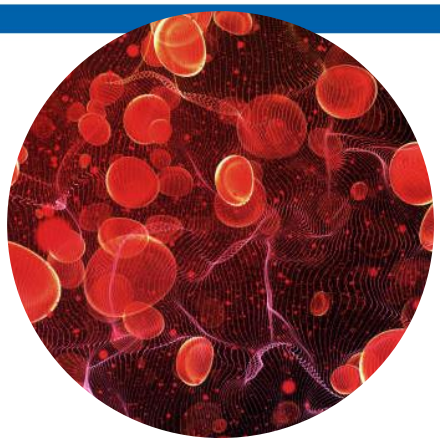


TN-1325

Rapid LC-MS/MS Method for Monitoring Bio-Relevant Levels of Per- and Polyfluoroalkyl Substances (PFAS) in Serum

Pierre Negri, PhD[1], Scott Krepich[1], and Bryan Tackett, PhD[2]
[1]AB SCIEX LLC, 500 Old Connecticut Path, Framingham, MA 01701, USA
[2]Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501, USA

Introduction

Per- and polyfluoroalkyl substances (PFAS) are pervasive compounds used in a variety of industrial applications and found in a wide range of consumer products such as cookware, stain repellant, flame-retardant, and coatings. PFAS are considered environmental factors due to their persistent and bioaccumulating nature. A recent biomonitoring study conducted by the National Health and Nutrition Examination Survey (NHANES) on a nationally representative sample of the U.S. population found that more than 98 % of the people tested had multiple congeners of PFAS present in their bodies.

Bioaccumulation of PFAS in the human body resulting from environmental exposure is a growing public health concern. Recent studies have linked PFAS exposure to adverse health outcomes including childhood health complications, reduction in kidney functions, thyroid disease, hormone suppression, decreased fertility, increased cholesterol levels and diabetes, among others. Given the prevalence and ubiquitous nature of PFAS in the environment and every-day consumer products (including our drinking water supply), there is a critical need to develop quantitative tools capable of accurately and precisely detecting low-levels of PFAS in biological fluids to inform the extent of their bioaccumulation and overall impact on the human body. Close biomonitoring of PFAS levels will help evaluate their toxicity and further understand the health consequences associated with their bioaccumulation over time in exposed human populations.

In this technical note, a quantitative workflow for the analysis of 22 PFAS in serum was developed using a Gemini 3 µm C18 column coupled with the SCIEX® QTRAP® 6500+ System. This targeted screening workflow provides a fast analytical method capable of accurately quantifying sub-nanogram per mL levels of PFAS in the human body.

Sample Preparation

A total of 22 PFAS and 15 mass-labeled internal standards were prepared using Baker's HPLC-grade Methanol. A list of all the PFAS included in this panel is summarized in **Table 1**.

A 1 µg/mL stock standard solution mixture containing the 22 PFAS was prepared by diluting the stock standard solutions with Methanol. The resulting 1 µg/mL stock standard solution mixture was used to spike 50 µL of serum in order to prepare a series of 9 calibrator solutions covering concentrations ranging from 0.01 to 100 ng/mL. A 1 µg/mL stock internal standard solution mixture containing the mass-labeled internal standards was prepared similarly by diluting the stock standard solutions with Methanol. This solution mixture was used to prepared a 5 ng/mL mass-labeled internal standard solution in 0.1 M Formic Acid. High density polyethylene or polypropylene consumables were used to minimize PFAS contamination from external sources.

PFAS were extracted from 50 µL serum samples by using a protein precipitation procedure summarized below:

Step	Description
Load:	Add 50 µL of spiked serum into a 2 mL polypropylene tube.
Denaturation:	Add 100 µL of a 5 ng/mL mass-labeled internal standard solution in 0.1 M Formic Acid. Vortex for 5 seconds.
Precipitation:	Add 450 µL of cold (-20 °C) Acetonitrile to each tube. Vortex for 5 seconds.
Centrifuge:	Centrifuge at 12,500 x g for 5 min at room temperature.
Transfer:	Transfer a 100 µL aliquot of the supernatant into an HPLC polypropylene vial.
Add Buffer:	Add 100 µL of 20 mM Ammonium Acetate buffer (1:1 mixture) to the vial. Vortex for 5 seconds.
Inject:	Inject 10 µL onto instrument.

LC Conditions

Column: Gemini™ 3 µm C18
Dimensions: 50 x 2.0 mm
Part No.: 00B-4439-B0
Delay Column: Luna™ 5 µm C18(2)
Delay Column Dimensions: 30 x 3.0 mm
Delay Column Part No.: 00A-4252-Y0
Mobile Phase: A: 20 mM Ammonium Acetate in Water
B: 0.1 % Formic Acid in Methanol

Gradient:

Time (min)	%B
0	10
1	55
4.5	99
4.95	99
5	10
6	10

Flow Rate: 0.6 mL/min
Injection Volume: 10 µL
Temperature: 25 °C
LC System: SCIEX ExionLC™
Detection: MRM
Detector: SCIEX QTRAP 6500+, with IonDrive™ Turbo V™ Ion Source, Negative

In addition, the fluoroethylene and Teflon tubing on the ExionLC AC System pumps and degasser was replaced with PEEK tubing to minimized the impact of PFAS background contamination and leaching. The FEB and PTFE tubings from the rinse solvent lines, the needle seal, the sample holding loop, pump seal, pump lining and degasser unit were also replaced with PEEK tubing to avoid system-related interferences. Additionally, the PTFE frits and rotor seals were replaced with stainless-steel parts.

Revision: 0
PHEN-RUO-00187

Results and Discussion

PFAS are pervasive and persistent compounds that have the potential to accumulate and contaminate the LC systems used in analytical testing. As a result, measures were implemented to reduce the risk of outside, ambient, and system-related PFAS contamination. The most critical measure was the inclusion of a delay column between the autosampler and LC pumps to trap ambient and system-related PFAS and ensure they will be retained away from the sample signals.

Figure 1 summarizes the chromatographic response following the addition of the delay column and shows the extracted ion chromatograms (XICs) for PFHpA (top row) and PFHxS (bottom row) before and after the hardware modifications were made on the LC system.

Figure 1A shows the background signal resulting from a blank sample injection before the system modifications were made. Sharp PFAS peaks resulting from the ambient LC system contamination were observed throughout the chromatograms, including at the retention times at which the sample peaks were expected.

Figure 1B shows the background signal of the same blank sample injection after the system modifications were made (including the addition of the Luna™ C18(2) delay column). This configuration eliminated the PFAS interference peaks at the expected analyte retention times and produced a much broader and delayed contaminant peak caused by the system-related PFAS that were held up by the Luna C18(2) delay column.

Figure 1C shows the XICs resulting from a 10 ng/mL injection of a sample containing all the PFAS in the panel with the system modification. The XICs show a sharp peak resulting from the PFAS in the samples followed by the same broad and delayed contamination peak shown in **Figure 1B**. The addition of the delay column and the modifications made to the LC system components together minimized the impact of system-related PFAS contamination and ensured the analytical integrity of this quantitative workflow.

Chromatographic separation of PFAS, including the two compounds that contain both branched and linear isotopes (PFOS and PFHxS), is critical to ensure reliable and accurate quantification. **Figure 2** shows the chromatographic profile on an injection of the neat, 10 ng/mL standard solution containing the 22 PFAS onto a Gemini™ 5 µm C18 column. The choice of column, gradient, and optimized mobile phase composition resulted in the baseline separation that was needed to correctly distinguish all isomers. As seen in **Figure 2**, the delayed contamination peaks caused by the delayed column did not interfere with the PFAS sample peaks. Blank serum samples were spiked with the standard mixture containing the 22 PFAS at concentrations ranging from 0.01 to 100 ng/mL. These standard solutions were extracted using the protein precipitation procedure and injected in triplicate. Data were processed in the Analytics module in SCIEX® OS Software 2.0 using the MQ4 Algorithm.

Calibration curves were generated for each of the PFAS in the panel and plotted across 9 calibration levels ranging from 0.1 to 100 ng/mL to evaluate the linearity of the method for the serum-spiked control samples. **Figure 3** shows the resulting calibration curves for the native linear perfluoroalkylsulfonates (A) and the native linear perfluoroalkyl carboxylic acids (B). These calibration curves demonstrated excellent linearity covering 3 orders of magnitude with R^2 greater than 0.99 for all of the PFAS in the panel with the exception of PFODA. The system modifications implemented in this method were critical in attaining linearity achieved in this workflow.

The ability to accurately detect low levels of PFAS extracted from serum samples is critical to inform the extent of their accumulation in the human body. The series of calibrators was injected to evaluate the ability to quantify PFAS across a wide range of concentrations. **Figure 4** shows representative extracted ion chromatograms (XIC) for the two MRM transitions monitored for PFHxS and PFOA, two PFAS commonly measured in the exposed population. Five levels of calibrators were used to determine the ion ratio criteria for the quantifier and qualifier ions of PFHxS and PFOA. The series of XIC traces for the two compounds showed a high level of consistency and precision, as evidenced by the acceptance criteria (20% or less) of all the ion ratios across the calibration series ranging from 0.5 (LLOQ) to 100 ng/mL. However, the majority of the PFAS showed detectable signal below the LLOQ. **Figure 5** shows the XICs for PFHxS for the matrix blank (left), at 0.1 ng/mL (middle) and 0.5 ng/mL (right). The signal at 0.1 ng/mL is the limit of detection (LOD) for PFHxS as it is well above the blank signal. Similar peaks were observed below the LLOQ for the PFAS in the panel. In addition, the assay showed great reproducibility over the course of the two consecutive days the data were acquired (inter-day peak area variations of 5% or less were observed for the 22 PFAS across the calibration range).

The lower limit of quantification (LLOQ) for each of the PFAS included in the panel was determined based on the lowest concentration at which the integrated peak area of the analyte was quantifiable, with calculated concentration accuracy between 80 and 120 %, precision (% bias) below 20 %, ion ratio acceptance criteria of less than 20 % and maintaining a linear calibration curve with an R^2 value of at least 0.99.

Table 1 summarizes the quantitative performance of the workflow and includes the calibration range, linear correlation coefficient (R^2 value), LLOQ, as well as the accuracy and precision values at the LLOQ for each of the 22 PFAS monitored in this workflow. PFBS and PFODA are the only two PFAS in the panel that proved challenging to quantify accurately due to their ubiquitous presence in the analytical system. This was evidenced by their detection in blank and solvent injections and as a result, their pervasiveness impacted their detection limits in this workflow. PFBS was quantified accurately from 5 to 100 ng/mL however PFODA could not be quantified.

The assay as a whole demonstrated excellent reproducibility, linearity, precision, and accuracy for all the other PFAS in the panel. This method achieved the required levels of robustness and qualitative performance necessary to accurately measure sub ng/mL levels of PFAS from serum samples.

Table 1. Statistical Results for the 22 PFAS Monitored in the Workflow..

Compound	Calibraiton Range (ng/mL)	Linear Correlation (R^2)	LLOQ (ng/mL)	Accuracy at LLOQ (%)	Precision at LLOQ (%)
PFBS 1	0.5 – 100	0.99876	0.5	101.70	10.16
PFPeA1	0.1 – 100	0.99896	0.1	99.41	12.21
PFBS 1	5 – 100	0.99647	5	99.55	5.51
PFBS 2	5 – 100	0.99689	5	101.50	3.68
PFHxA 1	0.5 – 100	0.99753	0.5	109.93	19.61
PFHxA 2	0.5 – 100	0.99791	0.5	102.34	11.19
PFPeS 1	0.5 – 100	0.99793	0.5	90.54	8.97
PFPeS 2	0.5 – 100	0.99846	0.5	106.62	6.88
PFHpA 1	0.5 – 100	0.99331	0.5	114.43	6.60
PFHpA 2	0.5 – 100	0.99685	0.5	111.71	6.76
PFHxS 1	0.5 – 100	0.99780	0.5	84.15	5.96
PFHxS 2	0.5 – 100	0.99741	0.5	80.98	1.33
PFOA 1	0.5 – 200	0.99885	0.5	96.41	10.31
PFOA 2	0.5 – 200	0.99570	0.5	104.74	3.12
PFHpS 1	0.5 – 100	0.99359	0.5	116.49	1.59
PFHpS 2	0.5 – 100	0.99386	0.5	115.21	3.58
PFNA 1	0.5 – 100	0.99151	0.5	110.17	0.88
PFNA 2	0.5 – 100	0.99237	0.5	99.73	9.95
FOSA 1	0.5 – 100	0.99702	0.5	89.92	1.35
PFOS 1	0.5 – 100	0.99294	0.5	104.67	16.54
PFOS 2	0.5 – 100	0.99932	0.5	108.33	14.24
PFDA 1	0.5 – 100	0.99557	0.5	100.17	7.09
PFDA 2	0.5 – 100	0.99002	0.5	91.69	2.00
PFNS 1	0.5 – 100	0.99214	0.5	101.09	11.33
PFNS 2	0.5 – 100	0.99721	0.5	99.06	15.41
PFUdA 1	0.5 – 100	0.99835	0.5	94.08	9.71
PFUdA 2	0.5 – 100	0.99524	0.5	107.37	3.33
PFDS 1	0.5 – 100	0.99477	0.5	94.23	1.89
PFDS 2	0.5 – 100	0.99651	0.5	111.42	0.23
PFDoA 1	0.5 – 100	0.99634	0.5	107.00	3.59
PFDoA 2	0.5 – 100	0.99950	0.5	99.49	16.56
PFTrDA 1	0.5 – 100	0.99732	0.5	93.24	3.47
PFTrDA 1	0.5 – 100	0.99495	0.5	102.89	16.07
PFTeDA 1	0.5 – 100	0.99761	0.5	96.96	19.47
PFTeDA 2	1 – 100	0.99443	1	90.17	11.51
PFHxDA 1	0.5 – 100	0.99465	0.5	111.05	2.38
PFHxDA 2	10 – 100	0.99481	1	95.32	19.95
PFODA 1	N/A	N/A	N/A	N/A	N/A
PFODA 2	N/A	N/A	N/A	N/A	N/A
PFDoS 1	1 – 100	0.99263	1	96.55	7.40
PFDoS 2	5 – 100	0.99680	5	100.08	16.05

Figure 1. Benefits of Using a Luna™ Omega C18(2) as a Delay Column for PFAS Analysis.

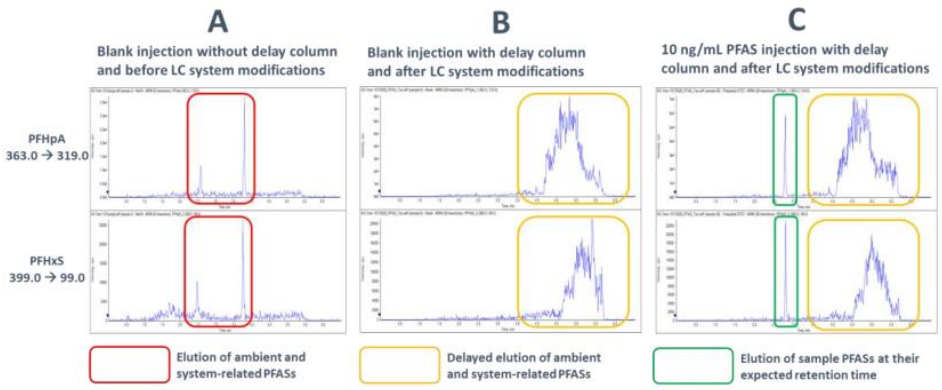


Figure 2. Chromatographic Profile Using a Gemini™ C18 Column of the 22 PFAS Monitored in this Study.

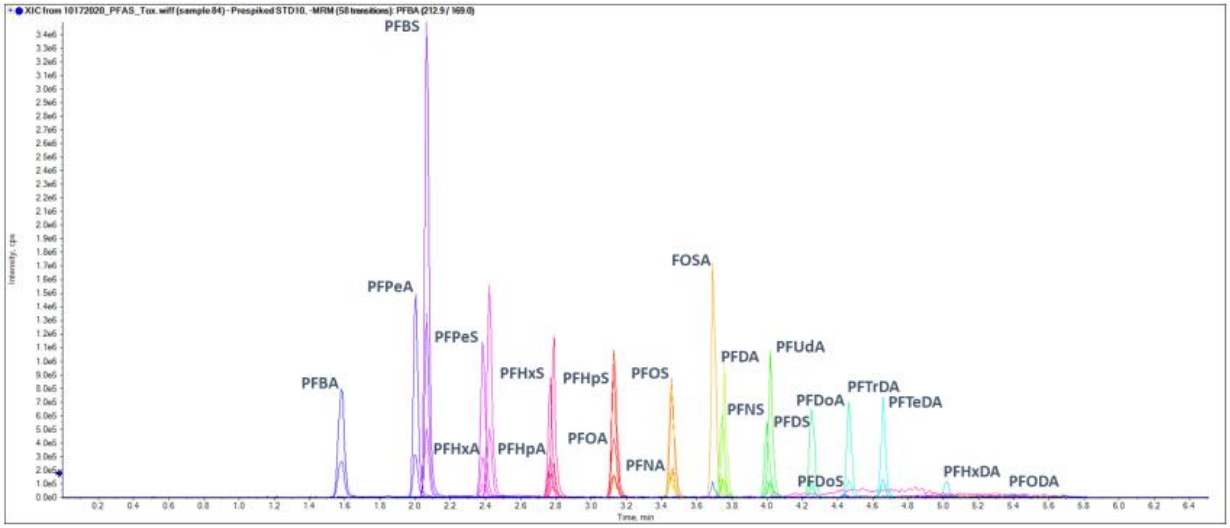


Figure 3. Excellent Linearity for the 22 PFAS.

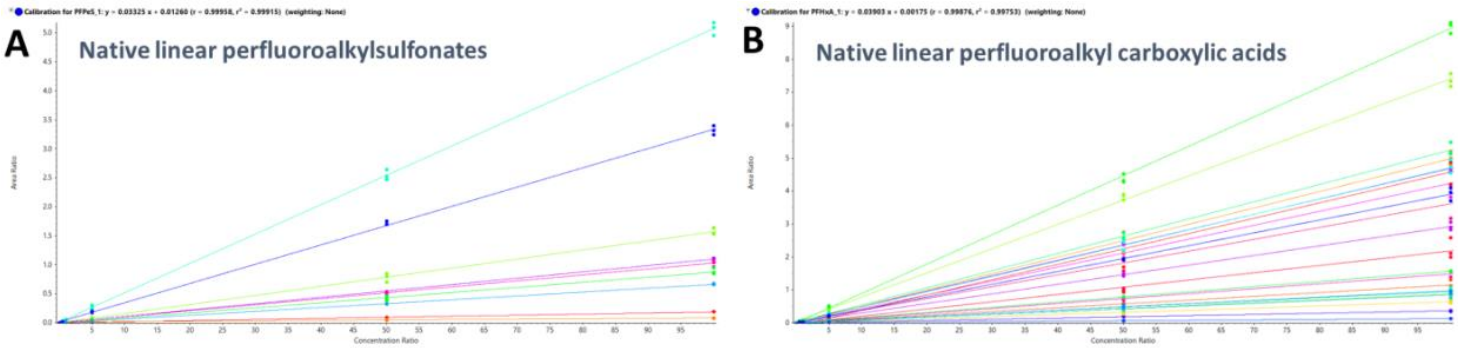


Figure 4. Accurate Quantification of Bioaccumulating PFAS in Serum.

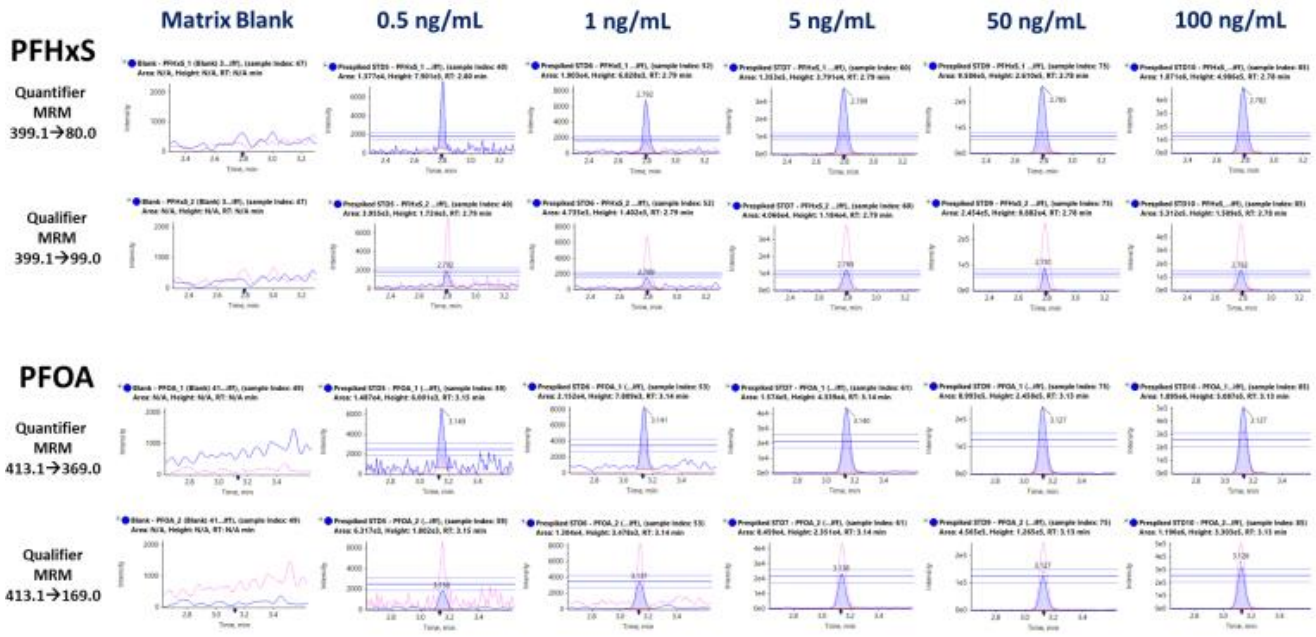
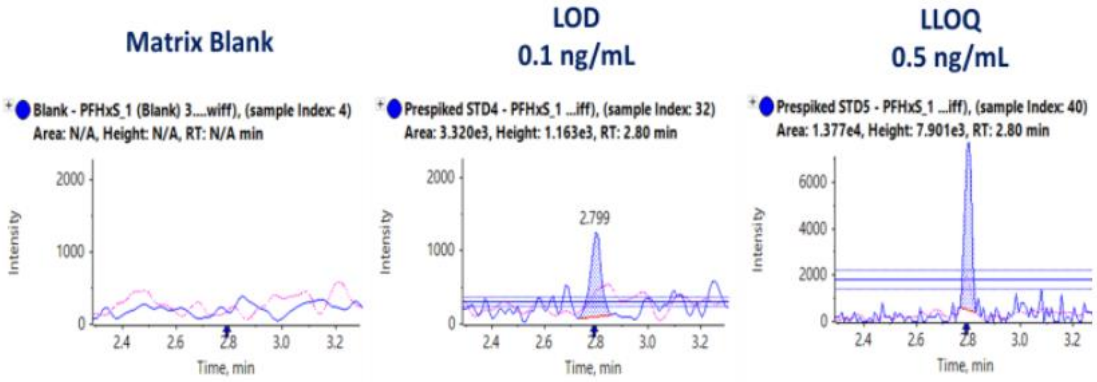


Figure 2. Accurate and Sensitive Detection of PFAS Extracted from Serum Samples.



Conclusion

A robust and sensitive workflow for the detection of PFAS in serum samples using the SCIEX QTRAP 6500+ System was successfully developed. The addition of a delay column and the modifications made to the LC system components reduced the risk of system-related PFA interferences. The combination of a simple sample preparation procedure with a fast LC separation enabled accurate and sensitive detection of 22 PFAS down to sub ng/mL levels. The assay showed excellent reproducibility, precision, accuracy ,and linearity, with an LLOQ of 0.5 ng/mL, LOD of 0.1 ng/mL, and an R^2 of greater than 0.99 for the vast majority of the PFAS in the panel with the exception of PFBS and PFODA. The excellent precision and accuracy observed at the LLOQ is highlighted in Table 1.

Overall, the developed method provides a robust and accurate method for bio-monitoring of low-levels of PFAS in biological fluids. Therefore, the presented workflow is readily adaptable for high-throughput toxicology investigations aimed at determining the extent of PFAS bio-accumulation and its broader impact on human health.

Gemini™ Ordering Information

3 µm Microbore, Minibore and MidBore™ Columns (mm)										SecurityGuard™ Cartridges (mm)
Phases	**50 x 1.0**	**20 x 2.0**	**30 x 2.0**	**50 x 2.0**	**100 x 2.0**	**150 x 2.0**	**50 x 3.0**	**100 x 3.0**	**150 x 3.0**	**4 x 2.0*/10pk**
C18	00B-4439-A0	00M-4439-B0	00A-4439-B0	00B-4439-B0	00D-4439-B0	00F-4439-B0	00B-4439-Y0	00D-4439-Y0	00F-4439-Y0	AJ0-7596
C6-Phenyl	—	—	—	00B-4443-B0	00D-4443-B0	00F-4443-B0	00B-4443-Y0	00D-4443-Y0	00F-4443-Y0	AJ0-7914
NX-C18	00B-4453-A0	00M-4453-B0	00A-4453-B0	00B-4453-B0	00D-4453-B0	00F-4453-B0	00B-4453-Y0	00D-4453-Y0	00F-4453-Y0	AJ0-8367

for ID: 2.0 – 3.0 mm

Luna™ Ordering Information

5 µm MidBore and Analytical Columns (mm)								SecurityGuard Cartridges (mm)	
Phases	**30 x 3.0**	**50 x 3.0**	**150 x 3.0**	**250 x 3.0**	**30 x 4.6**	**50 x 4.6**	**75 x 4.6**	**4 x 2.0***	**4 x 3.0***
								/10pk	**/10pk**
Silica(2)	—	—	—	—	—	00B-4274-E0	—	AJ0-4347	AJ0-4348
C5	—	—	00F-4043-Y0	—	—	00B-4043-E0	—	AJ0-4292	AJ0-4293
C8(2)	—	00B-4249-Y0	00F-4249-Y0	00G-4249-Y0	00A-4249-E0	00B-4249-E0	00C-4249-E0	AJ0-4289	AJ0-4290
C18(2)	00A-4252-Y0	00B-4252-Y0	00F-4252-Y0	00G-4252-Y0	00A-4252-E0	00B-4252-E0	00C-4252-E0	AJ0-4286	AJ0-4287
CN	—	00B-4255-Y0	00F-4255-Y0	00G-4255-Y0	00A-4255-E0	00B-4255-E0	00C-4255-E0	AJ0-4304	AJ0-4305
Phenyl-Hexyl	—	00B-4257-Y0	00F-4257-Y0	00G-4257-Y0	00A-4257-E0	00B-4257-E0	—	AJ0-4350	AJ0-4351
NH2	—	00B-4378-Y0	00F-4378-Y0	00G-4378-Y0	—	00B-4378-E0	—	AJ0-4301	AJ0-4302
SCX	—	—	00F-4398-Y0	—	—	00B-4398-E0	—	AJ0-4307	AJ0-4308
HILIC	—	—	00F-4450-Y0	—	—	—	—	AJ0-8328	AJ0-8329
PFP(2)	—	—	00F4448-Y0	—	—	00B-4448-E0	—	AJ0-8326	AJ0-8327

for ID: 2.0 – 3.0 mm 3.2 – 8.0 mm

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

PFAS CRM Native Standards. All analytes at the same concentration in acid form for easy calculation and dilution.

Product	Part	Volume	Concentration
EPA 533 mix	AL0-101838	1 mL	2 µg/mL in Methanol
EPA 537.1 mix	AL0-101839	1mL	2 µg/mL in Methanol
EPA 533 + 537.1 mix	AL0-101840	1 mL	2 µg/mL in Methanol

Custom CRMs available. Contact Phenomenex for details.

Other Recommended Products for Your PFAS Methods

Description	Part No.
Luna™ Omega Column 3 µm PS C18 50 x 3 mm	00B-4758-Y0
Kinetex™ EVO Column 5 µm C18 100 x 2.1 mm	00D-4633-AN
Strata™ PFAS (WAX/GCB) SPE 200 mg, /50 mg, /6mL tubes, 30/pk	CS0-9207
Strata SDB-L 500 mg/6mL tubes, 30/pk	8B-S014-HCH
Verex™ Vial, 9 mm Screw, PP, 1.7 mL, 1000/pk	AR0-39P0-13
Verex Vial, 9 mm Screw, PP, 300 µL, 1000/pk	AR0-39P2-13
Verex Vial, 9 mm Screw, PP, 700 µL, 1000/pk	AR0-39P1-13
Vial Cap Verex Cert+ Cap (one piece), 9 mm, PE w/ Starburst pre-Slit, 2mL, 1000/pk	AR0-89P6-13-C

Columns and vials available in multiple sizes. Contact Phenomenex for details.

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic
t: +420 272 017 077
cz-info@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia
t: +62 21 5019 9707
indoinfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Japan
t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 6559 4364
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

All other countries/regions
Corporate Office USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com