A sensitive method for the quantitation of per- and polyfluoroalkyl substances (PFAS) in pharmaceutical packaging containers

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This technical note demonstrates a sensitive quantitation method for PFAS using the SCIEX 7500+ system. Baseline chromatographic separation was achieved for the analyzed 34 PFAS compounds (Figure 1). Accurate and highly reproducible quantitative performance was achieved. The method was applied to measure PFAS in example pharmaceutical packaging containers, achieving an overall recovery of ≥96.7% across 34 compounds.

Currently, government agencies worldwide have established stringent safety limits and regulations for PFAS in drinking water to protect public health.[1] More recently, the involvement and impact of PFAS in pharmaceutical applications have started to attract attention. As interest in this area continues to grow, the accurate evaluation of PFAS in pharmaceutical products will require highly sensitive and selective analytical methods to ensure reliable detection and quantitation. In this technical note, a sensitive quantitation method was developed using 34 PFAS compounds as model analytes for this assay. The method was subsequently applied to pharmaceutical packaging containers, in which no PFAS compounds were detected.

Key benefits for quantitation of PFAS in pharmaceutical packaging containers using the SCIEX 7500+ system

- **Baseline chromatographic separation:** Achieve baseline separation of 34 PFAS compounds using a simple LC gradient method
- **Sensitive quantitation of PFAS compounds:** Limit of quantitation (LOQ) ranging between 0.001 and 0.005 ng/mL was achieved with an accurate and highly reproducible (%CV <14) quantitative performance across 34 PFAS using a low injection volume (3 µL)
- **Method applicability to pharmaceutical packaging containers:** An overall recovery of ≥96.7% was achieved when measuring 34 PFAS compounds in pharmaceutical packaging containers
- **Excellent quantitative performance:** A wide linear dynamic range (LDR) spanning ≥3.2 orders of magnitude was reached with a coefficient of determination (r^2) of ≥0.99 for all PFAS analyzed on the SCIEX 7500+ system

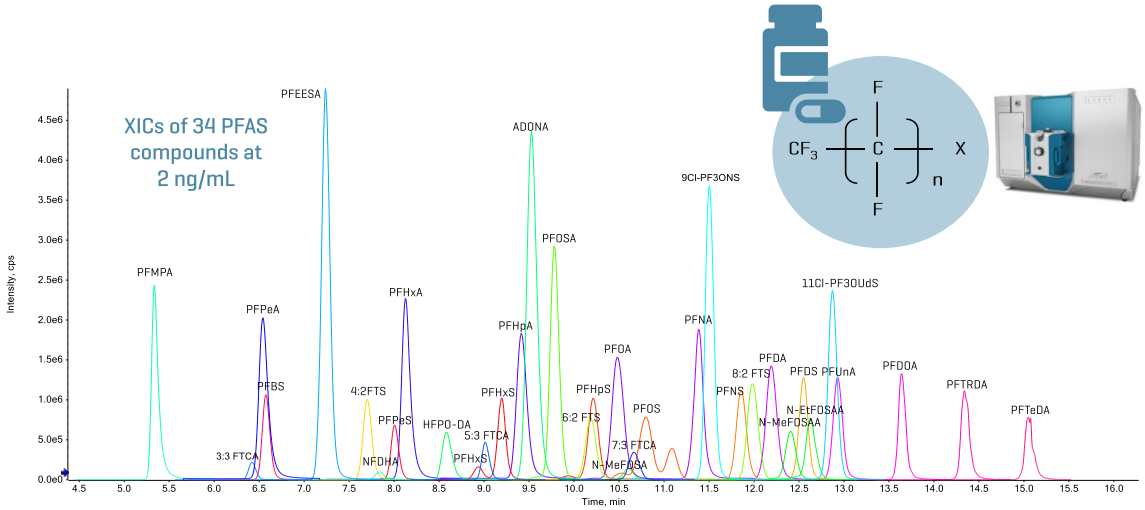


Figure 1. Good baseline separation across 34 PFAS compounds was achieved as shown in the representative extracted ion chromatograms (XICs) above. A scheduled MRM (sMRM) method was applied for analysis at a concentration of 2 ng/mL.

Introduction

Pharmaceutical packaging containers may inadvertently harbor trace levels of PFAS, making sensitive and reliable measurement essential. Quantitation of PFAS at ultra-trace levels is required to safeguard drug product quality and patient safety, especially given PFAS' persistence and potential health risks.

Under the evolving REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) framework, European authorities are proposing expanded restrictions on PFAS, which may impact pharmaceutical packaging, excipient materials, and container closure systems.[2] Following the FDA's revocation of PFAS use in food contact materials, concerns have intensified regarding the use of fluoropolymer materials in pharmaceutical packaging containers. Due to the progression of regulations, there is a potential need for sensitive methods to quantify PFAS in pharmaceutical containers for safe packaging and transport.

This study evaluated the LC-MS-based quantitative performance of 34 PFAS compounds and the method applicability in pharmaceutical containers.

Methods

Samples and reagents: PFAS mixture (PFAS-C-ES Mixture) was purchased from LGC Standards.

Sample preparation: A working stock of 20 ng/mL was prepared in an 80:10 (v/v) methanol/water mixture and serially diluted to prepare the calibration curve samples. The concentrations ranged between 0.001 and 10 ng/mL. For the extraction procedure, a 10 mL aliquot was added to the pharmaceutical packaging container (ophthalmic drug container). The weight of the container was 3.85 g. The container was spiked with 0.1 ng/mL of PFAS and 10 mL of methanol as the extraction solvent. The samples were sonicated for 1 hour at 50°C. After sonication, 0.2 mL of water was added to the samples and vortexed.[4] The samples were transferred into the autosampler vials and injected for analysis.

Chromatography: Chromatographic separation was achieved on an ExionLC AD system (SCIEX). A delay column (Phenomenex Luna Omega PS C18 column; 3 x 50 mm, 5 µm, 100 Å) was used after the LC mixer for chromatographic delay of any possible PFAS contamination coming from mobile phase solvents. A Phenomenex Luna omega PS C18 column (2.1 x 100 mm, 3 µm, 100 Å) was used to achieve baseline analytical separation of the PFAS compounds. The column temperature was set at 50°C. A flow rate of 0.6 mL/min was used. Mobile phase A was 2 mM ammonium acetate in water and mobile phase B was 0.05% acetic acid in methanol. An injection volume of 3 µL was used for the analysis.

The chromatographic gradient conditions are summarized in Table 1.

Table 1. LC gradient conditions.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	90	10
1.5	90	10
2.0	60	40
12.5	5	95
16.5	5	95
16.7	90	10
20	90	10

Mass spectrometry: Data was acquired using the SCIEX 7500+ system. The optimized source and gas parameters are listed in Table 2. An sMRM method was applied for the analysis; final transitions are listed in Table 3.

Table 2. List of compounds and methodology used for analysis.

Parameters	Value
Polarity	Negative
Ion source gas 1	70 psi
Ion source gas 2	80 psi
Curtain gas	45 psi
Source temperature	500°C
Ion spray voltage	1600 V
CAD gas	8

Data processing: Data collection and analysis were performed using the SCIEX OS software, version 3.4. Peaks were integrated using the MQ4 algorithm, and a weighting of $1/x^2$ was used for quantitation.

Table 3. MRM transitions and MS conditions.

Analyte	Q1 (m/z)	Q3 (m/z)	CE(V)	QoD (V)
PFPeA	262.95	218.9	-12	10
PFHxA	313	269	-12	0
PFHpA	363.1	319	-14	10
PFOA	413	369	-15	10
PFNA	463	419	-15	-10
PFDA	512.9	469	-16	0
PFUnA	563.1	519	-17	-20
PFDoA	613	569	-18	-10
PFTrDA	663	168.9	-36	-20
PFTeDA	713.1	669	-20	10
PFBS	298.7	79.9	-65	-50
PFPeS	349	79.9	-120	-10
PFHxS	398.7	79.9	-85	-20
PFHpS	262.98	79.9	-55	0
PFOS	498.9	79.9	-114	-20
PFNS	548.8	79.9	-130	-30
PFDS	599	79.9	-140	-10
4:2 FTS	327.1	307	-29	10
6:2 FTS	427.1	407	-35	-10
8:2 FTS	527.1	507	-40	-20
PFOSA	498	78	-90	10
N-MeFOSA	512	219	-25	-60
N-MeFOSAA	569.9	418.9	-30	-20
N-EtFOSAA	583.9	418.9	-30	-20
HFPO-DA	284.9	168.9	-12	-10
ADONA	376.9	250.9	-16	10
PFMPA	229.1	85	-18	-10
PFMBA	279.1	85	-16	-10
9Cl-PF3ONS	530.9	350.9	-37	-30
11Cl-PF3OUdS	630.9	450.9	-38	-20
PFEESA	314.9	134.9	-31	10

3:3 FTCA	240.9	176.9	-12	10

Quantitative performance

This technical note demonstrates the quantitation of PFAS at sub-ng/mL levels using the SCIEX 7500+ system. Baseline separation was achieved for the 34 PFAS compounds (Figure 1) using a simple gradient system.

An LOQ of ≤0.005 ng/mL was achieved for all 34 PFAS compounds. Figure 2 shows the XICs at the LOQ levels for 8:2 FTS, 4:2 FTS, PFOSA and ADONA. No background interference was observed in the blank samples. Figure 3 shows quantitative performance for 8:2 FTS, 4:2 FTS, PFOSA and ADONA. For the 4 representative PFAS compounds, the LDR was ≥3.3 orders of magnitude with an r^2 of ≥0.993 and an assay accuracy of ±12% (of the nominal concentration) with %CV <6.

Table 4 summarizes quantitation performance for the 34 PFAS compounds. LOQs ≤0.005 ng/mL were achieved with LDRs spanning a ≥3 orders of magnitude. Linearity was achieved with an r^2 of ≥0.993. The assay accuracy was within ±15% (of the nominal concentration) with %CV <9 for 34 PFAS compounds at the LOQ level.

Figure 4 shows representative XICs of the blank samples from the pharmaceutical packaging container and at 0.1 ng/mL of spiked in 8:2 FTS, 4:2 FTS, PFOSA and ADONA. No PFAS compounds were detected in the pharmaceutical packaging container as represented in the blank XICs.

Recovery was evaluated by comparing the peak area of each PFAS compound at 0.1 ng/mL in a pre-spiked pharmaceutical packaging container against the peak area of each PFAS compound at 0.1 ng/mL in the post-spiked sample (Table 4). Testing was performed using 3 replicates for all 34 PFAS compounds. Good assay recovery was achieved with an overall recovery of ≥96.7%.

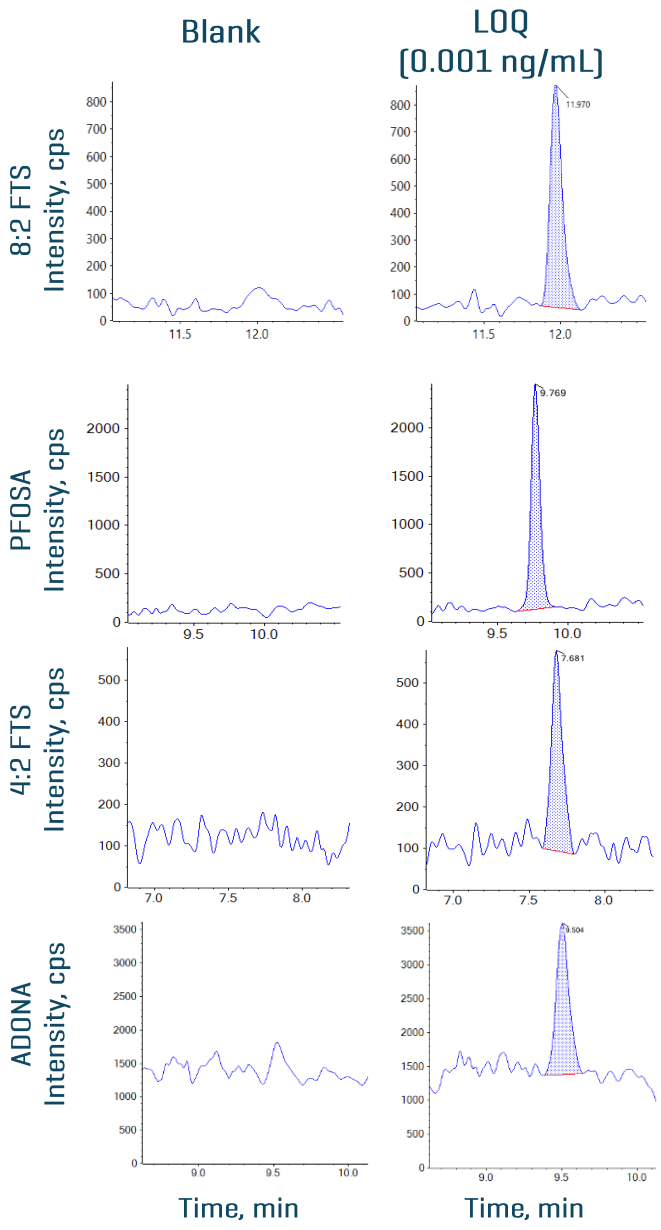
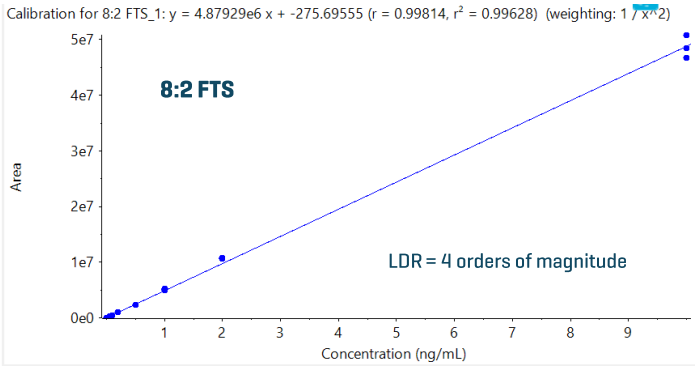
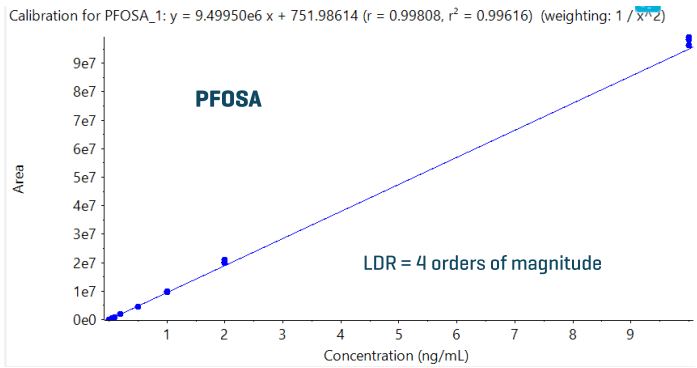


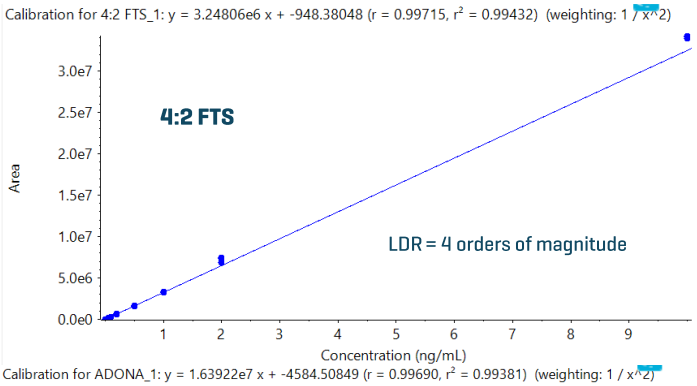
Figure 2. Representative XICs of 8:2 FTS, PNOSA, 4:2 FTS and ADONA. LOQs of 0.001 ng/mL were achieved for 4 PFAS. No interference was observed at the retention time of the analyte.



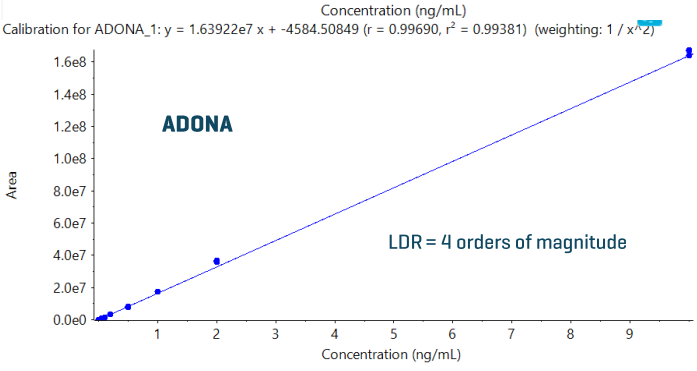
Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
1	8:2 FTS_1	0.001	3 of 3	0.001	0.000	2.34	104.
2	8:2 FTS_1	0.002	3 of 3	0.002	0.000	4.45	93.1
3	8:2 FTS_1	0.050	3 of 3	0.047	0.000	0.955	94.4
4	8:2 FTS_1	0.100	3 of 3	0.099	0.002	2.47	98.6
5	8:2 FTS_1	0.200	3 of 3	0.204	0.002	0.779	102.
6	8:2 FTS_1	0.500	3 of 3	0.476	0.011	2.41	95.2
7	8:2 FTS_1	1.000	3 of 3	1.035	0.030	2.91	103.
8	8:2 FTS_1	2.000	3 of 3	2.196	0.024	1.07	110.
9	8:2 FTS_1	10.000	3 of 3	9.975	0.415	4.16	99.8



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
1	PFOSA_1	0.001	3 of 3	0.001	0.000	8.81	102.
2	PFOSA_1	0.002	3 of 3	0.002	0.000	6.00	95.4
3	PFOSA_1	0.050	3 of 3	0.046	0.002	3.44	92.3
4	PFOSA_1	0.100	3 of 3	0.099	0.002	1.54	98.7
5	PFOSA_1	0.200	3 of 3	0.205	0.005	2.34	102.
6	PFOSA_1	0.500	3 of 3	0.479	0.011	2.39	95.8
7	PFOSA_1	1.000	3 of 3	1.027	0.015	1.46	103.
8	PFOSA_1	2.000	3 of 3	2.147	0.072	3.35	107.
9	PFOSA_1	10.000	3 of 3	10.300	0.156	1.52	103.



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
1	4:2 FTS_1	0.001	3 of 3	0.001	0.000	2.72	105.
2	4:2 FTS_1	0.002	3 of 3	0.002	0.000	1.64	89.9
3	4:2 FTS_1	0.050	3 of 3	0.046	0.002	4.88	91.4
4	4:2 FTS_1	0.100	3 of 3	0.097	0.002	1.57	97.5
5	4:2 FTS_1	0.200	3 of 3	0.200	0.003	1.45	99.8
6	4:2 FTS_1	0.500	3 of 3	0.489	0.011	2.18	97.9
7	4:2 FTS_1	1.000	3 of 3	1.025	0.018	1.77	103.
8	4:2 FTS_1	2.000	3 of 3	2.216	0.096	4.34	111.
9	4:2 FTS_1	10.000	3 of 3	10.502	0.039	0.370	105.



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
1	ADONA_1	0.001	3 of 3	0.001	0.000	5.59	106.
2	ADONA_1	0.002	3 of 3	0.002	0.000	2.21	89.4
3	ADONA_1	0.050	3 of 3	0.045	0.000	0.372	90.3
4	ADONA_1	0.100	3 of 3	0.097	0.003	2.92	97.3
5	ADONA_1	0.200	3 of 3	0.203	0.001	0.691	102.
6	ADONA_1	0.500	3 of 3	0.488	0.003	0.580	97.5
7	ADONA_1	1.000	3 of 3	1.063	0.012	1.10	106.
8	ADONA_1	2.000	3 of 3	2.213	0.023	1.05	111.
9	ADONA_1	10.000	3 of 3	10.136	0.109	1.07	101.

Figure 3. Calibration curves and quantitative performance of 8:2FTS, PFOSA, 4:2FTS and ADONA on the SCIEX 7500+ system. The area between PFAS and its respective concentration was used to generate calibration curves. Each concentration level was run in triplicate. Linearity was achieved between the ranges denoted on the graph. Linearity spanned an LDR of >3 orders of magnitude with an r^2 >0.995 on the SCIEX 7500+ system (left). Exceptional reproducibility and accuracy were achieved for all PFAS analyzed (right).

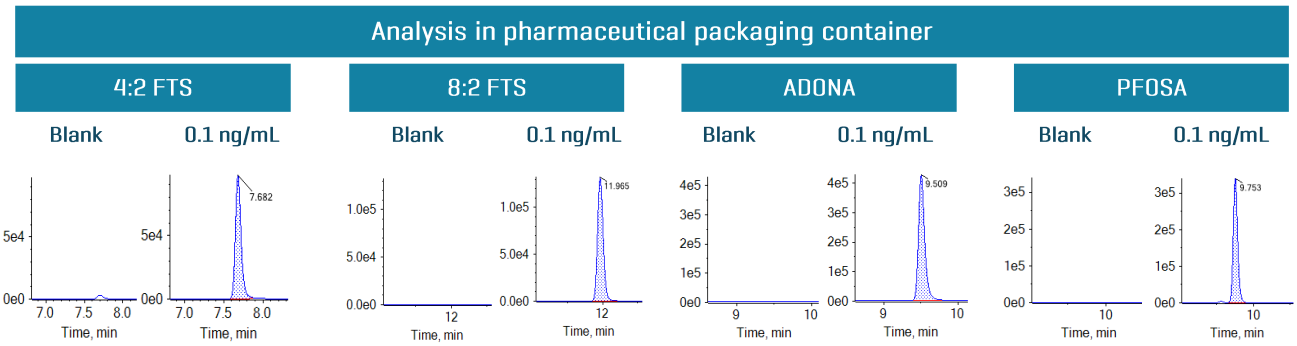


Figure 4. Representative XICs of 4 PFAS in a pharmaceutical container at extracted blank and spiked 0.1 ng/mL levels. No PFAS compounds were detected in the blank.

Table 4. Quantitative performance and recovery of the 34 PFAS compounds.

Compound	LOQ (ng/mL)	Linear range (ng/mL)	r^2	LDR	%CV at LOQ	%Accuracy at LOQ	%Recovery (n=3)
PFPeA	0.005	0.005-10	0.994	3.2	12.1	103	98.2
PFHxA	0.005	0.005-10	0.994	3.2	6.06	102	99.2
PFHpA	0.002	0.002-10	0.993	3.5	1.63	101	91.9
PFOA	0.005	0.005-10	0.994	3.2	9.11	100	102
PFNA	0.005	0.005-10	0.989	3.2	7.42	103	96.8
PFDA	0.005	0.005-10	0.992	3.2	6.05	100	115
PFUnA	0.005	0.005-10	0.994	3.2	11.3	107	110
PFDoA	0.005	0.005-10	0.993	3.2	5.13	104	109
PFTrDA	0.002	0.002-10	0.993	3.5	2.19	104	105
PFTeDA	0.005	0.005-10	0.991	3.2	9.99	106	106
PFBS	0.005	0.005-10	0.993	3.2	2.9	106	100
PFPeS	0.005	0.005-10	0.993	3.2	3.59	108	102
PFHxS	0.002	0.002-10	0.992	3.5	6.1	104	100
PFHpS	0.005	0.005-10	0.993	3.2	3.74	106	102
PFOS	0.005	0.005-10	0.994	3.2	8.79	101	102
PFNS	0.002	0.002-10	0.992	3.5	11.5	104	102
PFDS	0.001	0.001-10	0.994	4	5.24	104	102
4:2 FTS	0.001	0.001-10	0.994	4	2.7	105	102
PFPeA	0.001	0.001-10	0.993	4	2.13	104	90.1
6:2 FTS	0.001	0.001-10	0.996	4	1.9	104	102
8:2 FTS	0.001	0.001-10	0.996	4	7.56	102	102
PFOSA	0.002	0.002-10	0.995	3.5	13.6	101	102
N-MeFOSA	0.002	0.002-10	0.993	3.5	7.77	91.7	102
N-MeFOSAA	0.005	0.005-10	0.994	3.2	3.38	104	100
N-EtFOSAA	0.002	0.002-10	0.994	3.5	6.41	106	99.5
HFPO-DA	0.001	0.001-10	0.994	3.5	3.65	106	100
ADONA	0.002	0.002-10	0.993	4	3.43	106	100
PFMPA	0.002	0.002-10	0.993	3.5	3.52	108	100
NFDHA	0.002	0.002-10	0.993	3.5	6.43	106	102
9Cl-PF3ONS	0.002	0.002-10	0.992	3.5	6.67	107	101
11Cl-PF3OUdS	0.001	0.001-10	0.989	3.5	5.76	106	97.3
PFEESA	0.005	0.005-10	0.996	3.2	9.45	102	102
3:3 FTCA	0.005	0.005-10	0.994	3.2	5.88	105	99.3
5:3 FTCA	0.002	0.002-10	0.994	3.5	1.14	101	96.7

Conclusions

- Good baseline separation was achieved between 34 PFAS compounds using a simple gradient system.
- Sub-ng/mL levels of LOQs were achieved for 34 PFAS compounds analyzed on the SCIEX 7500+ system.
- The method demonstrated seamless applicability to measuring PFAS in pharmaceutical packaging containers.
- Excellent quantitative performance was achieved with an r^2 of ≥0.99 for all PFAS and LDR spanning ≥3.2 orders of magnitude achieved on the SCIEX 7500+ system.
- An overall recovery of ≥96.7% was achieved for all PFAS tested in the pharmaceutical packaging container.

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