Characterizing PFAS in cosmetic products using non-targeted acquisition and Molecule Profiler software

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This technical note outlines the analysis of PFAS in cosmetics using non-targeted acquisition (NTA) with compound identification through suspect screening and diagnostic fragment ion confirmation (Figure 1). The ZenoTOF 7600 system used data-dependent acquisition (DDA) to obtain TOFMS precursor and TOFMSMS fragmentation spectra. SCIEX OS software was used for initial compound identification through library matching suspect analytes with the SCIEX Fluorochemical HR-MS/MS Spectral Library. Additional manual screening showed the presence of several PAPs-like compounds, known ingredients in certain cosmetic products. The Molecule Profiler software was employed to rapidly find precursor compounds that shared a diagnostic PAPs fragment ion, reducing the time to detect structurally similar novel PFAS in the cosmetics samples.

Key benefits of PFAS characterization in cosmetics using the ZenoTOF 7600 system

- **Rapid filtering of non-targeted acquisition results for positive detections.** SCIEX OS qualitative rules ("traffic lights") are used to quickly identify PFAS detections
- **Improved confidence in PFAS identification through MS/MS library matching.** SCIEX Fluorochemical HR-MS/MS Spectral Library was used to confirm identification by comparison to the library database
- **Detection of novel PFAS compounds through diagnostic fragment ion screening in Molecule Profiler.** Rapid screening of non-targeted data sets to discover new PFAS

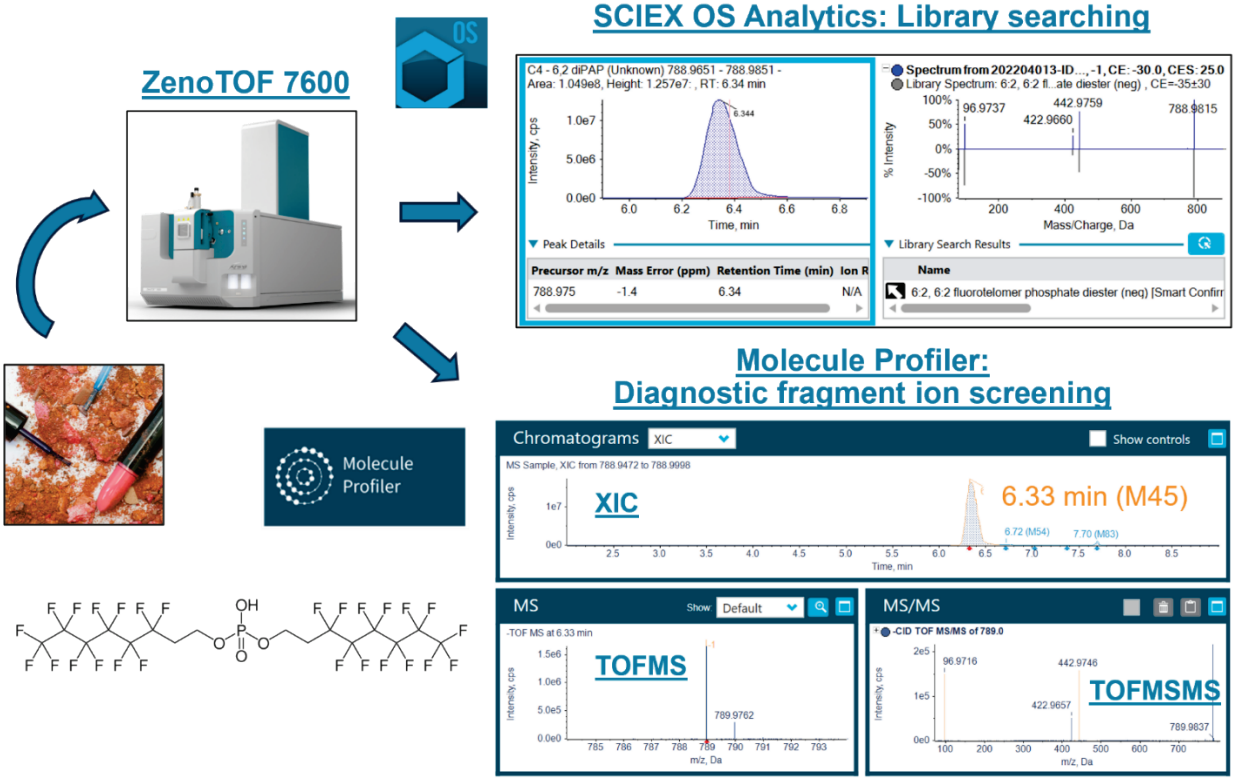


Figure 1. Instrumental and data processing workflow for the characterization of PFAS in cosmetics.

Introduction

PFAS are widely used in cosmetic and personal care products[1-4], due to their ability to impart waterproof ability and improve durability. PFAS in consumer products, including cosmetics, have been under increasing regulatory pressure with bans or proposed restrictions in the European Union, several countries, and individual states within the United States.[5] PFAS in cosmetics represent an exposure pathway through dermal absorption and ingestion and, ultimately, a potential human health risk.[6]

Previous studies characterizing PFAS in cosmetics have shown that targeted analytical methods do not capture a large portion of the total and extractable organic fluorine content.[1] This is because targeted methods typically only monitor ~20-30 PFAS, representing only a tiny fraction of the estimated >10,000 PFAS used in commercial products. Further, targeted methods are limited by the availability of analytical standards. Non-targeted methods, using accurate mass spectrometry MS/MS fragmentation patterns, can be used to detect novel PFAS and for confirmation of suspected PFAS.

Methods

Sample preparation. Various cosmetic products were purchased, including foundations, concealers, and creams. All products were identified as containing some PFAS, but compound-specific details were unknown. Extraction procedures included sonication in basic methanol, followed by clean-up with an ENVI-Carb SPE cartridge, extract concentration under a gentle stream of nitrogen gas, and reconstituting in methanol for instrumental analysis[3].

Chromatography. An Exion AD system was used that had been modified to replace the fluoropolymer tubing with PEEK. In addition, a delay column was used between the pumps and autosampler to reduce the PFAS contamination originating from the LC system. Both the analytical (100 x 2.1 mm, 1.6 µm) and delay (50 x 3 mm, 5 µm) columns were the Phenomenex Luna Omega PS C18. Mobile phases were water ("A", modified with 10mM ammonium acetate) and methanol ("B", modified with 10mM ammonium acetate). The flow rate was 0.6 mL/min and chromatography was performed using the gradient conditions described in **Table 1**. The column oven was set to 40°C, and the injection volume was 10 µL.

Table 1: Chromatographic gradient for the non-targeted acquisition of PFAS in cosmetics using the ZenoTOF 7600 system.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	80	20
0.5	80	20
7.0	1	99
8.0	1	99
8.1	80	20
12.0	80	20

Mass spectrometry. Samples were analyzed using negative mode electrospray ionization on the ZenoTOF 7600 system. Data was collected using DDA with the Zeno pulsing turned on, and MS/MS triggered on the top 30 precursor ions. Source and gas conditions are presented in **Table 2**. Fragmentation was performed using collision-induced dissociation (CID) using the parameters described in **Table 3.**

Table 2: Source and gas parameters.

Parameter	Value
Polarity	Negative
Ion source gas 1	50 psi
Ion source gas 2	50 psi
Curtain gas	35 psi
Source temperature	450°C
Ion spray voltage	-4500 V
CAD gas	10 psi

Table 3: DDA conditions used for the non-targeted acquisition of PFAS in cosmetics using the ZenoTOF 7600 system.

Parameter	TOFMS	TOFMSMS
Scan mode	TOFMS	Zeno IDA
Start/stop mass range	100-1200 Da	80-1200 Da
Accumulation time	0.1 s	0.01 s
Declustering potential (DP)	-50 V	-50 V
Collision energy (CE)	-5 V	-30 V
Collision energy spread (CES)	0 V	±25 V

Data processing and workflow. Data were processed using SCIEX OS, version 3.4. The SCIEX Fluorochemical HR-MS/MS Spectral Library 2.0 was used for library searching. The Molecule

Profiler software, version 1.3.2, within SCIEX OS was used to identify novel PFAS using diagnostic fragments.

Suspect screening with MS/MS library searching for known PFAS compounds

The data set was initially processed using a suspect screening approach with compound confirmation based on precursor mass accuracy (<5 ppm), isotope ratio difference (<5%), and MS/MS library "fit" score (>70) against the SCIEX Fluorochemical High Resolution MS/MS Spectral Library. The suspect screening list was built from known PFAS, such as perfluoroalkyl carboxylates (PFCAs) and sulfonates (PFSAs), as well as PFAS previously detected in cosmetic samples, including the mono- (monoPAP) and di-substituted (diPAP) fluorotelomer phosphate esters, saturated (FTCA) and unsaturated (FTUCA) fluorotelomer carboxylic acids. **Figure 2** shows the detection of perfluorohexanoic acid (PFHxA) in sample #7. The SCIEX OS software allows the user to quickly review the Results table for potential detections using the using qualitative "traffic light" rules of mass error, retention time, isotope and library confidence **(Figure 2, panel A)**. As shown by the green checkmarks, all four qualitative criteria were achieved for PFHxA in sample #7. The TOFMSMS spectrum in **Figure 2, panel B** shows an identical match to the library MS/MS spectrum, confirming the compound identification. Also shown is the precursor XIC and associated mass error, and the TOFMS spectrum with the predicted isotope pattern based on the compound formula (mirrored in grey lines).

Table 4 shows the most frequently detected PFAS compounds in the six cosmetic samples with the highest PFAS abundance. Overall, the samples contained several chain-length homologues of PFCAs, mono- and di-PAPs, FTCAs and FTUCAs. The PFSAs and perfluorinated sulfonamides were not detected in any sample. Similarly, none of the novel ether acids, such as 8:2 Cl-PFESA, were detected and the FTS compounds were not detected with exception of the 6:2 FTS in sample #1.

A. Results table

Index	Sample Name	Sample Type	Com... Name	Area	Precursor Mass	Found At Mass	Mass Error (...	Retent... Time	Expected RT	Library Hit	Library Score	Isotope Ratio...	Mass Error...	RT Confi...	Isotope Confi...	Library Confi...
3	blank	Blank	PFHxA	N/A	312.973	N/A	N/A	N/A	3.14		N/A	N/A				
62	C1	Unknown	PFHxA	3.043e6	312.973	312.9726	-0.8	3.13	3.13	PFHxA (perf...	100.0	2.7	✓			✓
121	C2	Unknown	PFHxA	1.704e5	312.973	312.9718	-3.3	3.12	3.14	No Match	0.0	9.8	✓			●
180	C4	Unknown	PFHxA	1.043e6	312.973	312.9724	-1.5	3.12	3.14	PFHxA (perf...	100.0	12.4	✓			✓
239	C5	Unknown	PFHxA	4.098e5	312.973	312.9723	-1.7	3.12	3.14	PFHxA (perf...	100.0	45.6	✓			✓
298	C6	Unknown	PFHxA	1.151e6	312.973	312.9724	-1.4	3.07	3.14	PFHxA (perf...	100.0	6.4	✓			✓
357	C7	Unknown	PFHxA	1.482e6	312.973	312.9730	0.5	3.09	3.14	PFHxA (perf...	100.0	4.7	✓			✓

B. Peak review

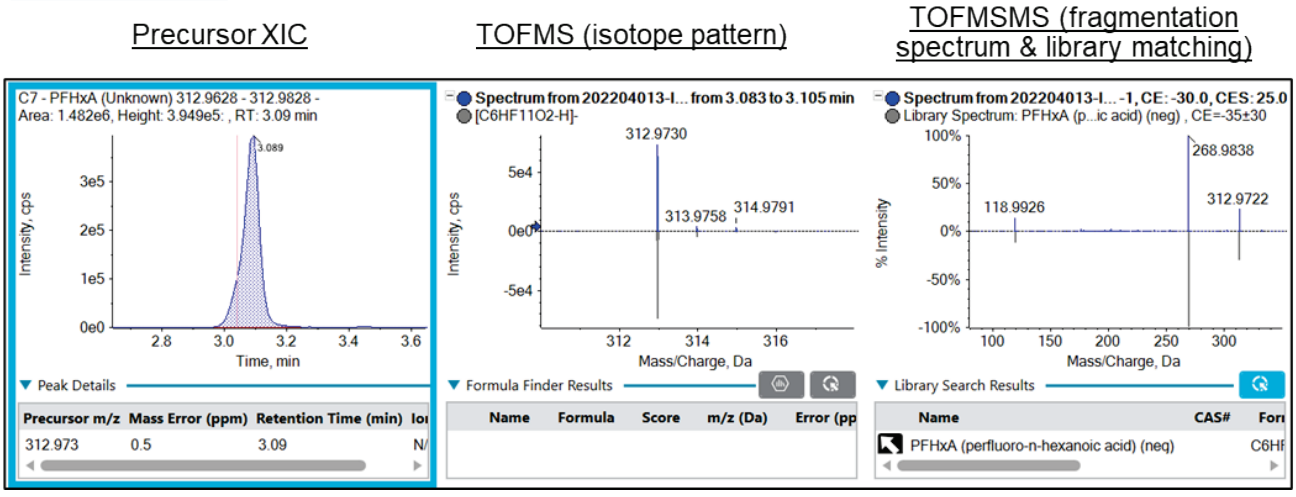


Figure 2. Detection of PFHxA in cosmetics sample #7 using the ZenoTOF 7600 system with DDA and suspect screening data processing. The top panel ("A") shows the results table output and the use of the qualitive rules for rapid filtering of positive hits. The bottom panel ("B") shows the extracted ion chromatogram (XIC), the TOFMS spectrum with match to the theoretical isotope pattern, and the TOFMSMS spectrum with match to the MS/MS spectrum from the SCIEX Fluorochemical HR-MS/MS Spectral Library 2.0.

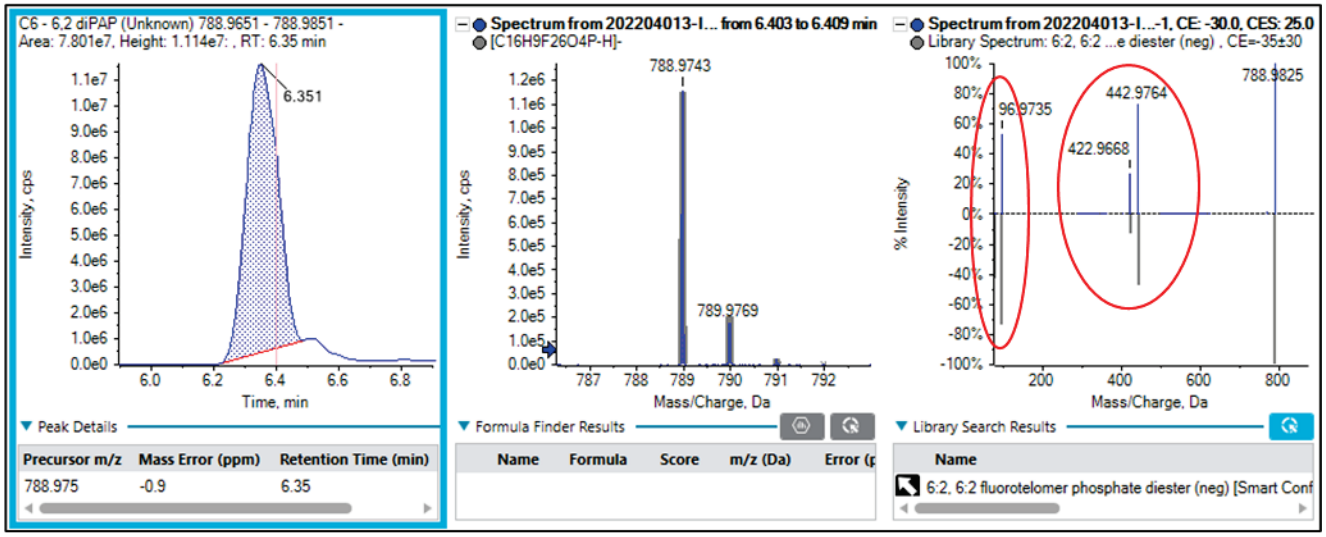
Use of diagnostic fragment ions for PFAS compound identification

The screening list was expanded to include additional chain-length compounds from the PFAS classes that were detected during the initial suspect screening. PFAS are frequently observed in homologous series and the detection of at least one PFAS compound typically indicates the presence of other chain-lengths. Since these compounds were not contained in the SCIEX Fluorochemical MS/MS library, diagnostic fragment ion matching was used for confirmation. For example, the 6:2 diPAP was detected in several cosmetic samples and confirmed by the SCIEX OS software, which showed a positive match to the MS/MS library. DiPAP compounds typically show characteristic fragmentation patterns which contain the $[PO_4H_2]^-$ fragment (m/z 96.9696 Da), as well as loss of one of the polyfluorinated alkyl "arms" and subsequent neutral loss of HF **(Figure 3, top panel)**. Using the expanded suspect list, **Figure 3** shows the 4:2, 6:2 diPAP detection in cosmetics sample #6. The precursor mass error was -2.0 ppm, the isotope ratio difference was 0.9%, and the MS/MS spectrum contained the m/z 96.9696 Da $[PO_4H_2]^-$ fragment, loss of the 4:2 and 6:2 polyfluoroalkyl chains (observed m/z 442.9719 Da and m/z 342.9785 Da, respectively) and subsequent HF neutral loss (observed m/z 422.9651 Da and m/z 322.9721 Da, respectively).

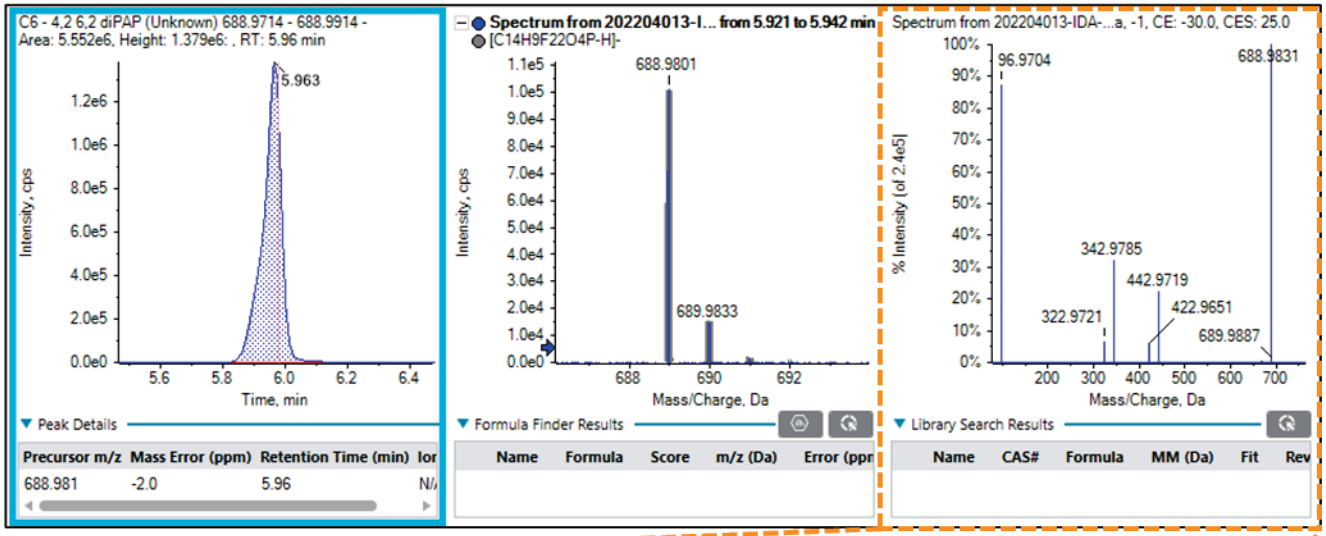
Table 4. PFAS compounds detected in cosmetic samples #1–6. The check-mark "✓" indicates that the compound was detected, "bl" indicates a peak was detected but it was below the procedural blank level, and "nd" indicates that no peak was found.

Compound	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
PFBA	✓	✓	✓	✓	✓	✓
PFPeA	✓	✓	✓	✓	✓	✓
PFHxA	✓	✓	✓	✓	✓	✓
PFHpA	✓	nd	✓	✓	✓	✓
PFOA	✓	nd	bl	bl	bl	bl
PFNA	✓	nd	nd	nd	nd	bl
PFDA	✓	nd	nd	nd	nd	nd
PFUdA	✓	nd	nd	nd	nd	nd
PFDoA	✓	nd	nd	bl	nd	nd
PFTrA	✓	nd	nd	nd	nd	nd
PFTeDA	✓	nd	nd	nd	nd	nd
4:2 monoPAP	nd	nd	nd	nd	nd	nd
6:2 monoPAP	✓	bl	✓	✓	✓	✓
8:2 monoPAP	✓	✓	✓	✓	✓	✓
10:2 monoPAP	✓	✓	✓	✓	✓	✓
4:2 diPAP	✓	nd	✓	nd	nd	nd
4:2, 6:2 diPAP	✓	nd	✓	nd	✓	✓
6:2 diPAP	✓	bl	✓	✓	✓	✓
6:2, 8:2 diPAP	✓	✓	✓	✓	✓	✓
8:2 diPAP	✓	✓	b	✓	✓	✓
8:2, 10:2 diPAP	✓	✓	b	✓	✓	✓
10:2 diPAP	✓	✓	nd	✓	✓	nd
6:2 FTCA	✓	nd	nd	✓	✓	✓
8:2 FTCA	✓	bl	bl	bl	bl	✓
10:2 FTCA	✓	bl	bl	nd	bl	bl
6:2 FTUCA	✓	nd	✓	✓	✓	✓
8:2 FTUCA	✓	✓	bl	bl	bl	bl
10:2 FTUCA	✓	nd	nd	nd	nd	nd

A. 6:2 diPAP



B. 4:2, 6:2 diPAP



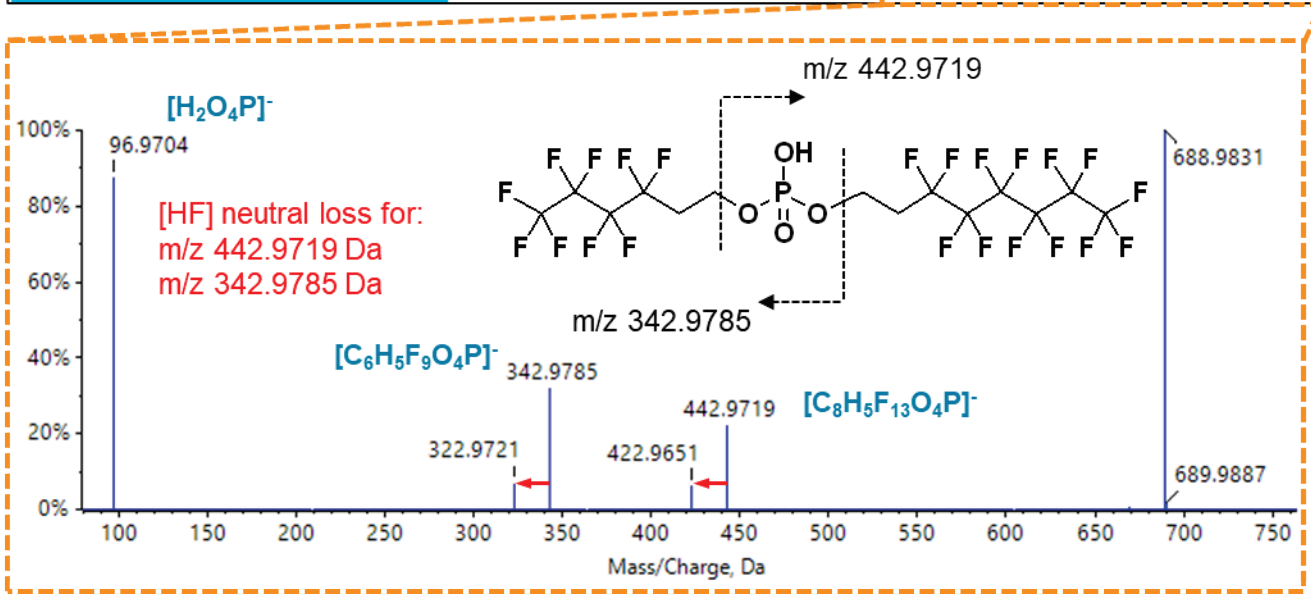


Figure 3. Detection of 6:2 diPAP and 4:2, 6:2 diPAP in cosmetic sample #6 using non-targeteded acquisition on the ZenoTOF 7600 system. The 6:2 diPAP was confirmed through matching of the TOFMSMS spectrum to the SCIEX Fluorochemical HR-MS/MS Spectral Library. The 4:2, 6:2 diPAP was confirmed through matching diagnostic fragments.

Detection of novel PFAS using Molecule Profiler software

Identifying novel compounds in large non-targeted datasets is complex and time-consuming. These novel compounds are usually not found in MS/MS libraries or suspect lists, so screening non-targeted data is often a manual and error-prone process due to the subjective compound identification criteria. Therefore, Molecule Profiler (MP) in SCIEX OS was used to screen the non-targeted data in an automated and systematic manner, identifying precursor compounds that contained a user-specified diagnostic PFAS fragment.

Manual screening of the non-targeted data showed several precursor compounds with PAPs-like characteristics but did not have precursor masses corresponding to known compounds. These features were not on our suspect screening list and were not in the SCIEX Fluorochemical MS/MS library. Specifically, these unknown PAPs-like PFAS showed a negative mass defect, indicating a highly fluorinated chemical and their MS/MS spectra contained the diagnostic m/z 96.9696 Da $[PO_4H_2]^-$ fragment, an unknown intermediate fragment and subsequent [HF] neutral loss.

Therefore, the MP processing method was set to search for precursors that contained the m/z 96.9696 Da $[PO_4H_2]^-$ fragment within the TOFMSMS spectra. The MP results table displayed the list of precursors with the diagnostic fragment, which was rapidly screened to confirm the detection of unknown PAPs-like compounds. This confirmation screening was much faster and less tedious than reviewing the original unknown feature list since it contained only a subset of features with the diagnostic fragment. For example, the MP software found the m/z 720.9859 Da (RT = 5.87 min) feature in sample #1 as an unknown of interest (**Figure 4**). The MP software also displayed the XIC and TOFMS and TOFMSMS spectra for the feature, which confirmed the presence of the 96.9696 Da $[PO_4H_2]^-$ fragment. In addition, the TOFMSMS spectrum showed the m/z 274.9909 Da fragment and the associated [HF] neutral loss fragment of m/z 254.9836 Da. Interestingly, the m/z 542.9632 Da fragment was also observed, characteristic of PAPs compounds with 8:2 fluorotelomer phosphate linkages, such as the 8:2 monoPAP, diPAP and triPAP compounds. Presumably, the associated [HF] neutral loss was not observed due to the low abundance of the m/z 542.9632 Da fragment.

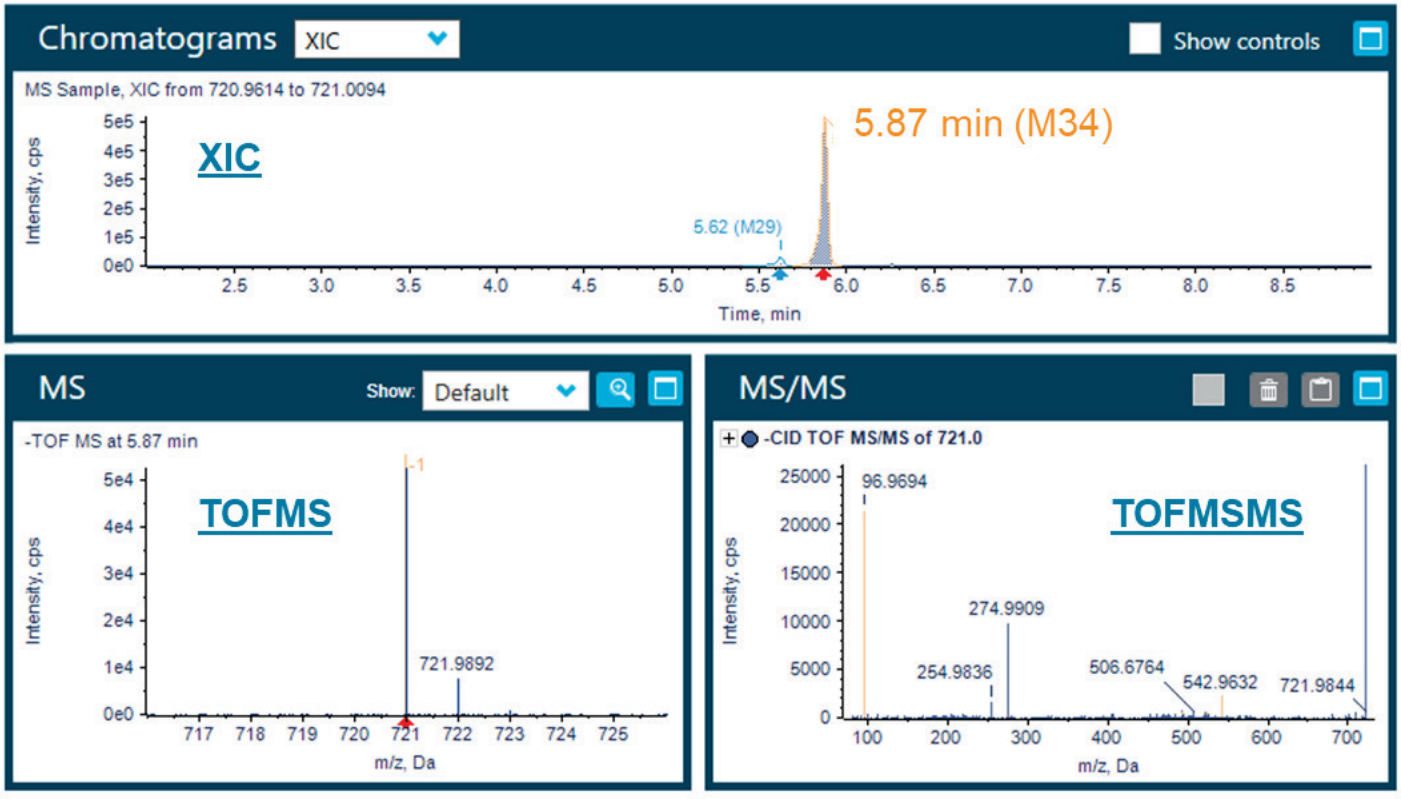


Figure 4. Molecule Profiler output showing the XIC, and TOFMS and TOFMSMS spectra for the identification of feature m/z 720.9859 Da in cosmetics sample #1.

Conclusions

- PFAS characterization in cosmetic products was performed using data-dependent acquisition (DDA) with the ZenoTOF 7600 system
- Initial compound identification was carried out using suspect screening in SCIEX OS for these samples. The confirmation was performed through library matching against the SCIEX Fluorochemical HR-MS/MS Spectral Library.
- Suspect screening results were rapidly filtered using the SCIEX OS qualitative rules ("traffic lights"), reducing time for data review
- Molecule Profiler software was used to identify new PFAS compounds with PAPs-like properties using diagnostic fragment ion screening, resulting in significant time savings compared to manually screening non-targeted datasets.

References

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