Application note | 003388

Pharma

Comprehensive PFAS screening in pharmaceutical packaging and medical devices by LC-HRAM-MS

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Keywords

Perfluoroalkyl substances (PFAS), E&L, Orbitrap Exploris 120 mass spectrometer, fluorinated ethyl propylene (FEP), Vanquish Horizon UHPLC system

Application benefits

- Combined targeted quantitation and non-targeted screening for PFAS compounds from one injection is achieved.
- One LC-MS method provides both PFAS-specific and general extractables screening.
- Targeted analysis of a list of PFAS compounds yields unequivocal identification and quantification down to sub-ppb levels.
- Non-targeted analysis reveals additional PFAS contaminants in the sample extracts that could be quantified using surrogate standards.
- Use of the PFAS analysis kit and delay column minimizes background interference and increases confidence in the analytical results.
- Use of Thermo Scientific™ Chromeleon™ CDS provides a 21 CFR Part 11 compliance-ready solution for data acquisition and quantitative analysis in extractables screening.

Goal

Demonstrate the sensitive detection and identification of known and unknown PFAS compounds in extracts of common pharmaceutical manufacturing materials by employing a combined targeted and non-targeted approach on the Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer.

Introduction

Per- and polyfluoroalkyl substances (PFAS) are known for their persistence in the environment and in the human body, leading to potential health issues. Regulatory agencies like the FDA and EPA have set stringent guidelines and limits for PFAS. For example, on April 10, 2024, the EPA announced the final National Primary Drinking Water Regulation (NPDWR) requiring monitoring for six PFAS in the nation's public water supplies.[1] The EPA expects that over many years the final rule will prevent PFAS exposure in drinking water for approximately 100 million people, prevent thousands of deaths, and reduce tens of thousands of serious PFAS-attributable illnesses. However, there is still no regulatory guidance on PFAS levels present in pharmaceutical products and medical devices, which could compromise product safety and efficacy for drug products. As such, medical device and pharmaceutical companies should be proactive by staying up to date with current and future regulations and develop risk mitigation strategies to avoid costly product recall or delays in approvals. To that end, having the ability to detect and quantitate PFAS in various pharmaceutically relevant test materials is essential.

Here, we report an LC-MS based analytical strategy to test for PFAS that could be extracted from manufacturing components and containers as part of extractables screening. To demonstrate its utility, it was applied to extracts of two fluorine-containing polymer components in collaboration with the E&L group at SGS Health Science, Fairfield, NJ.

Experimental

Reagents and consumables

- Fisher Chemical™ Ammonium acetate, Optima™ LC/MS grade (P/N A114-50)
- Thermo Scientific™ Methanol, UHPLC-MS grade (P/N A458-1)
- Thermo Scientific™ Water, UHPLC grade, 1 L (P/N W8-1)
- Thermo Scientific™ Vials and caps, 600 µL, polypropylene, integrated membrane (P/N 00109-99-00049)
- Native PFAS solution (Wellington Laboratories), see Table 1 (P/N PFAC-MXB)

Sample preparation

Two test materials constructed from fluorinated ethylene propylene (FEP)—a 50 mL bottle with a cap, and tubing material—were obtained and extracted as follows, using both 50:50 ethanol/water (v:v) and isopropanol, respectively.

For the bottle, an aliquot of 10 mL of each extraction solvent was added to the bottle, and it was closed with the cap. The extraction was performed via incubation with agitation at 50 °C for 72 hours per ISO 10993-12 recommendation.[2] Extraction blanks were stored in a glass bottle with an inert cap, under the same incubation conditions as the corresponding sample.

For tubing, due to the rigidness of the tubing material, a 1 ft section was measured, cut into pieces, and placed in a clean glass bottle. An aliquot of 15 mL of each extraction solvent was added to submerge the tubing pieces at the recommended surface area-to-volume ratio (SA/V) of at least 6 cm^2/mL as cited in USP <665> and in the BioPhorum Operations Group (BPOG) recommendation.[3,4] An inert cap was placed on each bottle immediately to avoid evaporation of solvents. The extraction was performed via incubation with agitation at 40 °C for 24 hours.[3,4] An extraction blank was created using each extraction solvent, placed in a glass bottle with an inert cap, and under the same incubation conditions as the corresponding sample.

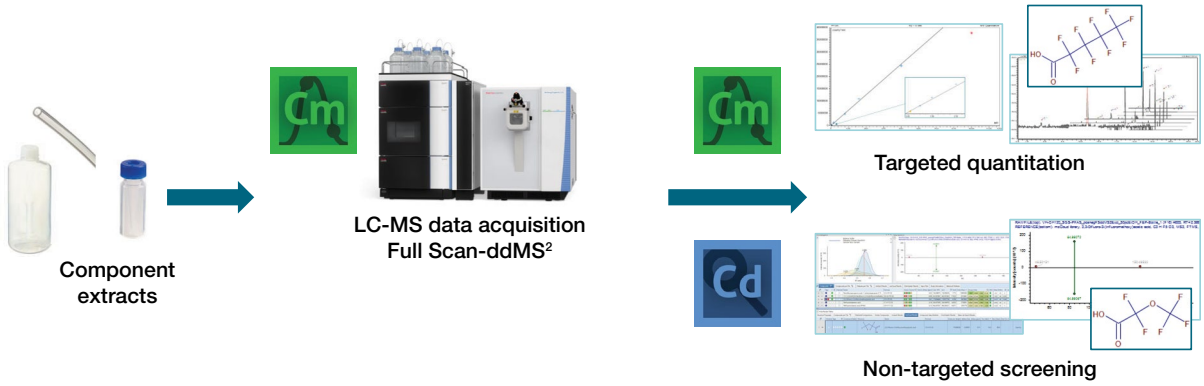

Figure 1. Overview of the LC-MS analytical strategy for detection of targeted and non-targeted PFAS as part of E&L analysis of pharmaceutical packaging and processing material extracts

After the extraction was completed, each extract from the individual bottles was transferred into separate glassware for storage, and aliquots were transferred into polypropylene autosampler vials for LC-MS analysis.

Standards

Seventeen target PFAS analytes were obtained as a mixture from Wellington Laboratories to evaluate the quantitative performance of the developed assay and used to build an external calibration curve in the targeted quantitative analysis of the test material extracts. Table 1 lists the identities and properties of the included standards. A serial dilution series was created by diluting the stock mixture (2 µg/mL in methanol) using 50% ethanol to prepare calibration standards at 0.1, 0.5, 1, 2, 20, 100, 200, and 500 ppb, along with a dilution blank.

Instrumentation

The LC-MS analysis was performed using a Thermo Scientific™ Vanquish™ Horizon UHPLC system coupled to an Orbitrap Exploris 120 high-resolution mass spectrometer (P/N BRE725531) equipped with the Thermo Scientific™ OptaMax™ NG source housing and using the heated electrospray ionization (HESI) probe.

The Vanquish Horizon UHPLC system consisted of:

- Vanquish System Base (P/N VH-S01-A-02)
- Vanquish Binary Pump H (P/N VH-P10-A-01)
- Vanquish Sampler HT (P/N VH-A10-A-02)
- Vanquish Column Compartment H (P/N VH-C10-A-02)
- Vanquish Diode Array Detector FG (P/N VF-D11-A-01) equipped with Standard flow cell, path length 10 mm (13 µL, SST) (P/N 6083.0510)
- PFAS Analysis Kit (P/N 80100-62142)

Notably, the system was fitted with the PFAS Analysis Kit that replaces wetted Teflon surfaces with comparable PEEK components as well as a PFAS delay column placed in-line between the solvent mixer and the autosampler needle, to further prevent background signal from potential PFAS sources in the analytical system and mobile phase solvents.

The chromatographic conditions and mass spectrometry source and method parameters used for the analysis are detailed in Tables 2–4.

Table 1. PFAS analytes present in the standard mixture

Name	Acronym	Formula	RT (min)	$[M-H]^-$ (*m/z*)	Stock concentration (µg/L)
Perfluoro-n-butanoic acid	PFBA	$C_4HF_7O_2$	5.56	212.9792	2000
Perfluoro-n-pentanoic acid	PFPeA	$C_5HF_9O_2$	10.72	262.976	2000
Perfluoro-1-butanesulfonic acid	PFBS	$C_4HF_9O_3S$	11.72	298.943	1770*
Perfluoro-n-hexanoic acid	PFHxA	$C_6HF_{11}O_2$	13.35	312.9728	2000
Perfluoro-n-heptanoic acid	PFHpA	$C_7HF_{13}O_2$	14.92	362.9696	2000
Perfluoro-1-hexanesulfonic acid	PFHxS	$C_6HF_{13}O_3S$	15.08	398.9366	1900**
Perfluoro-n-octanoic acid	PFOA	$C_8HF_{15}O_2$	16.03	412.9664	2000
Perfluoro-n-nonanoic acid	PFNA	$C_9HF_{17}O_2$	16.92	462.9632	2000
Perfluoro-1-octanesulfonic acid	PFOS	$C_8HF_{17}O_3S$	16.97	498.9302	1920**
Perfluoro-n-decanoic acid	PFDA	$C_{10}HF_{19}O_2$	17.65	512.96	2000
Perfluoro-1-decanesulfonic acid	PFDS	$C_{10}HF_{21}O_3S$	18.27	598.9238	1930**
Perfluoro-n-undecanoic acid	PFUdA	$C_{11}HF_{21}O_2$	18.27	562.9568	2000
Perfluoro-n-dodecanoic acid	PFDoA	$C_{12}HF_{23}O_2$	18.78	612.9537	2000
Perfluoro-n-tridecanoic acid	PFTrDA	$C_{13}HF_{25}O_2$	19.22	662.9505	2000
Perfluoro-n-tetradecanoic acid	PFTeDA	$C_{14}HF_{27}O_2$	19.58	712.9473	2000
Perfluoro-n-hexadecanoic acid	PFHxDA	$C_{16}HF_{31}O_2$	20.2	812.9409	2000
Perfluoro-n-octadecanoic acid	PFODA	$C_{18}HF_{35}O_2$	20.64	912.9345	2000

*Supplied as potassium salt; **supplied as sodium salt

Table 2. Chromatographic conditions

Parameter	Value
Analytical column	Thermo Scientific™ Hypersil GOLD™ VANQUISH™ C18 column (2.1 × 100 mm, 1.9 µm, P/N 25002-102130-V)
PFAS delay column	Thermo Scientific™ Hypersil GOLD™ C18 Selectivity (4.6 × 50 mm, 1.9 µm, P/N 25002-054630)
Mobile phases	A: 10 mM ammonium acetate in water B: methanol
Gradient	Time (min) % A % B 0 95 5 1 95 5 20 1 99 30 1 99 30.1 95 5 35 95 5
Flow rate	0.4 mL/min
Column temperature	40 °C
Autosampler temperature	4 °C
Autosampler needle wash solvent	50:50 water:methanol
Injection volume	2 µL
Diode array detector settings	200–400 nm
Divert valve	Flow to waste at 0–0.5 min and 31–35 min

Table 3. MS Instrument source settings overview

Parameter	Value
Sheath gas	50 a.u.
Aux gas	12 a.u.
Sweep gas	0.5 a.u.
Vaporizer temperature	225 °C
Ion transfer tube temperature	250 °C
Spray voltage	+3.4 / –1.0 kV
RF lens	70%

Table 4. MS Method parameter overview

Parameter	Value
Data acquisition type	Full Scan + data-dependent (dd) MS^2
Orbitrap resolution (MS^1/MS^2)	60,000/15,000 @ *m/z* 200
MS^1 scan range	*m/z* 150–1,000
Polarity	Positive/Negative Switching
Internal mass calibration	RunStart Easy-IC™
TopN	4
Dynamic exclusion	6 s
MS^2 intensity threshold filter	1e5
MS^2 isolation window	1.6 Da
HCD collision energies	15, 35, 55 V
MS^2 inclusion list	17 PFAS standards, see Table 1 for *m/z*
MS^2 exclusion list	Top 50 ions from averaged solvent blank run in each polarity

Software

The Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.3.2 was used for data acquisition and quantitative analysis of the LC-MS data. For qualitative MS data processing and differential analysis, data were imported into Thermo Scientific™ Compound Discoverer™ 3.3 SP3 software for spectral deconvolution and compound identification using the workflow template "PFAS Unknown ID w Database Search and Molecular Networks" with modifications to also include positive mode data and search against the Epoxidized Soybean Oil Library[5] and a custom E&L-specific library in the mzVault node, as well as a mass list generated from the PFAS standard mixture including retention times.

Results and discussion

Method development

The development of a suitable LC-MS method for the combined targeted and non-targeted screening for PFAS compounds as a part of extractables testing used the established chromatographic conditions from prior work as a starting point.[6] Briefly, the chromatographic separations took place on a Hypersil GOLD VANQUISH C18 UHPLC column using (A) 10 mM ammonium acetate in water and (B) methanol as the mobile phases for the gradient elution within 30 minutes, with a 5 minute re-equilibration step. The ion source was equipped with a HESI probe. A native PFAS standard mixture containing 17 common perfluoroalkyl acids and perfluoroalkylsulfonates (Table 1) was used to optimize the source parameters and instrument method for the sensitive detection of PFAS compounds.

The instrument method was created using a Full Scan with data-dependent MS^2 acquisition (FS-ddMS^2). While the majority of PFAS readily ionize in negative mode, a rapid polarity switching method was used to retain the ability of simultaneous detection of other extractable compounds from the FEP extracts in one injection. Quantitation was performed on the precursor mass in Full Scan, while fragmentation data acquisition was used to enable the identification of unknowns in the non-targeted analysis portion.

As shown in Figure 2, the existing chromatographic method resulted in excellent separation of the perfluoroalkyl acids with chain lengths ranging from four to eighteen carbons and gave symmetrical peak shapes for all analytes. For their sensitive detection, it was found to be beneficial to lower the source temperatures and negative mode spray voltage compared to the default suggested parameters (i.e., Vaporizer temperature = 350 °C; Ion transfer tube temperature = 325 °C; Negative mode spray voltage = -2.75 kV; provided by the Orbitrap Exploris 120 MS Tune editor based on the LC flow rate).

As shown in Table 5, this was particularly beneficial for the detection of short-chained PFAS species, with increased peak heights up to 600% and 176% on average. To ensure minimal to no impact of the changed source parameters on other analytes in extractable screening samples, a mixture of common extractables was analyzed under both conditions in a separate experiment. The results showed that the average peak intensity decreased by 11% with the PFAS-optimized source conditions relative to the default settings for 10 compounds in positive mode, but it increased by 10% for 9 compounds in negative mode. From these data, it was concluded that the PFAS-optimized source conditions were also suitable for simultaneous detection of other extractable compounds from the test materials (data not shown).

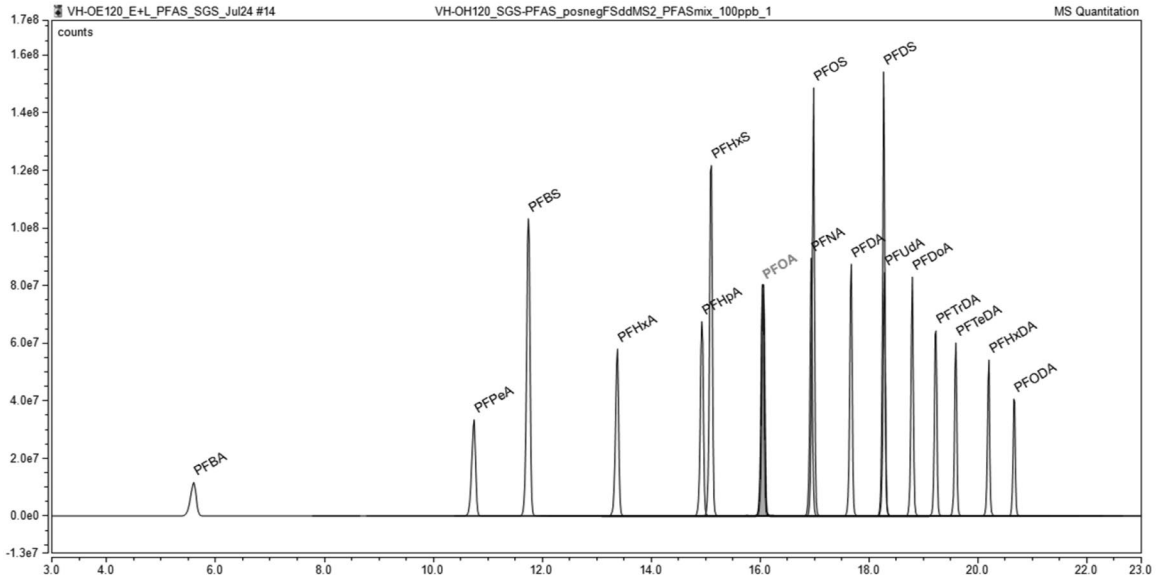

Figure 2. Elution profile of the PFAS standard mixture in the 100 ppb calibration standard

Table 5. Comparison of peak heights using the "default" and optimized source conditions for the detection of the PFAS standard mixture components from injection of the 100 ppb dilution standard. (Default: Vaporizer temperature = 350 °C; Ion transfer tube temperature = 325 °C; Negative mode spray voltage = -2.75 kV)

Standard	RT (min)	Height (counts) (default source conditions)	Height (counts) (optimized source conditions)	Relative intensity (optimized/default)
PFBA	5.56	2.00E+06	1.20E+07	600%
PFPeA	10.72	6.90E+06	3.30E+07	478%
PFBS	11.72	1.10E+08	1.00E+08	91%
PFHxA	13.35	1.80E+07	5.80E+07	322%
PFHpA	14.92	3.20E+07	6.80E+07	213%
PFHxS	15.08	1.40E+08	1.20E+08	86%
PFOA	16.03	4.20E+07	8.00E+07	190%
PFNA	16.92	5.10E+07	9.00E+07	176%
PFOS	16.97	1.60E+08	1.50E+08	94%
PFDA	17.65	6.60E+07	8.70E+07	132%
PFDS	18.27	1.60E+08	1.50E+08	94%
PFUdA	18.27	6.30E+07	8.50E+07	135%
PFDoA	18.78	7.90E+07	8.30E+07	105%
PFTrDA	19.22	8.50E+07	6.40E+07	75%
PFTeDA	19.58	8.60E+07	6.00E+07	70%
PFHxDA	20.2	7.50E+07	5.40E+07	72%
PFODA	20.64	8.00E+07	4.10E+07	51%
			Mean	**176%**

Quantitative performance

To evaluate the quantitative performance of the LC-MS system for PFAS compounds using the established polarity-switching method, a dilution series of the PFAS mixture was prepared with calibration standards at 0.1, 0.5, 1, 2, 20, 100, 200, and 500 ppb, along with a calibration blank.

The LOQ and linearity range values were obtained using the criteria of $R^2 > 0.99$ and %Diff < 25 for all calibration points. Excellent sensitivity was achieved for all PFAS compounds investigated here, with LLOQs below 1 ppb for all but one standard.

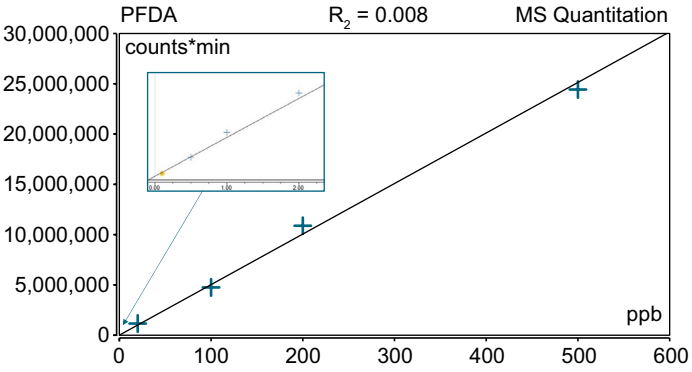

Figure 3. Calibration curve for PFOA with a linear range of 0.1–500 ppb using 1/X weighting and R^2 = 0.998

Table 6. Calibration figures of merit for the PFAS standards determined from injections of the dilution series with concentrations ranging from 0.1 to 500 ppb

Standard	LOD (ppb)	LLOQ (ppb)	ULOQ (ppb)	R^2
PFBA	0.5	1	500	0.999
PFPeA	0.1	0.5	500	0.998
PFBS	0.1	0.1	500	0.999
PFHxA	0.1	0.5	500	0.999
PFHpA	0.1	0.5	500	0.998
PFHxS	0.1	0.5	500	0.996
PFOA	0.1	0.1	500	0.998
PFNA	0.1	0.1	200	0.998
PFOS	0.1	0.1	200	0.998
PFDA	0.1	0.1	200	0.999
PFDS	0.1	0.1	200	0.999
PFUdA	0.1	0.1	200	0.998
PFDoA	0.1	0.5	200	0.996
PFTrDA	0.1	0.5	200	0.996
PFTeDA	0.1	0.5	500	0.998
PFHxDA	0.1	0.5	500	0.998
PFODA	0.1	0.5	500	0.997

The fast polarity-switching of the Orbitrap Exploris 120 MS maintained excellent mass accuracy and scan speed to enable adequate sampling of the chromatographic peak (≥7 scans/peak) for the quantitation of eluting targets, as highlighted in Figure 4.

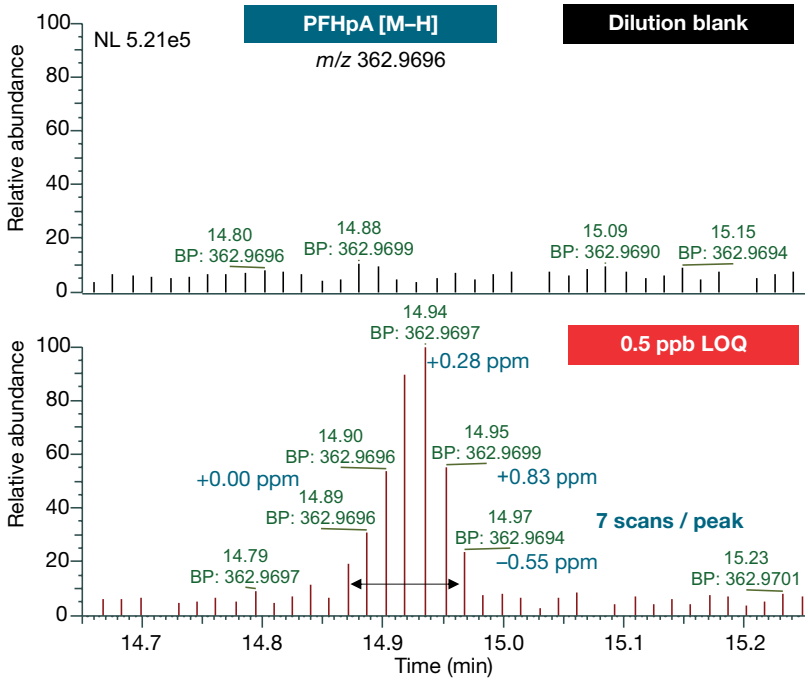

Figure 4. XICs for PFHpA [M-H]⁻ in the dilution blank and LOQ level, demonstrating scan speed and mass accuracy of the polarity-switching method

Targeted analysis of test material extracts

The established method was then used to analyze two extracts (50% ethanol or isopropanol) of pharmaceutical-grade bottle and tubing samples, both constructed from FEP. Using the calibration curves created from the dilution series of the PFAS standard mixture discussed above, the concentration of the 17 targeted PFAS compounds could be readily determined in the test material extracts using Chromeleon CDS. Figure 5 shows the extracted ion chromatograms (XICs) of the compounds found in the 50% ethanol extract of the FEP tubing sample monitored with the list of compounds in the PFAS standard mixture, where the highlighted peak for PFPeA was found to be present at 0.61 ppb. The results of the targeted screening for all 4 extracts and the respective extraction blanks are summarized in Table 7. Notably, none of the 17 compounds were found to be present above 1 ppb, and the short chain PFAS compounds, which were more polar, were present at higher concentration in the more polar 50% ethanol extract samples.

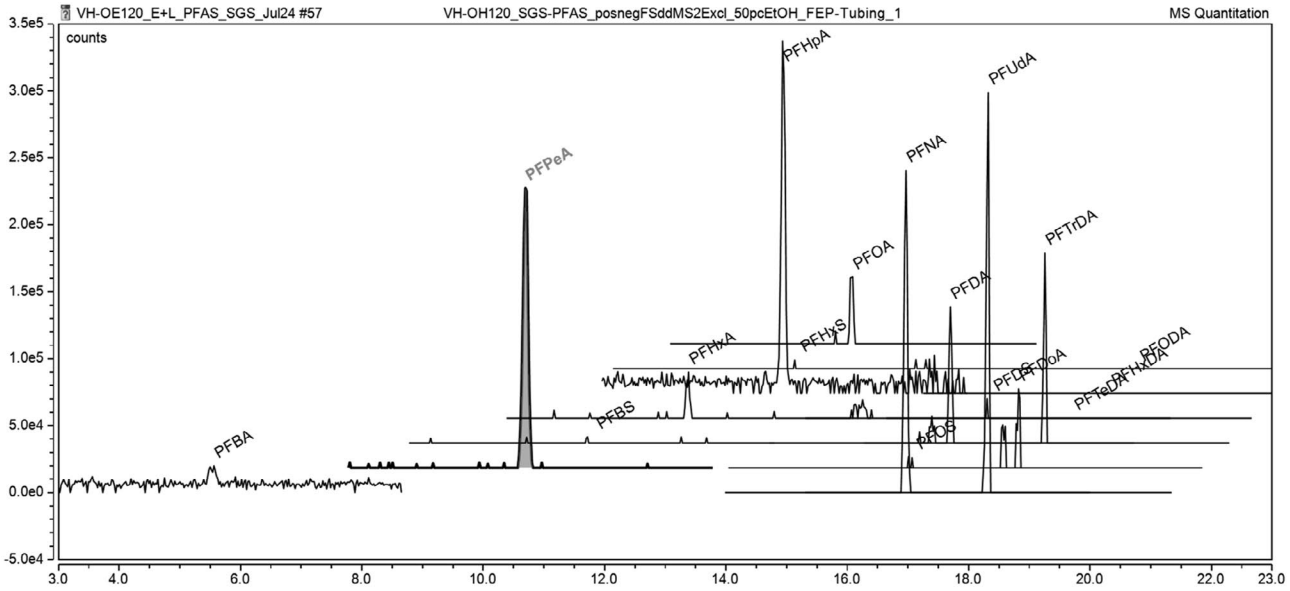

Figure 5. XICs of the 17 PFAS standards for the 50% ethanol extract of a pharmaceutical-grade FEP tubing sample, with the peak of PFPeA highlighted

Table 7. Quantitative results of the targeted PFAS screening in the different extract samples (n.d. = not detected above LOD)

	50% Ethanol extraction			Isopropanol extraction		
Compound	Blank (ppb)	Bottle (ppb)	Tubing (ppb)	Blank (ppb)	Bottle (ppb)	Tubing (ppb)
PFBA	n.d.	<1	<0.5	n.d.	<0.5	<0.5
PFPeA	<0.1	<0.5	0.609	n.d.	<0.1	<0.5
PFBS	n.d.	n.d.	n.d.	<0.1	n.d.	<0.1
PFHxA	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1
PFHpA	n.d.	n.d.	<0.5	n.d.	n.d.	<0.5
PFHxS	n.d.	n.d.	n.d.	<0.1	n.d.	<0.1
PFOA	n.d.	<0.1	<0.1	<0.1	<0.1	<0.1
PFNA	n.d.	<0.1	0.233	<0.1	<0.1	0.321
PFOS	n.d.	n.d.	n.d.	n.d.	n.d.	<0.1
PFDA	n.d.	<0.1	<0.1	n.d.	n.d.	<0.1
PFDS	n.d.	n.d.	n.d.	<0.1	n.d.	n.d.
PFUdA	n.d.	<0.1	0.281	n.d.	<0.1	0.309
PFDoA	n.d.	n.d.	<0.1	n.d.	n.d.	<0.1
PFTrDA	n.d.	<0.1	<0.5	n.d.	<0.5	<0.5
PFTeDA	n.d.	<0.5	n.d.	n.d.	<0.5	n.d.
PFHxDA	n.d.	n.d.	n.d.	n.d.	<0.5	n.d.
PFODA	n.d.	n.d.	n.d.	n.d.	<0.5	n.d.

Non-targeted analysis of test material extracts

To investigate the presence of other potential PFAS outside of the panel of targeted standards in the test material extracts, the data was exported from Chromeleon CDS and processed with Compound Discoverer software using the non-targeted PFAS analysis workflow, which is described in more detail in a separate application note.[7] Briefly, after non-targeted compound detection and elemental composition determination based on the HRAM data and isotopic peak pattern, annotation was carried out by first searching MS^2 data against authentic reference standard data in the Thermo Scientific™ mzCloud™ online spectral library, as well as the NIST™ 2023 Tandem MS/MS spectral library and an *in silico* PFAS spectral library[8] using the mzVault node. Secondly, the MS^2 data was searched for characteristic PFAS product ions using the Compound Class node and accurate monoisotopic mass and formula of the unknowns were used to search against several mass lists containing known and suspected PFAS structures. The resulting compound table was filtered for those giving one or more matches to the above, using the Boolean filtering logic depicted in Figure 6.

The non-targeted analysis found several PFAS compounds already detected using the targeted screening approach described above, such as PFPeA and PFNA, which also yielded MS^2 spectral matches to the mzCloud and NIST spectral libraries. Additionally, five other putative PFAS extractables were present in one or both of the test materials. In the case of the compounds with MW 163.9897 eluting at 1.72 min and MW 179.9847 at 2.55 min, spectral matching to either the *in silico* PFAS library or the mzCloud library, respectively, as exemplified for the latter in Figure 7. This allowed their annotation as pentafluoropropanoic acid and 2,2-difluoro-2-(trifluoromethoxy)acetic acid with an annotation confidence level of 3 and 2, respectively.[9]

Table 8 summarizes the results of the non-targeted PFAS screening, including four PFAS also included in the targeted screening (confidence level 1), and three suspected PFAS extractables in addition to the two discussed above. Notably, the level 1 annotations were supported by fragmentation spectral matches obtained at sub-ppb levels, as determined in Table 7.

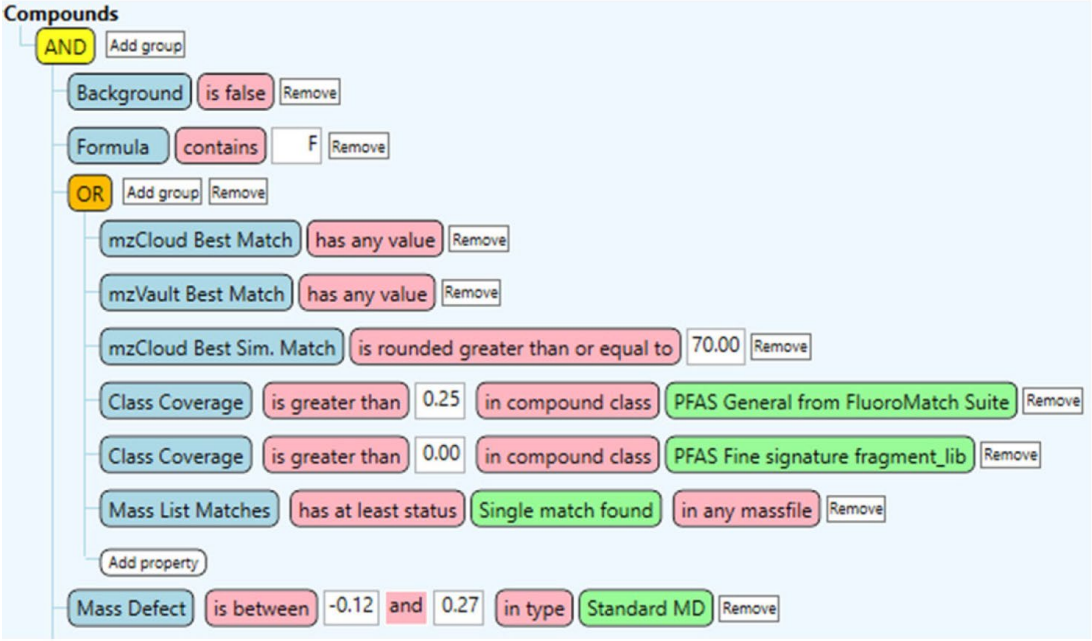

Figure 6. Result filter used for initial data reduction to display putative PFAS compounds in the data based on MS^1-based (Formula, Mass Defect, Mass List Match) and/or MS^2-based (mzCloud Match, mzVault Match, Class Coverage) filtering approaches

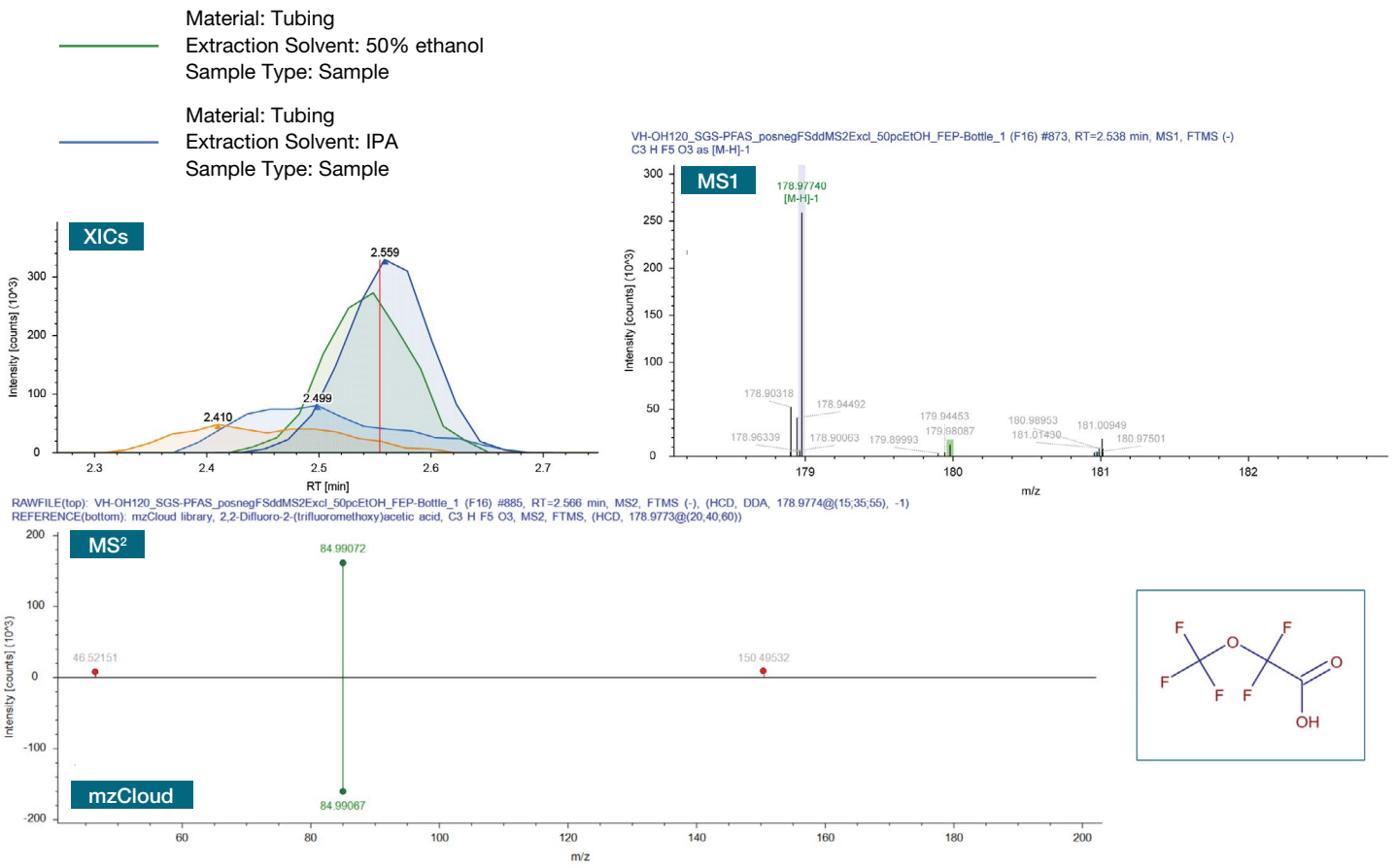

Figure 7. Confident identification of the compound with *m/z* 178.9774 at 2.5 min found in the tubing extracts as 2,2-difluoro-2-(trifluoromethoxy)acetic acid based on MS² match to the mzCloud library and matching isotopic pattern

Table 8. Summary of suspected PFAS extractables found in the tubing and bottle materials, listing their primary annotation source and annotation confidence level based on the criteria established by Charbonnet *et al.*, ordered by maximum peak area[9]

Entry	RT (min)	*m/z*	Calc. MW	Formula	ΔMass (ppm)	Name annotation	Annotation confidence level	Annotation source
1	1.72	162.98245	163.98972	$C_3HF_5O_2$	0.33	Pentafluoropropanoic acid	3	mzVault match (*in silico* library)
2	17.62	432.97263	433.97991	$C_8H_2F_{16}O_2$	-0.06	1,1,1,3,3,4,4,6,6,6-Decafluoro-2,5-bis(trifluoromethyl)hexane-2,5-diol	3	Class Coverage + MassList match
3	10.70	218.98629	263.98339	$C_5HF_9O_2$	0.4	Perfluoropentanoic acid (PFPeA)	1	Match to Reference Standard
4	2.55	178.97738	179.98466	$C_3HF_5O_3$	0.41	2,2-Difluoro-2-(trifluoromethoxy) acetic acid	2	mzCloud + NIST match
5	14.95	362.9697	363.97695	$C_7HF_{13}O_2$	0.16	Perfluoroheptanoic acid (PFHpA)	1	Match to Reference Standard + mzCloud
6	16.97	462.96331	463.97057	$C_9HF_{17}O_2$	0.13	Perfluorononanoic acid (PFNA)	1	Match to Reference Standard + mzCloud
7	18.31	562.95667	563.96399	$C_{11}HF_{21}O_2$	-0.24	Perfluoroundecanoic acid (PFUdA)	1	Match to Reference Standard + mzCloud
8	20.91	848.92603	849.9333	$C_{15}HF_{31}O_5$	0.48	Perfluoro 2,5,8,11-tetramethyl-3,6,9,12-tetraoxapentadecan-1-ol	4	Mass List Match
9	15.60	332.9791	333.98637	$C_6H_2F_{12}O_2$	0.16	Perfluoropinacol (Perfluoro 2,3-dimethylbutane-2,3-diol)	3	Class Coverage + Mass List match

To allow an estimation of the suspected PFAS extractables' concentration levels in the test material extracts, surrogate quantitation could readily be carried out in the Chromeleon CDS, using the closest-eluting authentic standard from the PFAS mixture, as shown in Table 9 (Relative response factor = 1). Notably, the short chained pentafluoropropanoic acid (PFPA) was found to be most abundant in the 50% ethanol extract of the FEP bottle, with its concentration estimated above 20 ppb, but also present at approximately 1.6 ppb in the tubing extract.

Table 9. Results of the estimated quantitation of the suspected PFAS extractables not already part of the targeted screening panel, using surrogate calibration based on the closest-eluting authentic standard

Entry	RT (min)	Name annotation	Surrogate standard	LOQ (ppb)	Estimated quantitation (ppb)			
					Bottle, 50% ethanol	Bottle, IPA	Tubing, 50% ethanol	Tubing, IPA
1	1.715	Pentafluoropropanoic acid	PFBA	1	21.1	2.1	1.6	<LOQ
4	2.554	2,2-Difluoro-2-(trifluoromethoxy)acetic acid	PFBA	1	1.9	<LOQ	1.5	<LOQ
9	15.602	Perfluoropinacol	PFOA	0.1	n.d.	n.d.	0.4	0.3
2	17.623	1,1,1,3,3,4,4,6,6,6-Decafluoro-2,5-bis(trifluoromethyl)hexane-2,5-diol	PFDA	0.1	n.d.	n.d.	0.5	0.7
8	20.912	Perfluoro 2,5,8,11-tetramethyl-3,6,9,12-tetraoxapentadecan-1-ol	PFODA	0.5	n.d.	n.d.	n.d.	<LOQ

Concurrent screening for non-fluorinated extractables

As described above, the data acquisition in this work was carried out using a polarity-switching method, which enabled the simultaneous detection and identification of other extractables originating from the tubing and bottle, respectively, in either ionization mode. Especially the more non-polar isopropanol extract was found to contain various plasticizers at appreciable levels, including trioctyl trimellitate (TOTM) and several epoxidized triglycerides—common constituents of epoxidized soybean oil (ESBO)—that could be identified with high confidence based on matching to the custom spectral library generated from such compounds in a separate application note (and included with the Compound Discoverer 3.3 SP3 software).[5]

Lastly, an additional benefit of the delay column was found for the detection of extractables in the sample that are frequently present in LC-MS systems or solvents, leading to large background interference, such as aliphatic acids (e.g., palmitic acid, stearic acid, or oleic acid) and surfactants (e.g., dodecylbenzene sulfonic acid). As shown in Figure 8, the system peak was shifted to later retention times with the delay column (positioned ahead of the autosampler in the flow path). This enabled the interference-reduced detection of the compounds originating from the sample, which might otherwise be filtered out in the data processing due to the peak area in the sample not significantly differing from that in the extraction blank, caused by the introduction from the system instead of being an actual extractable compound.

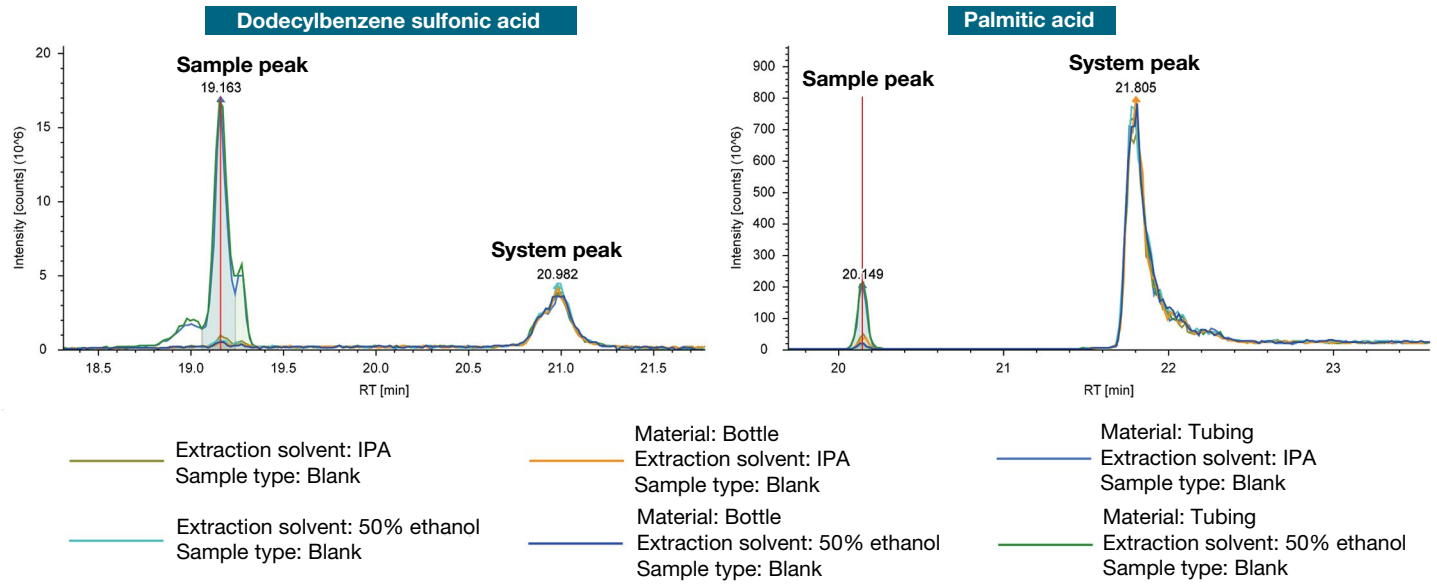

Figure 8. Impact of delay column on separation of extractable peaks originating from the sample from the contribution of the same compound also being present in the system blank

Conclusion

In this work, we have developed a comprehensive solution for the targeted and non-targeted screening for PFAS as part of the E&L analysis of pharmaceutical packaging and processing material components using the Vanquish Horizon UHPLC system coupled to the Orbitrap Exploris 120 mass spectrometer and the combination of Chromeleon CDS 7.3.2 and Compound Discoverer 3.3 software.

- The LC-MS analysis with a polarity switching Full Scan-ddMS^2 method allowed the simultaneous identification of known and unknown suspected PFAS, as well as unknown extractables, with high confidence due to the excellent sensitivity and mass accuracy of the Orbitrap detector.
- The screening and targeted quantitation of 17 common PFAS could be carried out with the Full Scan data with high sensitivity (LOQs ranging from 0.1 to 1 ppb) and minimal background interference from the analytical system with the use of the PFAS analysis kit.
- The result of the analysis of fluorinated test materials for pharmaceutical applications demonstrated the ability to detect and identify PFAS at low ppb to sub-ppb levels, including five suspected PFAS found in the non-targeted analysis.
- The use of the delay column also benefits the analysis of extractables that are frequent contaminants of LC-MS systems by separating the system peak from the sample peak.

The presented approach should have broad applicability to the screening for PFAS compounds in E&L, as well as other pharmaceutical testing and beyond.

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