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Concurrent Quantitation of Different PFAS Chain Lengths in Water Using a Single Direct Injection LC-MS/MS Method

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Introduction

Per-and polyfluoroalkyl substances (PFAS) are contaminants characterized by their long persistence and omnipresent existence in the environment. In response to the environmental and health concerns associated with long-chain PFAS, manufacturers have increasingly adopted short- and ultrashort-chain alternatives. Initially considered less toxic, common ultrashort-chain PFAS are highly polar, water-soluble, and environmentally persistent. These properties facilitate their buildup in aquatic and plant environments, leading to potential long-term exposure for both wildlife and humans. This is especially true for perfluoroalkyl acids (PFAAs) containing 1–3 perfluorinated carbon atoms (CF_n), such as trifluoroacetic acid (TFA), perfluoropropanoic acid (PFPrA), perfluoromethane sulfonic acid (PFMS), perfluoroethane sulfonic acid (PFEtS), and perfluoropropane sulfonic acid (PFPrS).

TFA has been frequently detected in both aqueous and non-aqueous matrices from sources such as industrial emissions and atmospheric degradation of chemicals like fluorinated gases. Although ultrashort-chain PFAS are not yet regulated, there is a growing awareness due to their potential impact on human health. Limited environmental data exist for ultrashort-chain PFAAs due to the analytical challenges posed by their low molecular weights and high polarity. These compounds are poorly retained on most reversed-phase liquid chromatography (LC) columns and can elute near or within the column's void volume, where the presence of early-eluting co-extractables increases the risk of matrix effects. This technical note presents a direct injection liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for the analysis of ultrashort-, short-, and long-chain PFAAs in tap water, lake water, and rainwater samples. The optimized method utilizes mixed-mode chromatography in both analytical and delay columns to stabilize retention times and achieve effective separation of ultrashort-chain PFAAs.

Sample Preparation

Lake water samples were collected from different regions in Ontario, Canada while tap water and rainwater samples were collected from the Toronto region. Each water sample was diluted 2-fold with Methanol and spiked with isotopically-labelled internal standards at in-vial concentrations of 2–10 ng/L for a final vial composition of Methanol / Water (50:50, v/v). All collected samples did not pass through a filtration step and were directly injected for this method.

LC Conditions

Column: Luna™ Omega 3 um PS C18
Dimensions: 150 x 2.1 mm
Part No.: 00F-4758-AN
Delay Column 1: Luna™ Omega 3 um PS C18
Delay Column Dimensions 50 x 3.0 mm
Part No.: 00B-4758-Y0
Delay Column 2 Biozen™ 2.6 um Glycan
Delay Column Dimensions 100 x 2.1 mm
Part No.: 00D-4773-AN
Mobile Phase: A: 0.1% Acetic Acid (v/v) in Water B: Acetonitrile/ Water with 10 mM Ammonium Acetate (90:10, v/v)

Gradient:

Time (min)	% B
0	2
0.5	2
3	30
7	70
7.5	96
12	96
12.1	2
20	2

Flow Rate: 0.4 mL/min
Injection Volume: 45 uL
Temperature: 45°C
LC System: Shimadzu® Prominence
Detection: MRM
Detector: SCIEX™ 7500

MRM Conditions

Ion Source: Electrospray Ionization
Polarity: Negative
Source Temperature: 380°C
GS1: 40 psi
GS2: 70 psi
CUR: 40 psi
IS: -2000 Voltage

TFA can often be found in reagents, solvent modifiers, and laboratory consumables, which pose a risk of introducing these contaminants, making accurate low-level quantitation challenging. Multiple suppliers of solvents and pipette tips were pre-screened to identify LC-MS grade methanol and water with the lowest PFAS concentrations for use in this study (**Figure 1**). It is imperative that all laboratory consumables — pipette tips, reagents, solid phase extraction (SPE) materials, vials, and caps are pre-screened for a diverse PFAS panel to ensure they are suitable for PFAS analysis.

Column Selection

The best delay column selection was the Luna Omega PS C18 column, 50 x 3 mm, 3 µm (part No. 00B-4758-Y0) and a Biozen Glycan column, 100 x 2.1 mm, 2.6 µm (part No. 00D-4773-AN) in series between the solvent mixer and the autosampler. The Luna Omega PS C18 column, featuring a positively charged surface chemistry with C18 functionalization, enables mixed-mode retention of both acidic and hydrophobic compounds. In contrast, the Biozen Glycan column offers a more polar stationary phase, enhancing selectivity for polar compounds and improving the retention of ultrashort-chain PFAS. The dual delay column setup demonstrated that PFAS from system contamination were delayed, resulting in clear separation from the target PFAS analytes in extracted samples (**Figure 2**).

Figure 1. Pre-screened consumables and solvents with contamination of TFA, PFBA and PFHxA.

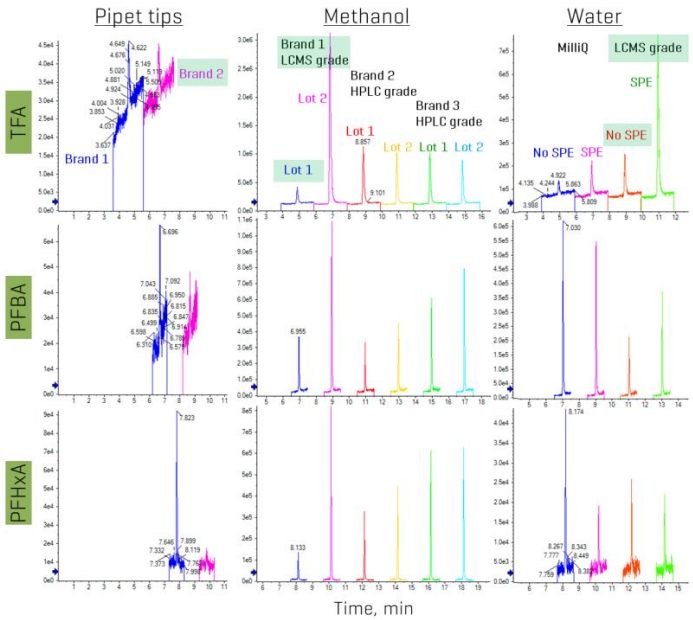
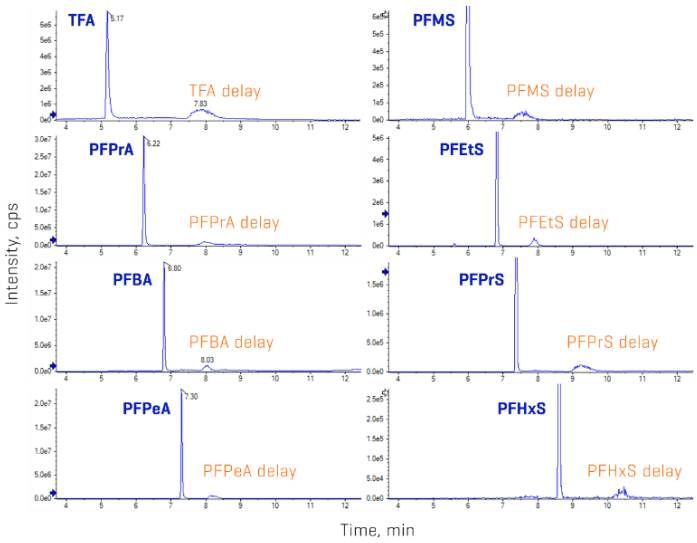


Figure 2. Chromatographic separation (delays) between the target analytes and LC system contamination.



Results and Discussion

To ensure reproducible retention and effective separation, extensive chromatographic optimization was conducted to address the key analytical challenges presented by ultrashort-chain PFAS. The chromatographic optimization included the selection of analytical and delay columns, mobile phase composition, and gradient conditions. Additionally, the incorporation of acetic acid into the mobile phase significantly enhanced the retention of ultrashort-chain PFAAs due to protonation, achieving elution times greater than 4.5 minutes.

In the extracted ion chromatogram (XIC) from Run 1 (top), the initial peaks appearing before 1 minute correspond to unretained ultrashort-chain PFAS under isocratic conditions with 99% organic mobile phase (**Figure 3**). In contrast, Run 2 (bottom) demonstrates that the final gradient conditions achieved effective separation of the ultrashort-chain PFAS from the void region of the analytical column.

Figure 3. Comparative separation of ultrashort-chain PFAS from the void region.

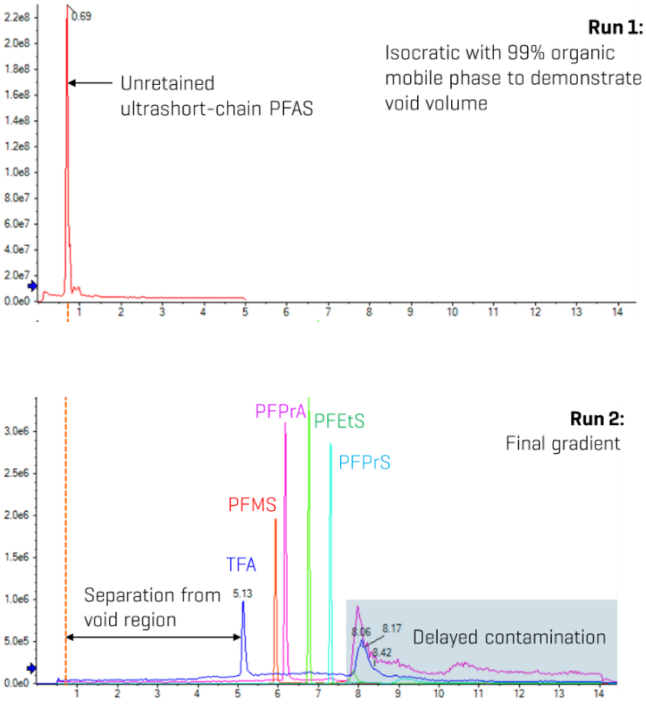


Figure 4 illustrates the full range of target analytes from different PFAS classes and chain lengths. Panel A displays perfluorocarboxylic acids (PFCAs, C1–C17), while panel B displays perfluoroalkane sulfonates (PFSAs, C1–C13) that were monitored. Although perfluorohexadecanoic acid (PFHxDA) and perfluorooctadecanoic acid (PFODA) were not quantified in this study, the gradient conditions are suitable for their analysis. Panels C and D further demonstrate the gradient's capability to achieve chromatographic separation of both routinely monitored PFAS and emerging analytes, including polyfluoroalkyl phosphate diesters (diPAPs) (**Figure 4**).

Figure 4. Chromatographic separation of the target PFAS using the Luna Omega PS C18 column.

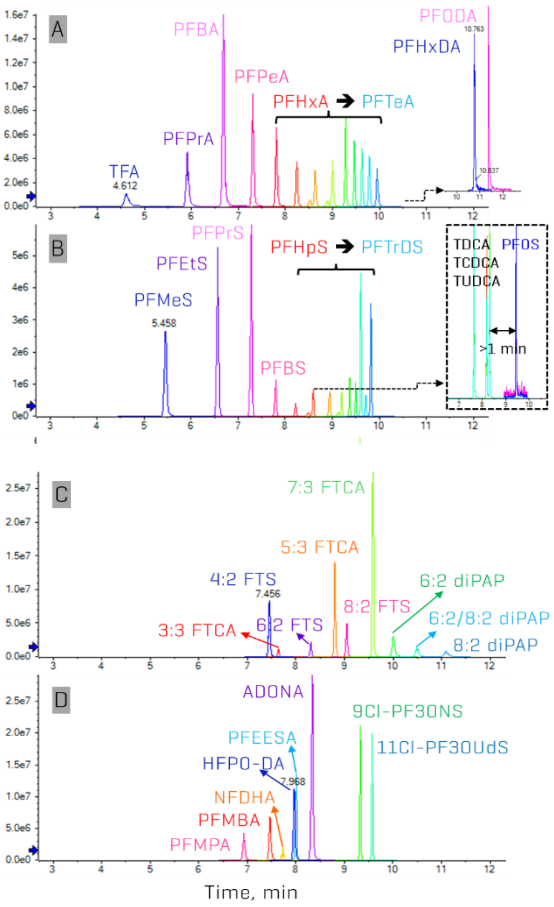


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Figure 5 depicts the blue and green bars that represent the recoveries obtained at a mid (5–500 ng/L) and high (10–5000 ng/L) spike levels in-sample, respectively. Each bar represents the mean recovery of 8–9 replicate spikes with error bars representing the standard error of the mean. The dotted orange lines represent the ±20% tolerance on recovery. The majority of target PFAS compounds had excellent recovery (±20%) and precision (coefficient of variation <20%) at the higher spiking levels, demonstrating solid quantitative performance from this configuration. In the environmental aqueous samples, PFAAs accounted for the majority of the distribution, with TFA being the predominantly measured analyte (**Figure 6**). TFA concentrations ranged from 250 ng/L to over 2000 ng/L, with the highest levels observed in lake and river water. PFMS emerged as the predominant PFAS, detected at sub-to-low nanogram per liter (ng/L) concentrations, ranging from 13 to 18 ng/L in tap water samples (**Figure 6**).

Figure 5. Recoveries in tap water matrix spiked at mid-to-higher levels above the method detection limit (MDL).

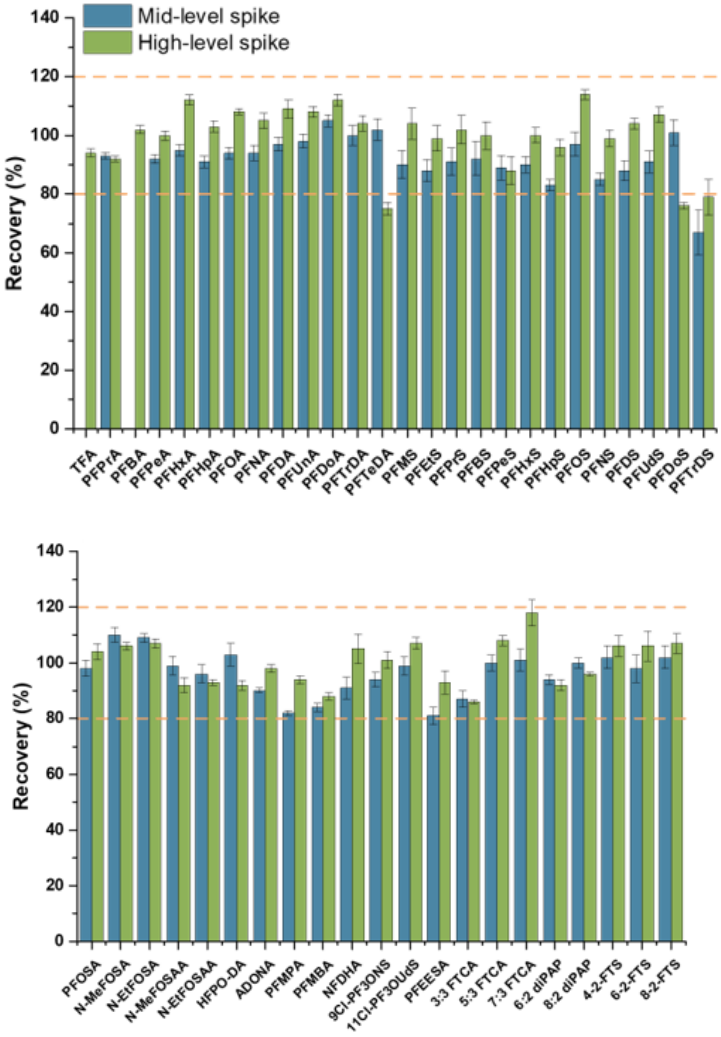

Figure 6. PFAAs distribution and detection in real world aqueous samples.

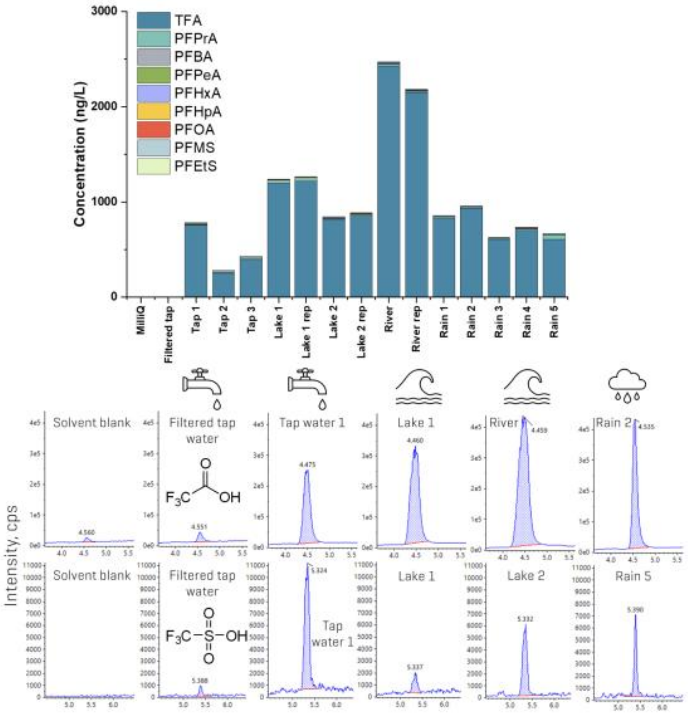

Table 1 presents all analytes listed with their in-sample calibration range (ng/L), linear regression coefficient (r2), accuracy (% CV) and precision at the in-sample limit of quantitation (LOQs) and MDL tap water spikes prepared at 1–5x of LOQ. This demonstrates that the method can meet current sensitivity requirements for common ultrashort-chain PFAS compounds.

Table 1. Quantitative performance of the solvent calibration standards and tap water spikes at the MDL (ng/L).

Compound	In-sample range (ng/L)	r^2	LOQ (In-sample)		Tap water MDL spike (In-sample)			
			Conc (ng/L)	Accuracy (%CV) (n = 3)	MDL spike (ng/L)	x-LOQ	Accuracy (%CV) (n = 8–9)	MDL (ng/L)
TFA	100 - 5000	0.9932	100	89 (10)	500	5	103 (12)	186.6
PFPrA	5 - 20000	0.9986	5	110 (9.2)	10	2	94 (14)	3.9
PFBA	100 - 10000	0.9937	100	81 (3.3)	500	5	99 (4.2)	63.0
PFPeA	0.5 - 5000	0.9966	0.5	92 (8.2)	2.5	5	108 (7.9)	0.6
PFHxA	0.5 - 5000	0.9959	0.5	96 (6.1)	1.25	2.5	110 (25)	1.0
PFHpA	0.5 - 5000	0.9980	0.5	92 (8.5)	1.25	2.5	105 (9.1)	0.4
PFOA	0.5 - 5000	0.9988	0.5	105 (5.4)	1.25	2.5	109 (9.6)	0.4
PFNA	0.5 - 5000	0.9957	0.5	107 (4.7)	1.25	2.5	95 (14)	0.5
PFDA	0.5 - 2500	0.9947	0.5	94 (16.5)	1.25	2.5	95 (9.1)	0.3
PFUnA	0.5 - 2500	0.9901	0.5	91 (21.4)	1.25	2.5	95 (11)	0.4
PFDoA	0.5 - 500	0.9963	0.5	96 (5.9)	2.5	5	94 (13)	0.9
PFTrDA	0.5 - 500	0.9934	0.5	91 (14)	2.5	5	104 (9.8)	0.7
PFTeDA	5 - 500	0.9914	5	81 (15)	12.5	2.5	95 (12)	4.3
PFMS*	0.2 - 10000	0.9932	0.2	94 (10)	-	-	-	-
PFEtS	0.5 - 5000	0.9948	0.5	111 (3.5)	1	2	116 (19)	0.7
PFPrS	0.5 - 10000	0.9956	0.5	92 (16)	2	4	90 (8.6)	0.4
PFBS	1.25 - 5000	0.9956	1.25	98 (9.6)	2.5	2	108 (12)	0.9
PFPeS	1.25 - 5000	0.9956	1.25	109 (5.8)	5	4	105 (17)	2.5
PFHxS	0.5 - 5000	0.9956	0.5	101 (10)	2.5	5	112 (7.8)	0.6
PFHpS	1.25 - 5000	0.9945	1.25	107 (3.9)	5	4	110 (4.8)	0.8
PFOS	1.25 - 2500	0.9943	1.25	103 (4.1)	5	4	97 (13)	1.8
PFNS	1.25 - 5000	0.9975	1.25	102 (13)	5	4	103 (6.7)	1.0
PFDS	1.25 - 5000	0.9955	1.25	102 (10)	5	4	114 (6.5)	1.1
PFUdS	2 - 2000	0.9918	2	95 (9.1)	5	2.5	94 (12)	1.6
PFDoS	2.5 - 500	0.9935	2.5	101 (12)	12.5	5	98 (12)	4.5
PFTrDS	2 - 2000	0.9912	2	111 (7.9)	5	2.5	92 (15)	2.0
4-2-FTS	0.2 - 50	0.9930	0.2	105 (8.9)	0.5	2.5	94 (12)	0.2
6-2-FTS	1 - 50	0.9897	1	98 (17)	5	5	96 (11)	1.6
8-2-FTS	5 - 100	0.9926	5	92 (17)	10	2	102 (12)	3.6
PFOSA	0.5 - 500	0.9978	0.5	111 (12)	1.25	2.5	106 (11)	0.4
N-MeFOSA	5 - 5000	0.9931	5	118 (5.5)	12.5	2.5	111 (6.6)	2.7
N-EtFOSA	5 - 5000	0.9879	5	122 (16)	12.5	2.5	115 (7.5)	3.2
N-MeFOSAA	2.5 - 5000	0.9936	2.5	88 (17)	12.5	5	92 (10)	3.5
N-EtFOSAA	2.5 - 5000	0.9949	2.5	104 (6.5)	12.5	5	92 (8.9)	3.1
HFPO-DA	0.5 - 500	0.9905	0.5	96 (10)	1	2	95 (9.9)	0.3
ADONA	0.2 - 2000	0.9962	0.2	104 (7.3)	0.5	2.5	108 (6.6)	0.1
PFMPA	0.5 - 2500	0.9985	0.5	114 (19)	2.5	5	94 (5.8)	0.4
PFMBA	0.5 - 5000	0.9986	0.5	124 (0.9)	2.5	5	89 (5.9)	0.4
NFDHA	2.5 - 1000	0.9820	2.5	120 (9.9)	2.5	1	117 (9.1)	0.8
9Cl-PF3ONS	0.2 - 2000	0.9945	0.2	112 (9)	0.5	2.5	109 (18)	0.3
11Cl-PF3OUdS	0.5 - 500	0.9924	0.5	92 (12)	2	4	111 (11)	0.7
PFEESA	2.5 - 2500	0.9951	2.5	92 (4.7)	2.5	1	97 (8.5)	0.6
3:3 FTCA	2.5 - 12500	0.9962	2.5	121 (11.7)	12.5	5	92 (6.3)	2.1
5:3 FTCA	12.5 - 12500	0.9975	12.5	113 (4.3)	31.25	2.5	100 (4.7)	4.4
7:3 FTCA*	31.25 - 6250	0.9943	31.25	86 (26.9)	-	-	-	-
6:2 diPAP	10 - 20000	0.9935	10	110 (14.4)	50	5	89 (9.6)	12.8
8:2 diPAP	10 - 20000	0.9991	10	98 (18.7)	50	5	89 (4.5)	6.0

Conclusions

Robust chromatographic separation was achieved across a broad range of PFAS chain lengths using mixed-mode chromatography in the analytical and delay column configuration, allowing for the concurrent analysis of ultrashort-, short-, and long-chain PFAAs. The method demonstrated good quantitative performance (accuracy ±25%, precision <25%) in both standards and spiked tap water. In environmental samples, PFAAs were detected from sub-ng/L to µg/L, with ultrashort-chain compounds like TFA and PFMS being most prevalent.

Ordering Information

Luna™ Omega

1.6 µm Minibore Columns (mm)			SecurityGuard™ ULTRA		Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
PS C18	00A-4752-AN	00B-4752-AN	00D-4752-AN	00F-4752-AN	AJ0-9508
				for ID:	2.1mm ID

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

5 µm Minibore Columns (mm)			SecurityGuard		Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	4 x 2.0* (10/pk)
PS C18	00A-4753-AN	00B-4753-AN	00D-4753-AN	00F-4753-AN	AJ0-7605
				for ID:	2.0 - 3.0 mm

5 µm MidBore™ Columns (mm)			SecurityGuard	Cartridges (mm)
Phases	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0* (10/pk)
PS C18	00B-4753-Y0	00D-4753-Y0	00F-4753-Y0	AJ0-7605
			for ID:	2.0 - 3.0 mm

5 µm Analytical Columns (mm)			SecurityGuard		Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0* (10/pk)
PS C18	00B-4753-E0	00D-4753-E0	00F-4753-E0	00G-4753-E0	AJ0-7606
				for ID:	3.1-8.0 mm

*SecurityGuard Analytical cartridges require holder, Part No.: KJ0-4282

bioZen Columns (mm)			Biocompatible Guard Cartridges	
	100 x 2.1	150 x 2.1	for 2.1 mm	Holder
bioZen 2.6 µm Glycan	00D-4773-AN	00F-4773-AN	AJ0-9800	AJ0-9000

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