

Chromatographic Performance Comparison in Ultrashort-Chain PFAS Analysis

Using the Agilent Altura Poroshell 120 PFAS column and other mixed-mode LC columns to analyze USC PFAS in food matrix extracts

Authors

Limian Zhao and
Chenchen He
Agilent Technologies, Inc.

Abstract

This application note presents a comparative evaluation of the chromatographic performance of ultrashort-chain (USC) and short-chain (SC) PFAS analytes in food matrix extracts using the novel Agilent Altura Poroshell 120 PFAS LC column and several commercially available LC columns designed for USC PFAS analysis. Four complex food matrices—baby food, protein powder, pet food, and shrimp—were extracted as matrix blanks and then post-spiked with a standard mixture containing USC and SC PFAS analytes. The spiked extracts were analyzed using the Altura Poroshell 120 PFAS column and three other mixed-mode LC columns marketed for USC PFAS applications.³

The results demonstrated that the Altura Poroshell 120 PFAS column provided substantially improved analyte retention over the other commercially available mixed-mode LC columns for USC PFAS analysis, achieving retention factors greater than 3 for all evaluated USC and SC analytes. Enhanced retention improved separation of target PFAS from food matrix interferences, reduced matrix effect, and thus increased detection sensitivity for both USC and SC compounds in matrix. In addition, the robust retention mechanism enabled direct injection of high-organic sample extract without compromising peak shape for early-eluting analytes.

Introduction

Determination of PFAS residues in food has become an increasing concern in recent years. In April 2023, the European Commission implemented regulations for four PFAS compounds—PFOS, PFOA, PFNA, and PFHxS—across multiple food categories.¹ In November 2023, AOAC released SMPR 2023.003, establishing performance requirements for the analysis of 30 PFAS compounds in 11 food categories.² Accurate and reliable analytical methods are therefore essential for monitoring PFAS levels in foods, supporting effective regulation and protecting public health. Recent interest has developed for the determination of polar USC and SC PFAS in high-water content foods and processed foods because of their mobility and bioavailability in aqueous environments and food production.

USC PFAS are small, highly polar compounds containing C1 to C3 carbon chains, such as trifluoroacetic acid (TFA, C2) and perfluoropropanoic acid (PFPrA, C3). SC PFAS typically include C4 to C7 species such as perfluorobutanoic acid (PFBA, C4) and perfluorobutane sulfonic acid (PFBS, C4). Their high polarity, strong water solubility, and small molecular size pose significant analytical challenges, from chromatographic performance to sample preparation. On conventional reversed-phase LC columns, USC and SC PFAS analytes exhibit insufficient retention and poor peak shape, suffer from severe matrix effects due to coeluted polar interferences, and ultimately show compromised detection sensitivity and quantitation accuracy and precision. As a result, these analytes often fail quantitative analysis, particularly in complex matrices.

The novel Agilent Altura Poroshell 120 PFAS LC columns combine P120 mixed-mode C18 particles with Altura hardware to deliver strong retention, symmetric peak shapes, high pH durability, and enhanced separation of USC and SC analytes. In this study, the chromatographic performance of the Altura Poroshell 120 PFAS column for USC analysis in food matrix extract was evaluated and compared with the other three commercially available mixed-mode LC columns that are marketed for USC PFAS analysis. The assessment and comparison focused on the analytes' chromatographic retention, solvent effect tolerance, separation of analytes from matrix interferences, and matrix effect mitigations.

Experimental

Chemicals and reagents

Native USC and SC PFAS individual stock solutions were purchased from Wellington Laboratories (Ontario, CA, U.S.A.). Methanol (MeOH), acetonitrile (ACN), and isopropyl alcohol (IPA) were from VWR (Randor, PA, U.S.A.). Acetic acid (AA) and ammonium acetate were procured from Millipore Sigma (Burlington, MA, U.S.A.).

A mixed spiking solution was prepared at 10 µg/mL by diluting the eight individual stock solutions with MeOH and stored in a 2 mL polypropylene (PP) vial at 4 °C. The standard spiking solution should always be brought up to ambient temperature and vortex thoroughly before use.

Sample extraction solvent was ACN with 1% acetic acid. For LC/MS/MS analysis, mobile phase A (MPA) consisted of 5 mM ammonium acetate (NH₄OAc) and 0.05% acetic acid in water, and mobile phase B (MPB) was 95:5 ACN:water. Needle wash solvents included 1:1 IPA/ACN, 90:10 MPA/MPB, and ACN.

Equipment and material

The study was conducted using an Agilent 1290 Infinity II LC system consisting of an Agilent 1290 Infinity II high-speed pump (G7120A), an Agilent 1290 Infinity III hybrid multisampler (G7137B), and an Agilent 1290 Infinity II multicolumn thermostat (G7116B). The LC system was coupled to an Agilent 6495D triple quadrupole LC/MS system equipped with an Agilent Jet Stream iFunnel electrospray ion source. Data acquisition and analysis were performed using Agilent MassHunter Workstation software.

The 1290 Infinity III LC system was modified using an Agilent InfinityLab PFAS analysis HPLC conversion kit (part number 5004-0006). Instead of the typical PFC delay column from the conversion kit, an Agilent Poroshell 120 PFAS delay column, 4.6 × 30 mm (part number 025403-007), was used. Chromatographic separation was performed using an Agilent Altura Poroshell 120 PFAS HPLC column, 2.1 × 50 mm, 2.7 µm (part number 227205-007).

Agilent consumables used for sample preparation included:

- Bond Elut QuEChERS EN extraction kit, EN 15662 method, buffered salts, ceramic homogenizers (p/n 5982-5650CH)
- Captiva EMR PFAS Food I cartridges, 6 mL, 340 mg (p/n 5610-2230)
- Captiva EMR PFAS Food II cartridges, 6 mL, 750 mg (p/n 5610-2232)
- Polypropylene (PP) screw cap vials and caps, 2 mL (p/n 5191-8121 and 5191-8151)
- Tubes and caps, 50 mL, 50/pk (p/n 5610-2049)
- Tubes and caps, 15 mL, 100/pk (p/n 5610-2039)

All the consumables used in the study were tested and verified for acceptable PFAS cleanliness.

LC/MS/MS instrument conditions

Table 1 presents the LC method conditions used in this study. Two LC gradient programs were applied to accommodate the markedly different retention behaviors of the analytes across the four LC columns. Gradient A was used for evaluation of the Altura Poroshell 120 PFAS column and commercial LC column 1, while gradient B was employed for assessment of commercial LC columns 2 and 3.

Table 2 presents the LC multisampler injection settings using the 1290 Infinity II hybrid multisampler.

Mass spectrometer (MS) acquisition parameters: Negative ion mode with constant fragmentor setting at 166 V and negative ionization mode. Table 3 summarizes the acquisition method conditions. The retention time of analytes varies by LC column and gradient.

Mass spectrometer electrospray ion (ESI) source settings include drying gas at 240 °C, 18 L/min; sheath gas at 350 °C, 11 L/min; nebulizer gas at 15 psi; capillary voltage at 2,500 V (NEG); and nozzle voltage at 0 V (NEG).

Table 1. LC pump conditions for LC/MS/MS analysis.

Parameter	Setting			
Mobile Phase A	5 mM NH ₄ OAc in water with 0.05% acetic acid			
Mobile Phase B	95:5 ACN/water			
Gradient A (for the Altura Column and Column 1)	Time (min)	A%	B%	Flow (mL/min)
	0.0	90	10	0.5
	0.5	90	10	0.5
	9.0	25	75	0.5
10.0	0	100	0.5	
Gradient B (for Columns 2 and 3)	Time (min)	A%	B%	Flow (mL/min)
	0.0	98	2	0.5
	2.0	98	2	0.5
	9.0	25	75	0.5
10.0	0	100	0.5	
Stop Time	12 min			
Post Time	3 min			
Column Temperature	40 °C			
Injection Volume	10 µL			

Table 2. LC injection program on Agilent 1290 Infinity II hybrid multisampler.

Parameter	Setting			
Feed Injection	Injection mode: Feed Injection volume: 10 µL Feed speed: Adaptive, 10% of pump flow Flush-out mode: Automatic			
Injection Path Cleaning	Inner wash mode: Extended Outer wash mode: Extended			
	Draw sample			
	Step	Task	Solution	Duration
	1	Outer wash	S1 = ACN	10 seconds
	2	Outer wash	S3 = 1:1 ACN/IPA	10 seconds
	Injection			
	Step	Task	Solution	Volume
	1	Inner wash	S2 = 90:10 MPA/MPB	150 µL
	2	Inner wash	S2 = 90:10 MPA/MPB	150 µL
	3	Seat wash	S1 = ACN	150 µL
4	Seat wash	S3 = 1:1 ACN/IPA	150 µL	
5	Reconditioning	S2 = 90:10 MPA/MPB		

Table 3. Acquisition method of USC and SC targets.

Target	Abbreviation	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)	Collision Cell Accelerator (V)	iFunnel Mode
Trifluoroacetic Acid	TFA	113	68.9	10	5	Fragile
Trifluoromethane Sulfonic Acid	TFMS	149	98.9 79.9	30 26	5	Fragile
Perfluoropropionic Acid	PFPrA	163	118.9	6	4	Fragile
Pentafluoroethanesulfonic Acid	PFETS	199	98.9 79.8	30 34	4 5	Fragile
Perfluorobutanoic Acid	PFBA	213	169	7	2	Standard
Perfluoropropane Sulfonic Acid	PFPrS	248.9	98.9 79.8	30 38	2	Fragile
Perfluoropentanoic Acid	PFPeA	263	219	7	2	Standard
Perfluorobutanesulfonic Acid	PFBS	293.9	99 80	36 43	2	Standard

Matrix sample preparation

Four food matrices were included in this study: Two types of baby food, whey protein powder, pet food, and shrimp. Samples were extracted following previously established protocols using QuEChERS extraction followed by EMR mixed-mode passthrough cleanup.^{4,5} Although this method has been validated for routine PFAS analysis in multiple food matrices, it has not been evaluated for USC PFAS analysis. In this study, the method was used solely to generate matrix-blank extracts for assessing matrix background effects on chromatographic performance. The EMR eluates were used directly for post-spiking without further treatment. Because of the differing matrix complexity, the extraction mass varied by sample type: 10 g for baby food puree, 5 g for homogenized shrimp, and 2 g for protein powder and pet food powder.

After collection of the EMR-cleaned extracts, a portion of each was spiked with a USC/SC standard spiking solution at 1 and 0.1 ng/mL. Both matrix blanks (MBs) and fortified QC samples were used to evaluate chromatographic performance. For the Altura Poroshell 120 PFAS column and commercial column 1, the MBs and QC samples were injected directly into the LC/MS/MS system for analysis. For commercial column 2 and 3, all matrix samples were further diluted with water at a 1:1 ratio prior to LC/MS/MS analysis. This was to accommodate the different solvent effect mitigation on four LC columns.

Results and discussion

LC gradients and sample solvent for injection

The sample extraction procedures commonly applied to solid food matrices typically result in final extracts containing a high percentage of organic solvent. Even when water-miscible organic solvents such as acetonitrile (ACN) and methanol (MeOH) are used, injection of extracts with high organic content onto a reversed-phase LC column often leads to peak distortion for early-eluting analytes and poor peak integration consistency, compromising reliable quantitation. As a result, strategies to mitigate solvent effects are required, with offline solvent exchange through drying and reconstitution being a commonly used approach. However, when analytes are volatile, evaporation steps should be avoided to prevent analyte loss. This is particularly relevant for USC PFAS analysis, as several USC compounds, especially TFA, are highly volatile.

Under these circumstances, dilution with water becomes the only viable solvent-effect mitigation strategy, which can be implemented either offline or online. Offline dilution, however, results in reduced detection and quantification sensitivity, presenting a significant analytical trade-off.

In this study, the Altura Poroshell 120 PFAS column was evaluated and compared with three commercially available mixed-mode LC columns marketed for USC PFAS analysis. All four columns had identical dimensions (2.1 × 50 mm) and were tested using the same mobile phase compositions. The initial experimental design applied a single LC gradient program (gradient A) to all columns using the same samples. However, commercial LC columns 2 and 3 exhibited unacceptable chromatographic performance for early-eluting analytes under these conditions. As a result, the LC gradient for these two columns was modified to include a higher initial aqueous composition and an extended hold time (gradient B) to enhance retention of polar analytes.

The food sample extracts evaluated in this study contained 90% to 100% ACN and were intended for direct injection to achieve the lowest possible detection limits. In addition, it was anticipated that the mixed-mode LC retention mechanisms would provide improved retention of polar USC analytes and more effective mitigation of solvent effects directly on column. However, despite application of the modified gradient B for commercial LC columns 2 and 3, chromatographic performance remained unacceptable for reliable and consistent comparison. Therefore, neat standards for these two columns were prepared in ACN/water (1:1, v/v), and food sample extracts were diluted with water at a 1:1 ratio prior to injection.

The methodological differences applied in the four-column comparative study were necessary to ensure acceptable chromatographic performance and generate reliable data for meaningful comparison. These necessary adjustments for comparison further indicate that the Altura Poroshell 120 PFAS column and commercial LC column 1 possess a higher capacity for mitigating solvent effects. This enhanced tolerance enables the use of gradients starting at lower aqueous composition and allows direct injection of samples in high organic solvent without compromising chromatographic performance. Table 4 summarizes the LC gradient programs and sample solvent compositions used for evaluation of each LC column.

Table 4. LC gradient and STD/sample solvent used for each LC column.

LC Column	Gradient Used	Organic % in Sample	Dilution Factor on Food Sample Extract	Injection Volume
Altura PFAS Column	Gradient A	90–100% ACN	No dilution	10 μ L
Column 1	Gradient A	90–100% ACN	No dilution	10 μ L
Column 2	Gradient B	50% ACN	2-fold	10 μ L
Column 3	Gradient B	50% ACN	2-fold	10 μ L

Both the Altura Poroshell 120 PFAS column and commercial LC column 1 demonstrated acceptable peak shapes and sufficient retention for all USC and SC PFAS analytes. In contrast, commercial LC columns 2 and 3 failed to deliver acceptable chromatographic performance, even when much milder LC gradients and weaker sample solvent composition were applied. The limited solvent-effect mitigation capability of these two columns resulted in inadequate chromatographic performance that did not meet the required analytical criteria.

Solvent effect mitigation

Given the necessary adjustments to the LC gradient and sample solvent composition, all four LC columns were initially evaluated using standard injections to enable direct comparison. Figure 1 shows the chromatograms obtained from injection of a 10 ppb neat standard (10 μ L injection volume) on each LC column using the standard injection mode.

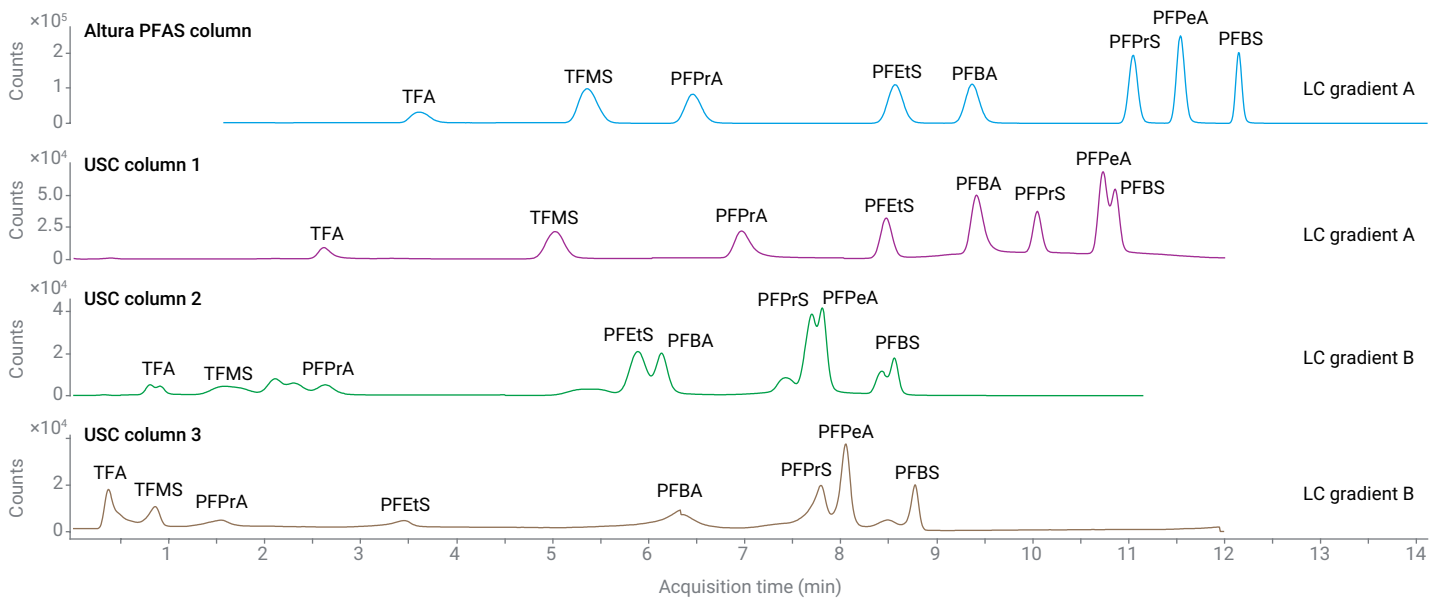


Figure 1. Chromatograms of neat standards (1 ng/mL) analyzed on four mixed-mode LC columns using standard injection on a standard multisampler.

To address these limitations, an online injection program was implemented to enhance solvent-effect mitigation for LC columns 2 and 3. Based on a previously reported approach¹, a feed-injection program was applied using a 1290 Infinity III Hybrid Multisampler. This highly effective online dilution strategy significantly improved chromatographic behavior on LC columns 2 and 3, resulting in acceptable peak integrity for early-eluting analytes. Figure 2 presents the chromatograms obtained by incorporating the feed-injection program on the 1290 Infinity III Hybrid Multisampler.

Although the combined approaches of applying a milder LC gradient, using a weaker sample-solvent composition, and implementing a feed-injection program provided the most effective mitigation of solvent effects and improved peak shape integrity for early-eluting compounds, these analytes still exhibited broader peak shapes with noticeable fronting or tailing when analyzed using commercial LC columns 2 and 3. In contrast, the Altura Poroshell 120 PFAS column and commercial LC column 1 demonstrated superior solvent-effect mitigation capability, offering greater tolerance and flexibility in LC gradient conditions, injection strategies, and sample solvent composition without compromising chromatographic performance.

Overall chromatographic assessment

The Altura Poroshell 120 PFAS column and commercial LC column 1 maintained robust chromatographic performance even under more aggressive conditions, including a stronger initial LC gradient, higher organic content in the sample solvent, and use of a standard injection without online dilution. These features provide both columns with greater flexibility in method development, enhanced accommodation of sample extract solvent composition, and reduced constraints on LC system requirements.

The Altura Poroshell 120 PFAS column provided the strongest analyte retention among the tested columns, with a minimum retention factor of 9.4 for the first-eluting analyte (TFA). All analytes displayed excellent peak symmetry, although peak widths were moderately broader. Baseline separation was achieved for all analytes, with a minimum resolution exceeding 2 for the most closely eluting peak pair. Analyte peaks were also evenly distributed across the chromatographic retention window, further supporting robust separation performance.

Commercial LC column 1 delivered overall performance comparable to the Altura Poroshell 120 PFAS column, albeit with slightly shorter analyte retention. However, several analytes—specifically TFA, PFPrA, and PFBA—exhibited more pronounced peak tailing on column 1. In addition, PFPeA and PFBS were not well resolved, with a resolution (R_e) of 0.48.

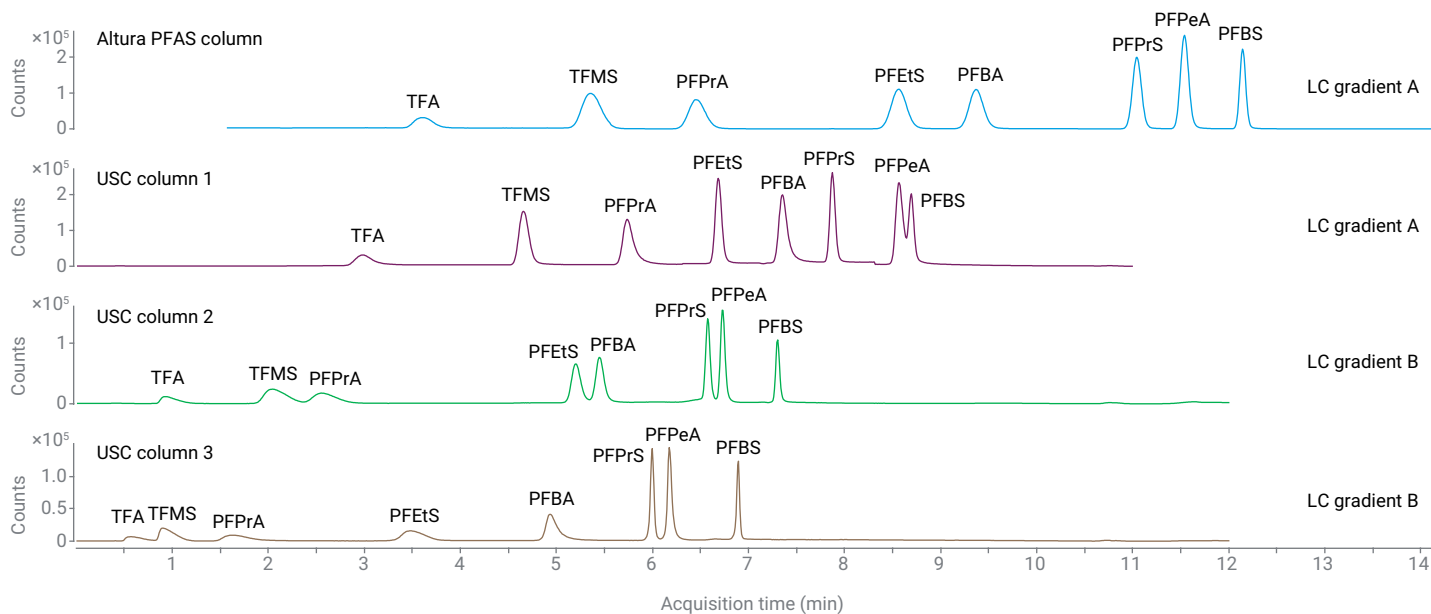


Figure 2. Chromatograms of neat standards (1 ng/mL) analyzed on four mixed-mode LC columns using the Agilent Feed Injection program on an Agilent 1290 Infinity II hybrid multisampler.

Commercial LC columns 2 and 3 exhibited less effective retention for early-eluting analytes, even when multiple solvent-effect mitigation strategies were applied. Nevertheless, these columns produced sharper peak shapes for later-eluting analytes, including PFPrS, PFPeA, and PFBS, which contributed to improved chromatographic resolution in the later portion of the run.

Commercial LC column 2 provided barely acceptable retention for TFMS and PFPrA, while retention for TFA remained marginal. Two analyte pairs (PFEtS/PFBA and PFPrS/PFPeA) eluted in close proximity but remained acceptably resolved due to sharp peak shapes, with resolution values of 0.97 and 1.08, respectively. Commercial LC column 3 demonstrated the weakest retention for TFA, TFMS, and PFPrA, with retention times ranging from 0.6 to 1.5 minutes. Although baseline separation was achieved for the remaining analytes, significant peak fronting or tailing was observed for early-eluting compounds, primarily attributable to solvent effects.

Table 5 summarizes the retention times, retention factors, and peak widths for all analytes across the four evaluated mixed-mode LC columns.

Matrix effect

Matrix effects were evaluated by comparing analyte responses in matrix-matched spiked samples with those obtained from neat standards at equivalent concentrations. Matrix effects for eight USC and SC PFAS analytes were calculated across five food matrix extracts, and the overall trends are summarized in Figure 3.

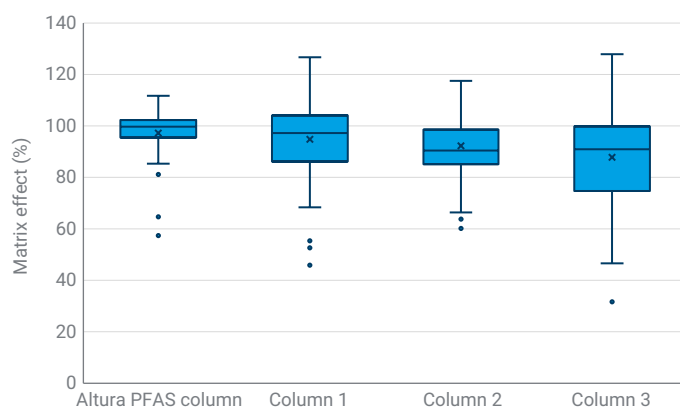


Figure 3. Matrix effects for USC/SC PFAS analytes in food matrices using four different mixed-mode LC columns.

The results demonstrate that the Altura Poroshell 120 PFAS column yielded matrix effects within 90% to 110% for all analytes except TFA, indicating minimal matrix-induced analyte response suppression or enhancement. This effective reduction in matrix suppression is attributed to the strong analyte retention on the Altura Poroshell 120 PFAS column, which enables more efficient chromatographic separation of analytes from polar matrix interferences, thereby minimizing ion suppression caused by coeluting species.

In contrast, the other three mixed-mode LC columns exhibited lower and more variable matrix-effect values for USC and SC PFAS analytes across the evaluated food matrices. These results indicate stronger and more heterogeneous matrix suppression, likely resulting from less effective chromatographic separation between the analytes and coextractive matrix interferences.

Table 5. Summary of retention times, retention factors (k'), and peak widths for all analytes across four mixed-mode LC columns.

Column	Parameter	Analyte							
		TFA	TFMS	PFPrA	PFEtS	PFBA	PFPrS	PFPeA	PFBS
Altura PFAS Column	RT (min)	3.603	5.364	6.461	8.582	9.373	11.055	11.547	12.149
	k'	9.4	14.49	17.65	23.78	26.06	30.92	32.34	34.08
	Width (min)	0.634	1.021	0.918	0.841	0.774	0.514	0.466	0.360
Column 1	RT (min)	2.527	4.856	6.833	8.336	9.294	9.943	10.643	10.764
	k'	6.3	13.02	18.73	23.07	25.83	27.71	29.73	30.08
	Width (min)	0.707	1.019	0.914	0.672	0.686	0.511	0.549	0.449
Column 2	RT (min)	0.930	2.044	2.557	5.206	5.448	6.584	6.733	7.305
	k'	1.69	4.9	6.38	14.03	14.73	18.01	18.44	20.09
	Width (min)	0.579	1.237	1.083	0.527	0.468	0.282	0.268	0.246
Column 3	RT (min)	0.623	0.903	1.624	3.484	4.936	5.987	6.170	6.873
	k'	0.80	1.61	3.69	9.06	13.25	16.29	16.81	18.84
	Width (min)	0.936	0.977	1.257	1.081	0.662	0.202	0.293	0.243

TFA—the first-eluting analyte—experiences the most severe ion suppression due to coelution with polar matrix interferences. Even after partial removal of polar components during sample preparation, the final extracts may still contain substantial amounts of polar coextractives, such as sugars and salts. Because these polar interferences typically elute very early with minimal retention on LC columns, effective chromatographic separation between TFA and these coeluting species is critical for minimizing matrix-induced ion suppression. Consequently, an LC column providing stronger retention for TFA can be highly advantageous for improving matrix effects.

Figure 4 illustrates this effect by comparing TFA responses in baby food extracts analyzed using the different LC columns. When the Altura Poroshell 120 PFAS column was applied, a marked improvement in TFA response was observed, attributable to its superior retention of TFA ($k' = 9.4$). Commercial LC column 1 exhibited the next highest retention for TFA and consequently produced the second-strongest response. In contrast, commercial LC columns 2 and 3 showed the poorest retention for TFA, resulting in either the lowest response (column 3) or coelution with isobaric interferences (column 2).

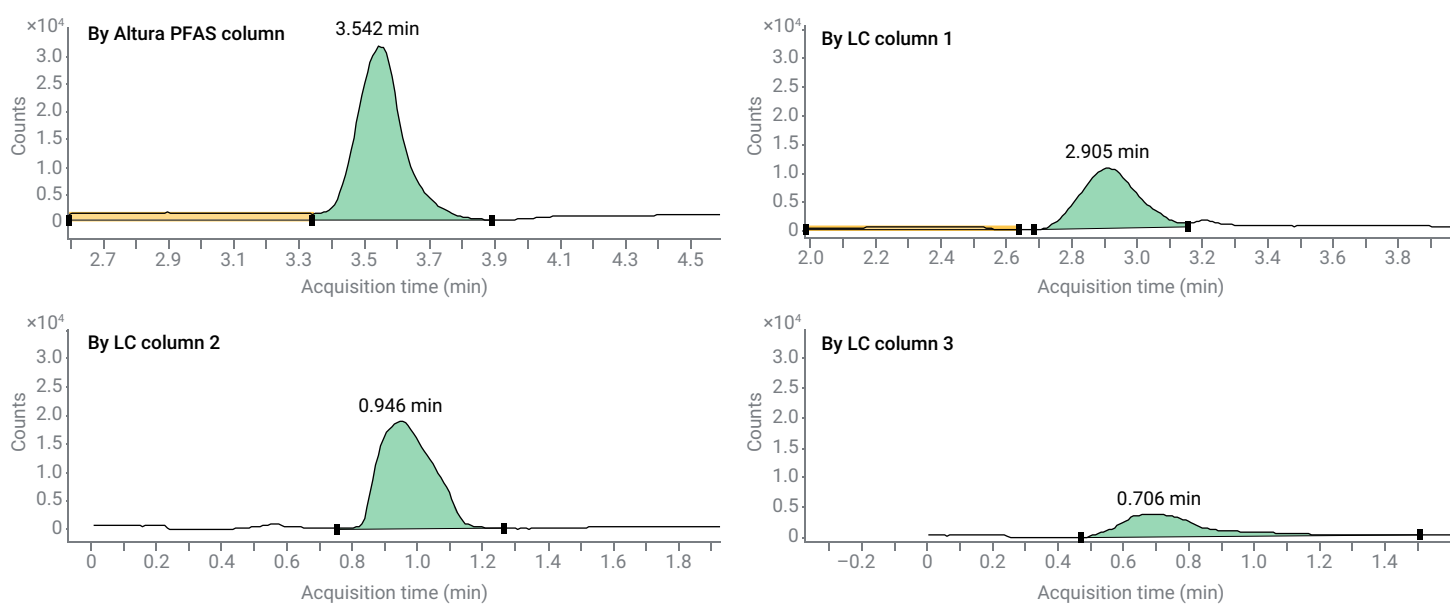


Figure 4. The responses of TFA in baby food extract analyzed by different LC columns.

Separation of analytes with matrix interferences

The stronger retention and more effective separation of analytes from polar matrix interferences not only reduce matrix effects but also improve resolution of isobaric matrix interference peaks. A notable example is PFBA analysis in plant-based food matrices. These matrices commonly contain a strong isobaric interference that elutes within the PFBA acquisition window. On traditional reversed-phase LC columns, this isobaric interference often coelutes with PFBA. Figure 5 compares the performance of the traditional reversed-phase C18 column and the Altura Poroshell 120 PFAS column. Baby food extracts with and without postspiked PFBA were used for evaluation. Because PFBA has only a single MRM transition and lacks a qualifier ion for secondary confirmation, complete coelution results in a high risk of false-positive PFBA detection and a high method LOQ as a result (Figure 5, left). In contrast, the Altura Poroshell 120 PFA column provides sufficient retention and selectivity to fully separate this interference peak from PFBA, ensuring accurate identification and quantitation (Figure 5, right).

In addition, the AOAC SMPR guidance requires secondary confirmation for PFBA and PFPeA due to the absence of suitable qualifier ions for these analytes.² High-resolution LC/MS is typically employed to achieve reliable confirmation. While effective, the high cost of high-resolution LC/MS instrumentation can present a significant barrier for many laboratories. The Altura Poroshell 120 PFAS column offers an orthogonal chromatographic separation for PFBA and PFPeA, providing a promising and more cost-effective alternative for confirmatory identification of these analytes in food matrices. This chromatographic confirmation approach will be further investigated in future studies.

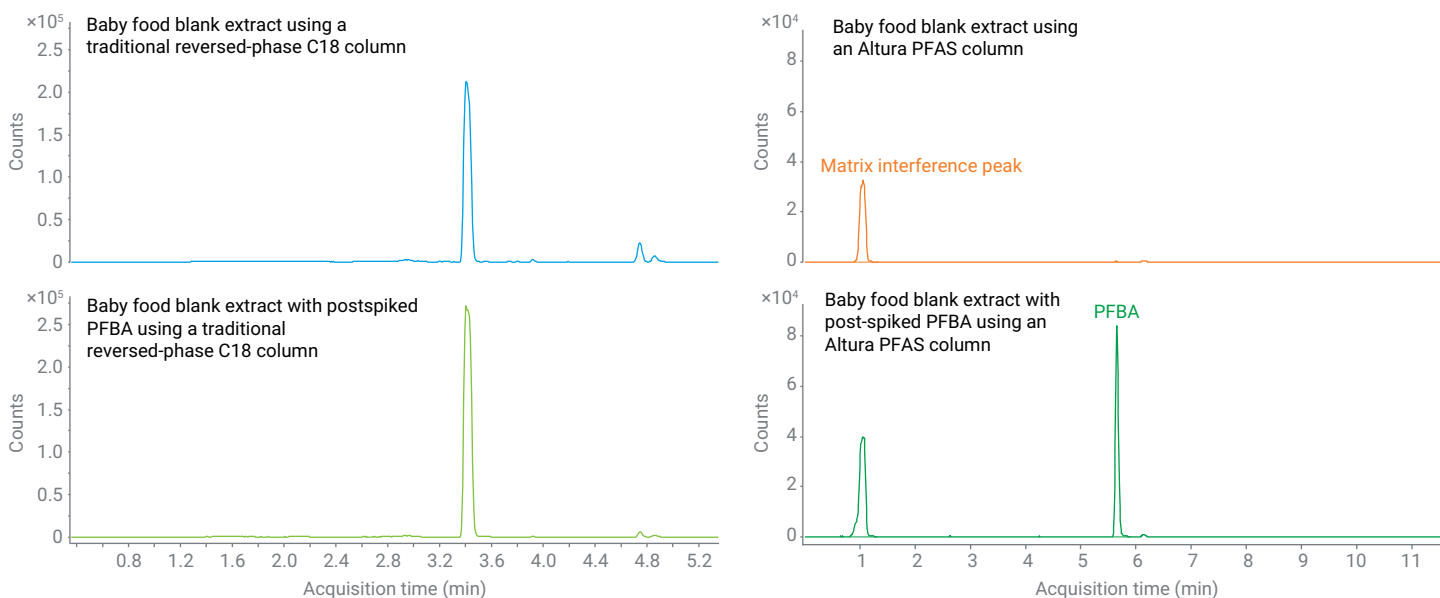


Figure 5. Comparison of performance in separating PFBA from isobaric interference on a traditional reversed-phase C18 (RP-C18) and an Agilent Altura Poroshell 120 PFAS column in baby food extract.

Conclusion

The novel, mixed-mode Agilent Altura Poroshell 120 PFAS column demonstrated substantially stronger retention and superior chromatographic separation for ultrashort-chain (USC) and short-chain (SC) PFAS analytes in food matrix extracts compared with other commercially available mixed-mode LC columns marketed for USC PFAS analysis. In addition, the Altura Poroshell 120 PFAS column provided excellent solvent-effect mitigation, enabling direct injection of high-organic food extracts and offering greater flexibility in LC gradient design and sample injection conditions. These capabilities simplify the sample preparation workflow while maintaining, or even improving, method detection sensitivity and selectivity through more adaptable method optimization.

The enhanced chromatographic performance of the Altura Poroshell 120 PFAS column effectively reduces matrix-induced ion suppression and improves separation of matrix-derived isobaric interferences from target analytes. Collectively, these advantages contribute to more accurate and reliable identification and quantitation of USC and SC PFAS analytes in complex food matrices.

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