

# Development of Screening Method for Targeted and Undiscovered Per- and polyfluoroalkyl Substances (PFAS) in Surface Water on Q-TOF Mass Spectrometer

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# Development of Screening Method for Targeted and Undiscovered Per- and polyfluoroalkyl Substances (PFAS) in Surface Water on Q-TOF Mass Spectrometer

## Overview

- A comprehensive screening method for targeted and undiscovered PFAS was established on Shimadzu Q-TOF LCMS-9030.
- PFOA and PFOS were found in a water sample by targeted screening using an in-house HRMS library consisting of 34 PFAS with RT, MS and MS/MS spectra.
- A new PFAS, perfluorohexyl sulfinic acid or its analogue, was identified successfully in the same water sample via a workflow proposed in this study.

## 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<sup>[1]</sup>. 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<sup>[1,2]</sup>. 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

## Experimental

34 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)
<b>Interface</b>	: <b>ESI Heated</b>
MS Mode	: Negative MS & DDA
CID gas	: Argon, 270 kPa
Block Temperature	: 300°C
DL Temperature	: 250°C
Interface Temperature	: 400°C
Nebulizing Gas Flow	: Nitrogen, 3 L/min
Drying Gas Flow	: Nitrogen, 10 L/min
Heating Gas Flow	: Zero Air, 10 L/min

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Table2. Mass accuracy of 34 PFAS standards on Q-TOF 9030

	Compound Name	Chemical Formula	RT/min	Theoretical m/z	Measured m/z	Mass Accuracy / ppm
1	PFBA	C <sub>4</sub> H <sub>7</sub> F <sub>7</sub> O <sub>2</sub>	4.85	212.9792	212.9787	2.2
2	PFPA	C <sub>5</sub> H <sub>9</sub> F <sub>9</sub> O <sub>2</sub>	8.78	262.976	262.9755	1.8
3	PFHxA	C <sub>6</sub> H <sub>11</sub> F <sub>11</sub> O <sub>2</sub>	11.58	312.9728	312.9725	1.2
4	PFHpA	C <sub>7</sub> H <sub>13</sub> F <sub>13</sub> O <sub>2</sub>	13.78	362.9696	362.9693	0.8
5	PFOA	C <sub>8</sub> H <sub>15</sub> F <sub>15</sub> O <sub>2</sub>	15.65	412.9664	412.9662	0.6
6	PFNA	C <sub>9</sub> H <sub>17</sub> F <sub>17</sub> O <sub>2</sub>	17.32	462.9632	462.9631	0.3
7	PFDA	C <sub>10</sub> H <sub>19</sub> F <sub>19</sub> O <sub>2</sub>	18.90	512.96	512.9598	0.4
8	PFUdA	C <sub>11</sub> H <sub>21</sub> F <sub>21</sub> O <sub>2</sub>	20.41	562.9568	562.9571	0.5
9	PFDoA	C <sub>12</sub> H <sub>23</sub> F <sub>23</sub> O <sub>2</sub>	21.88	612.9537	612.9537	0.1
10	PFTeDA	C <sub>13</sub> H <sub>25</sub> F <sub>25</sub> O <sub>2</sub>	23.32	662.9505	662.9506	0.2
11	PFTeDA	C <sub>14</sub> H <sub>27</sub> F <sub>27</sub> O <sub>2</sub>	24.71	712.9473	712.9471	0.3
12	PFHxDA	C <sub>16</sub> H <sub>31</sub> F <sub>31</sub> O <sub>2</sub>	27.40	812.9409	812.9409	0.0
13	PFODA	C <sub>18</sub> H <sub>35</sub> F <sub>35</sub> O <sub>2</sub>	29.87	912.9345	912.9355	1.1
14	PF-3,7-DMOA	C <sub>10</sub> H <sub>19</sub> F <sub>19</sub> O <sub>2</sub>	17.97	468.9702	468.9704	0.5
15	HPFHpA	CHF <sub>2</sub> (CF <sub>2</sub> ) <sub>5</sub> COOH	11.94	344.979	344.9789	0.3
16	PFBS	C <sub>4</sub> F <sub>9</sub> SO <sub>3</sub> H	11.63	298.943	298.9436	2.1
17	PFHxS	C <sub>6</sub> F <sub>13</sub> HO <sub>3</sub> S	16.26	398.9366	398.9371	1.2
18	PFHpS	C <sub>7</sub> H <sub>15</sub> O <sub>3</sub> S	18.07	448.9334	448.9343	1.9
19	L-PFOS	C <sub>8</sub> F <sub>17</sub> O <sub>3</sub> HS	19.74	498.9302	498.9308	1.2
20	L-PFDS	C <sub>10</sub> H <sub>21</sub> SO <sub>3</sub>	22.82	598.9238	598.9243	0.7
21	H4PFuNA	C <sub>11</sub> H <sub>21</sub> O <sub>2</sub>	20.41	562.9568	562.957	0.3
22	FOSA	C <sub>8</sub> H <sub>2</sub> F <sub>17</sub> NO <sub>2</sub> S	21.90	497.9462	497.946	0.5
23	N-MeFOSA	C <sub>9</sub> H <sub>4</sub> F <sub>17</sub> NO <sub>2</sub> S	18.78	511.9619	511.9608	2.0
24	N-EtFOSA	C <sub>10</sub> H <sub>6</sub> F <sub>17</sub> NO <sub>2</sub> S	19.39	525.9775	525.9774	0.2
25	N-MEeFOSAA	C <sub>11</sub> H <sub>6</sub> F <sub>17</sub> NO <sub>4</sub> S	18.78	569.9673	569.9674	0.2
26	N-EtFOSAA	C <sub>12</sub> H <sub>8</sub> F <sub>17</sub> NO <sub>4</sub> S	19.40	583.983	583.983	0.0
27	FHEA	C <sub>8</sub> H <sub>3</sub> F <sub>13</sub> O <sub>2</sub>	13.03	376.9853	376.985	0.7
28	FOEA	C <sub>10</sub> H <sub>3</sub> F <sub>17</sub> O <sub>2</sub>	16.45	476.9789	476.9783	1.3
29	6:2 FTS	C <sub>8</sub> H <sub>4</sub> F <sub>13</sub> SO <sub>3</sub> Na	14.89	426.9674	426.9679	1.3
30	8:2 FTS	C <sub>10</sub> H <sub>4</sub> F <sub>17</sub> SO <sub>3</sub> Na	18.13	526.961	526.9615	1.1
31	HFPO-DA	C <sub>6</sub> H <sub>11</sub> O <sub>3</sub>	12.45	328.9677	328.9672	1.8
32	9Cl-PF3ONS	C <sub>8</sub> F <sub>16</sub> O <sub>4</sub> SCl <sub>9</sub>	20.88	530.895	530.8961	2.1
33	11Cl-PF3OUdS	C <sub>10</sub> F <sub>20</sub> O <sub>4</sub> SCl <sub>11</sub>	23.88	630.8886	630.8896	1.5
34	ADONA	C <sub>7</sub> H <sub>2</sub> F <sub>12</sub> O <sub>4</sub>	14.51	376.9689	376.9689	0.0

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## Results and Discussion

### 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 the LabSolutions.

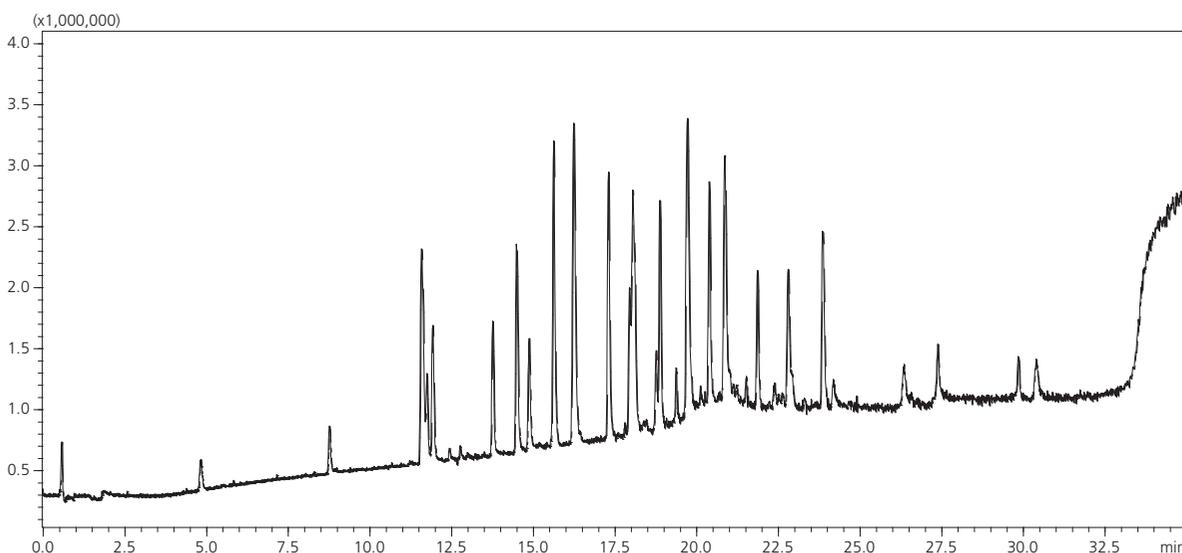


Figure 1: TIC in full mass range mode of a mixed standard of 34 PFASs with each at 100 ng/mL in Milli-Q water. Peak ID, RT and mass accuracy are shown in Table 2.

### Workflow for screening of undiscovered PFAS

In general, untargeted screening aiming to find undiscovered PFAS is challenging due to lack of information<sup>[2,3]</sup>. 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 (FOEA, (-) m/z 476.97888) to -76.2 mDa (L-PFDS, (-) m/z

598.92383). 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 -80 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/z 168.9893). Some functional groups have characteristic fragmentation patterns. For example, the sulfonic acid functional group ( $-SO_3H$ ) can produce fragments  $SO_3^-$  ions (m/z 79.9574),  $FSO_3^-$  ions (m/z 98.9558) 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.

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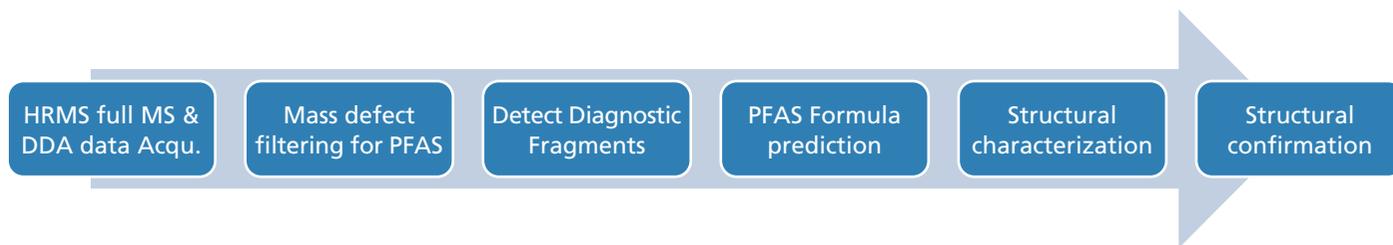


Figure 2: Workflow for screening and structural analysis of undiscovered PFASs by Q-TOF LCMS-9030

## Analysis of water sample using targeted and untargeted screening method

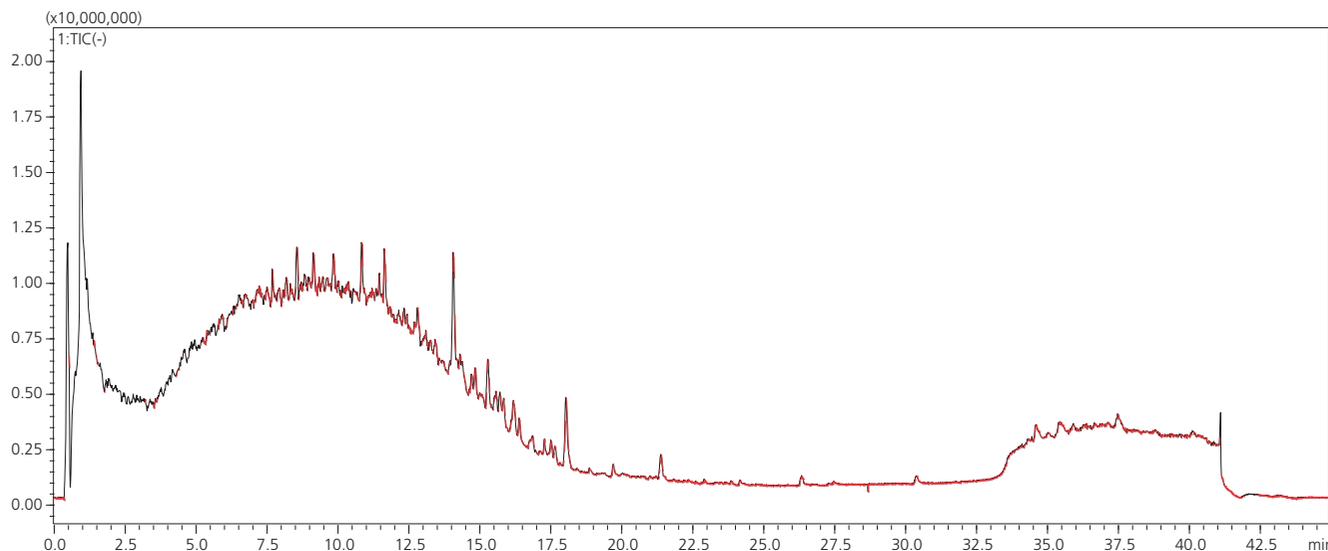


Figure 3: Chromatogram in full mass range of an unknown water sample W26 on LCMS-9030

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 water samples W26. Figure 3 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.

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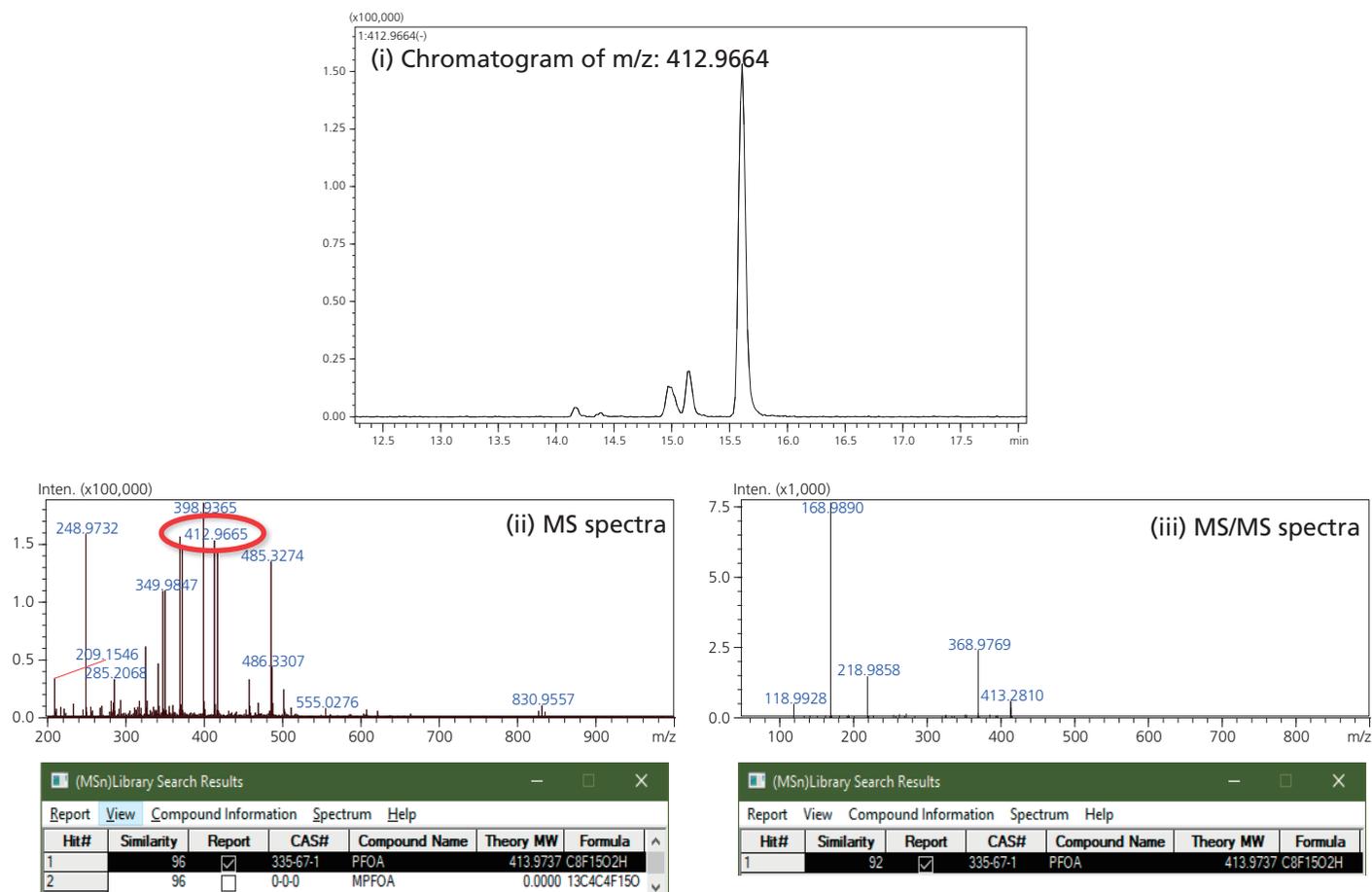


Figure 3: Identification of PFOA in a water sample. (i) Extracted chromatogram of FOAS; (ii) MS and library search result; (iii) MS/MS spectra and library search result

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 the LabSolutions are used in the subsequent structural elucidation and identification. A precursor ion of m/z 382.94179 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 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 analysis procedure is illustrated in details in Figure 4.

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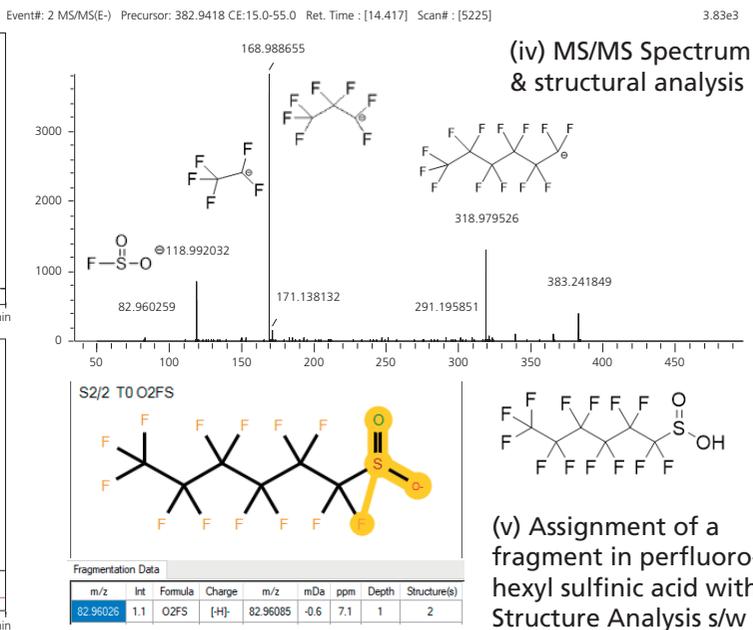
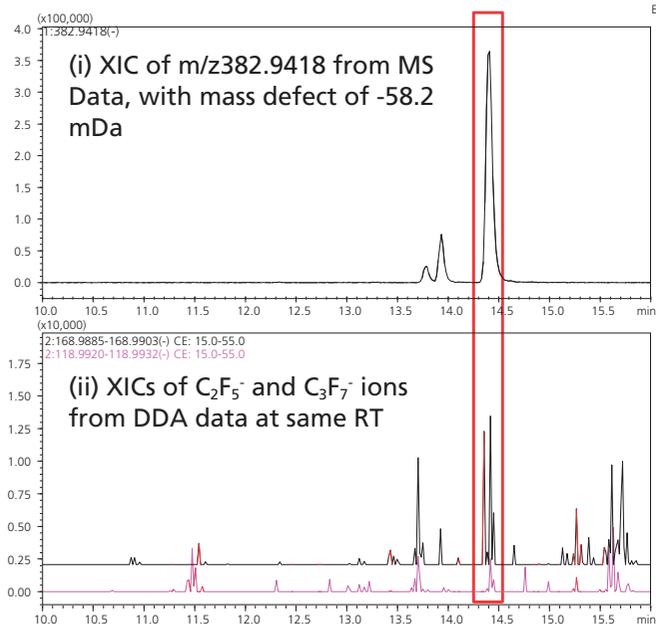
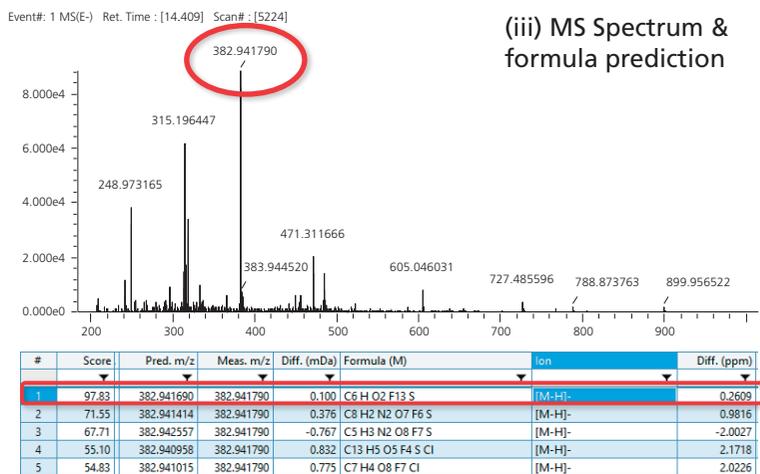


Figure 4: Illustration of finding an undiscovered PFAS: (i) XIC of m/z382.9417 from MS data via mass defect filtering; (ii) XIC of C<sub>2</sub>F<sub>5</sub><sup>-</sup> and C<sub>3</sub>F<sub>7</sub><sup>-</sup> ions from DDA data, (iii) MS spectrum and formula prediction, (iv) MS/MS spectrum and structural analysis; (v) assignment of fragment ion using Structural Analysis s/w.

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## 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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