH$	1	1
3	Perfluoro-n-hexanoic acid	PFHxA	307-24-4	C6HF11O2	$CF_3(CF_2)_4COOH$	1	1
4	Perfluoro-n-heptanoic acid	PFHpA	375-85-9	C7HF13O2	$CF_3(CF_2)_5COOH$	1	1
5	Perfluoro-n-octanoic acid	PFOA	335-67-1	C8HF15O2	$CF_3(CF_2)_6COOH$	1	1
6	Perfluoro-n-nonanoic acid	PFNA	375-95-1	C9HF17O2	$CF_3(CF_2)_7COOH$	1	1
7	Perfluoro-n-decanoic acid	PFDA	335-76-2	C10HF19O2	$CF_3(CF_2)_8COOH$	1	1
8	Perfluoro-n-undecanoic acid	PFUnDA	2058-94-8	C11HF21O2	$CF_3(CF_2)_9COOH$	1	1
9	Perfluoro-n-dodecanoic acid	PFDoA	307-55-1	C12HF23O2	$CF_3(CF_2)_{10}COOH$	1	1
10	Perfluoro-n-tridecanoic acid	PFTrA	72629-94-8	C13HF25O2	$CF_3(CF_2)_{11}COOH$	1	1
11	Perfluoro-n-tetradecanoic acid	PFTeA	376-06-7	C14HF27O2	$CF_3(CF_2)_{12}COOH$	1	1
12	Perfluorohexadecanoic acid	PFHxDA	67905-19-5	C16HF31O2	$CF_3(CF_2)_{14}COOH$	1	1
13	Perfluorooctane sulfonamide	FOSA	754-91-6	C8F17SO2NH2	$CF_3(CF_2)_7SOONH_2$	0	2
14	N-Methylperfluoro-1-octanesulfonamide	N-MeFOSA	31506-32-8	C9H4F17NO2S	$CF_3(CF_2)_7SOONHCH_3$	0	4
15	N-Ethylperfluorooctylsulfonamide (Sulfluramid)	N-EtFOSA	4151-50-2	C10H6F17NO2S	$CF_3(CF_2)_7SOONHC_2H_5$	0	6
16	N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	2355-31-9	C11H6F17NO4S	$CF_3(CF_2)_7SOON(CH_3)CH_2COOH$	1	6
17	N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtPFOSAA	2991-50-6	C12H8F17NO4S	$CF_3(CF_2)_7SOON(C_2H_5)CH_2COOH$	1	8
18	2H-Perfluoro-2-decenoic Acid	FOUEA	70887-84-2	C10H2F16O2	$CF_3(CF_2)_7CF{=}CHCOOH$	2	2
19	Perfluoro(2-methyl-3-oxahexanoic) acid	HFPO-DA	13252-13-6	C6HF11O3	$CF_3(CF_2)_2OCF(CF_3)COOH$	1	1
20	Perfluorobutane-1-sulfonic acid	PFBS	375-73-5	C4HF9O3S	$CF_3(CF_2)_3SO_2OH$	0	1
21	Perfluorohexane-1-sulfonic acid	PFHxS	355-46-4	C6HF13O3S	$CF_3(CF_2)_5SO_2OH$	0	1
22	Perfluoroheptanesulfonic acid	PFHpS	375-92-8	C7HF15O3S	$CF_3(CF_2)_6SO_2OH$	0	1
23	Perfluorooctane-1-sulfonic acid	PFOS	1763-23-1	C8HF17O3S	$CF_3(CF_2)_7SO_2OH$	0	1
24	Perfluorodecane-1-sulfonic acid	PFDS	335-77-3	C10HF21O3S	$CF_3(CF_2)_9SO_2OH$	0	1
25	1H,1H,2H,2H-Perfluorooctane sulfonic acid	6:2 FTS	27619-97-2	C8H5F13O3S	$CF_3(CF_2)_5CH_2CH_2SO_2OH$	0	5
26	1H,1H,2H,2H-Perfluorodecanesulfonic acid	8:2 FTS	39108-34-4	C10H5F17O3S	$CF_3(CF_2)_7CH_2CH_2SO_2OH$	0	5
27	Sodium dodecafluoro-3H-4,8-dioxanonanoate	NaDONA	2250081-67-3	C7H2F12O4	$CF_3O(CF_2)_3OCFHCF_2COOH$	1	2
28	Potassium 9-chlorohexadecafluoro-3-oxanone-1-sulfonate	9Cl-PF3ONS	73606-19-6	C8HClF16O4S	$Cl(CF_2)_6OCF_2CF_2SO_2OH$	0	1
29	Bis[2-(perfluorooctyl)ethyl] phosphate	8:2-DiPAP	678-41-1	C20H9F34O4P	$[CF_3(CF_2)_7CH_2CH_2O]_2POOH$	0	9

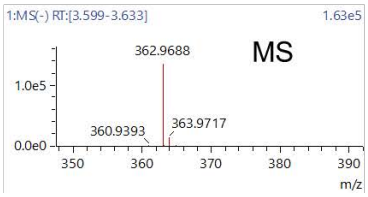
3. How to select correct formula if there are more than one candidates?

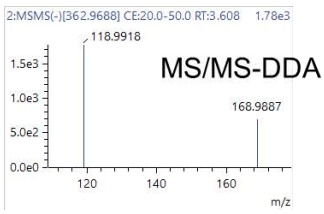
Using the restricted settings for PFAS, one or few candidates are generated. How to select the right formula if there are more than one candidates? We will use examples to demonstrate the selection based on DBE and H atom number.

Example 1: Precursor #22, (m/z 362.96879)

#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)	Iso Score	DBE
2	71.28	363.97690	362.96962	362.96880	-0.82	C7 H O2 F13	[M-H]-	-2.259	69.50	1.0
1	89.09	363.97640	362.96913	362.96880	-0.33	C7 H4 N2 O4 F8 S	[M-H]-	-0.909	99.44	3.0

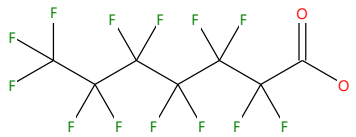






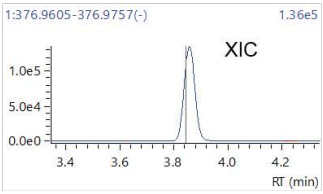
Remark:

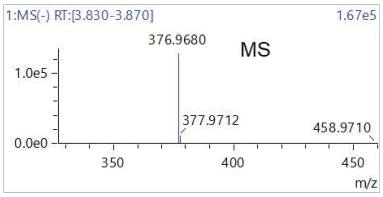
1) MS pattern: confirm [M-H]-
2) The formula with BDE=1, H=1 is correct PFAS, Perfluoroheptanoic acid
3) The other candidate with DBE=3, H=4, lower possibility
4) Library search: Perfluoroheptanoic acid, PFHpA, C7HF13O2

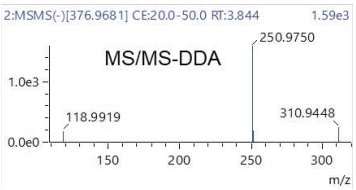


Example 2: Precursor #27, (m/z 376.9681)

#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)	Iso Score	DBE
2	74.83	377.97615	376.96887	376.96800	-0.87	C7 H2 O4 F12	[M-H]-	-2.308	73.48	1.0
1	98.79	377.97500	376.96773	376.96800	0.27	C10 H O3 F11	[M-H]-	0.716	99.00	5.0

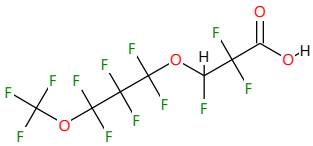






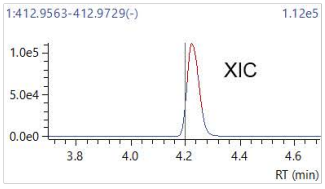
Remark:

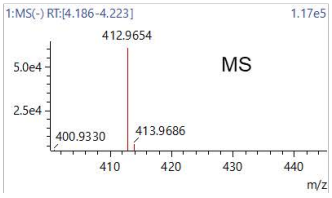
1) MS pattern: confirm [M-H]-
2) Formula with DBE=1, H=2 is the correct PFAS, NaDONA;
3) Formula with high DBE=5 is low possibility
4) Library search: NaDONA, C7H2F12O4

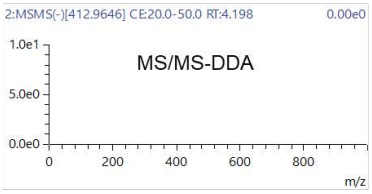


Example 3: Precursor #42, (m/z 412.9646)

#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)	Iso Score	DBE
2	72.34	413.97370	412.96643	412.96537	-1.06	C8 H O2 F15	[M-H]-	-2.567	70.95	1.0
1	87.63	413.97321	412.96593	412.96537	-0.56	C8 H4 N2 O4 F10 S	[M-H]-	-1.356	98.09	3.0

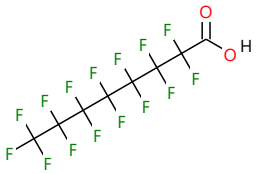






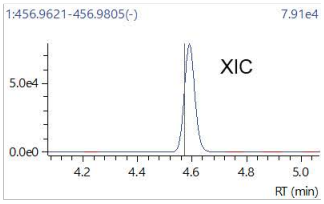
Remark:

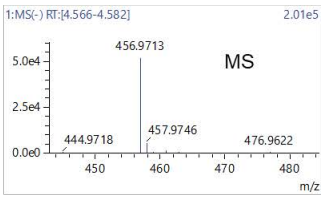
1) MS pattern: confirm [M-H]-
2) Formula with DBE=1, H=1 is correct PHOA
3) Formula with DBE=3 is less possibility
4) Library search: N.A. (due to lack of spectrum)
5) Assign-MOL search: Perfluorooctanoic Acid, C8HF15O2

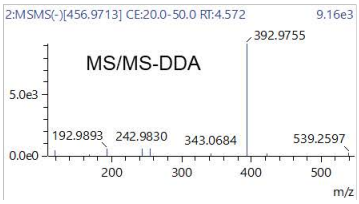


Example 4: Precursor #64, (m/z 456.9713)

#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)	Iso Score	DBE
4	8.50	457.97699	456.96972	456.97127	1.55	C8 H4 N2 O2 F14 S	[M-H]-	3.392	23.22	1.0
2	70.33	457.97993	456.97265	456.97127	-1.38	C10 H2 O2 F16	[M-H]-	-3.020	69.21	2.0
1	80.99	457.97944	456.97216	456.97127	-0.89	C10 H5 N2 O4 F11 S	[M-H]-	-1.948	91.14	4.0
3	20.70	457.97721	456.96993	456.97127	1.34	C10 H2 N2 O5 F12	[M-H]-	2.932	25.07	5.0

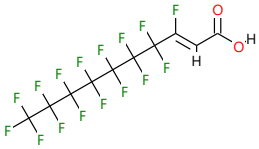






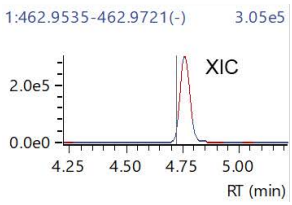
Remark:

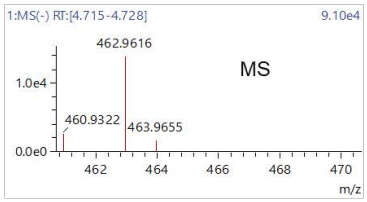
1) MS pattern: confirm [M-H]-
2) Formula with BDE=2, H=2 is correct (FOUEA, C10H2F16O2);
3) Library search: PFCA-perfluoroalkyl_Hsubstituted; C10H2F16O2
4) However, C8H4N2O2F14S, BDE=1, H=4, cannot be rejected.
5) MOL search for the formula in ChemSpider and PubChem, it shows no results

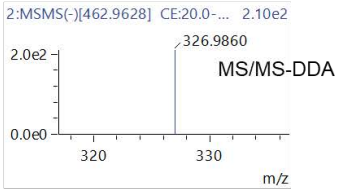


Example 5: Precursor #69, (m/z 462.9628)

#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)	Iso Score	DBE
4	43.63	463.96757	462.96029	462.96148	1.19	C7 H3 N2 O2 F15 S	[M-H]-	2.570	50.17	0.0
3	55.40	463.97051	462.96323	462.96148	-1.75	C9 H O2 F17	[M-H]-	-3.780	53.75	1.0
2	61.05	463.97002	462.96274	462.96148	-1.26	C9 H4 N2 O4 F12 S	[M-H]-	-2.722	69.68	3.0
1	75.16	463.96779	462.96051	462.96148	0.97	C9 H N2 O5 F13	[M-H]-	2.095	73.67	4.0

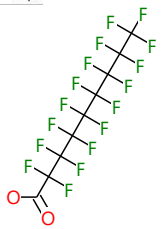


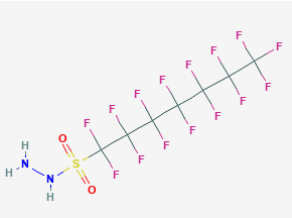




Remark:

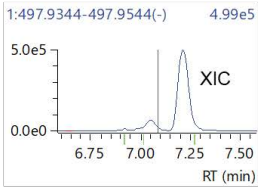
1) MS pattern: confirm [M-H]-
2) Formula with DBE=1, H=1 is correct (PFNA C9HF17O2);
3) Assign-MOL search: Perfluorononanoic acid, C9HF17O2
4) However, C7H3N2O2F15S, DBE=0 cannot be rejected
5) MOL search for the formula in ChemSpider and PubChem, it shows 1 result, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-Pentadecafluoroheptane-1-sulfonohydrazide

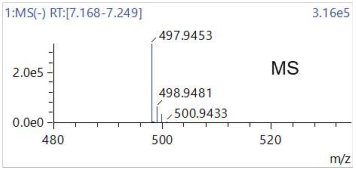


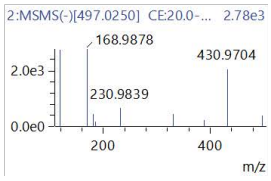


Example 6: Precursor #254, (m/z 497.9444)

#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)	Iso Score	DBE
2	45.89	498.95348	497.94620	497.94525	-0.95	C8 H2 N O2 F17 S	[M-H]-	-1.908	41.00	0.0
3	42.65	498.95076	497.94348	497.94525	1.77	C8 H2 N3 O5 F13 S	[M-H]-	3.555	39.18	3.0
1	74.64	498.95234	497.94506	497.94525	0.19	C11 H N O F16 S	[M-H]-	0.382	71.99	4.0



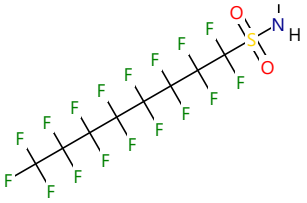




MS/MS-DDA

Remark:

1) MS pattern: confirm [M-H]-
2) Formula with DBE=0, H=2 is correct (PFOSA),
3) Library search: PFOSA, C8H2F17NO2S
4) Formula C8H2N3O5F13S with DBE=3 less possibility, MOL search for the formula in ChemSpider and PubChem, it shows NO result



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