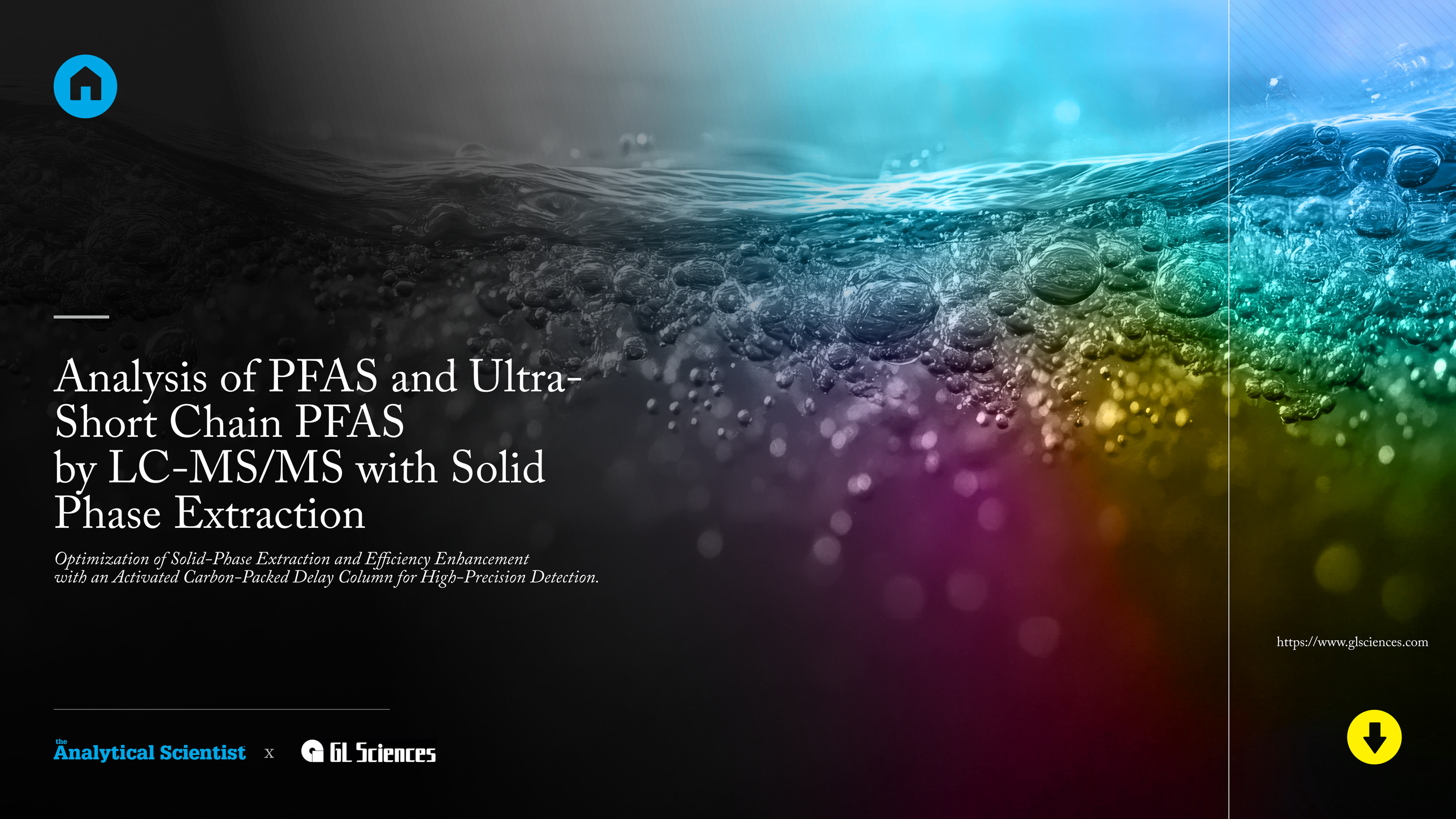


Analysis of PFAS and Ultra-Short Chain PFAS by LC-MS/MS with Solid Phase Extraction

Optimization of Solid-Phase Extraction and Efficiency Enhancement with an Activated Carbon-Packed Delay Column for High-Precision Detection.

https://www.glsciences.com

the Analytical Scientist x GL Sciences

Selecting SPE Cartridges for PFAS Analysis

When analyzing PFAS (Per- and Polyfluoroalkyl Substances) in water samples, choosing the appropriate solid-phase extraction (SPE) cartridge as a sample preparation step is critical for achieving optimal results. Commonly used SPE cartridges for this purpose include InertSep PLS-2, InertSep WAX FF, and InertSep MA-2. Each cartridge offers specific functionalities tailored to the nature of the target analytes and the sample matrix.

1. InertSep PLS-2

- Material: Styrene Divinylbenzene Polymer
- Function: Primarily used for reversed-phase extraction. This cartridge is designed to handle hydrophobic compounds, making it suitable for PFAS analytes with non-polar characteristics.

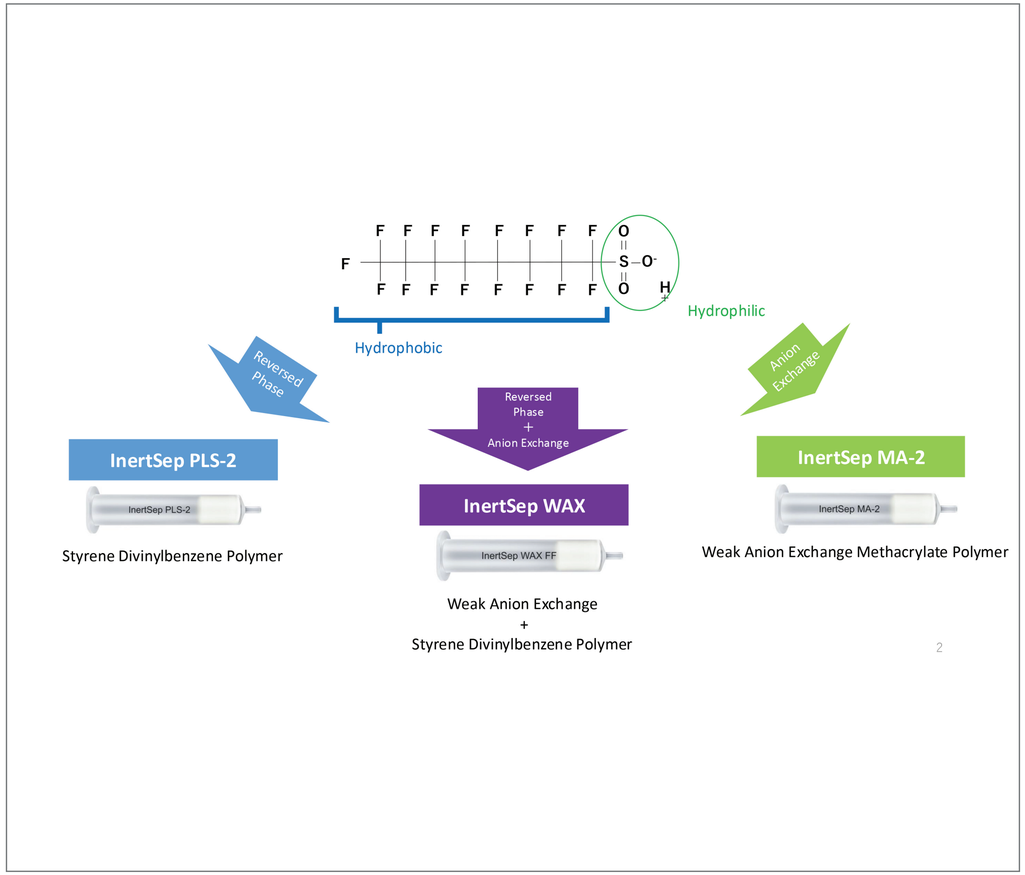
2. InertSep WAX FF

- Material: Weak anion exchange styrene-divinylbenzene polymer
- Function: A weak anion exchange cartridge designed for the extraction of anionic PFAS species. It effectively targets PFAS compounds by combining hydrophobic and ion exchange modes.

3. InertSep MA-2

- Material: Weak anion exchange methacrylate polymer
- Function: Operates in a pure ion exchange mode without reversed-phase characteristics. This unique feature allows selective retention of PFAS without trapping hydrophobic contaminants, resulting in superior cleanup efficiency. It also minimizes ion suppression in LC-MS/MS, ensuring highly accurate analysis.

These cartridges can be selected based on the specific requirements of EPA methods, such as Method 533 for anion exchange and Method 537.1 for reversed-phase extraction.



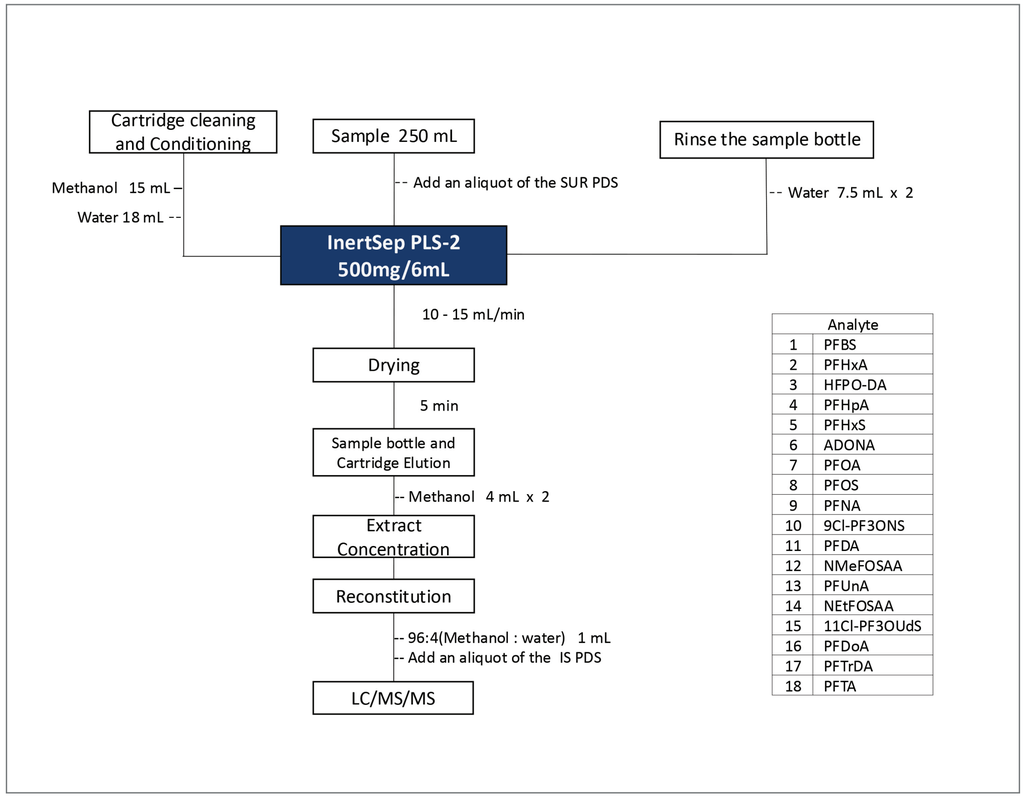
SPE Process for PFAS Analysis (EPA Method 537.1)

In PFAS analysis based on EPA Method 537.1, the solid-phase extraction (SPE) procedure begins with the preparation of a 250 mL water sample. The SPE cartridge (InertSep PLS-2) is conditioned with 15 mL of methanol and 18 mL of water. The prepared water sample is passed through the SPE cartridge at a flow rate of 10–15 mL/min, allowing PFAS compounds to be retained by the sorbent material in the cartridge. After sample loading, any residual water is removed.

The PFAS compounds are eluted in two steps using 4 mL of methanol each time. The resulting extract is concentrated and reconstituted in 1 mL of a 96:4 methanol-to-water solution. At this stage, an internal standard (IS) and surrogate recovery standard (SUR) are added to ensure accuracy and reliability. Finally, the prepared extract is analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS), providing detailed data on the PFAS compounds present in the sample.

Advantages of Using InertSep PLS-2

- High Retention for Hydrophobic PFAS: Its polymer structure ensures strong retention of hydrophobic PFAS compounds, enabling efficient extraction.
- EPA Method 537.1 Compatibility: Designed specifically for reverse-phase extraction under this method.
- Improved Recovery Rates: Enhances recovery for various PFAS analytes, ensuring reliable results.
- Excellent Permeability: The optimized frit and particle size distribution of the packing material ensure high sample permeability.



	Analyte
1	PFBS
2	PFHxA
3	HFPO-DA
4	PFHpA
5	PFHxS
6	ADONA
7	PFOA
8	PFOS
9	PFNA
10	9Cl-PF3ONS
11	PFDA
12	NMeFOSAA
13	PFUnA
14	NEtFOSAA
15	11Cl-PF3OUdS
16	PFDoA
17	PFTrDA
18	PFTA

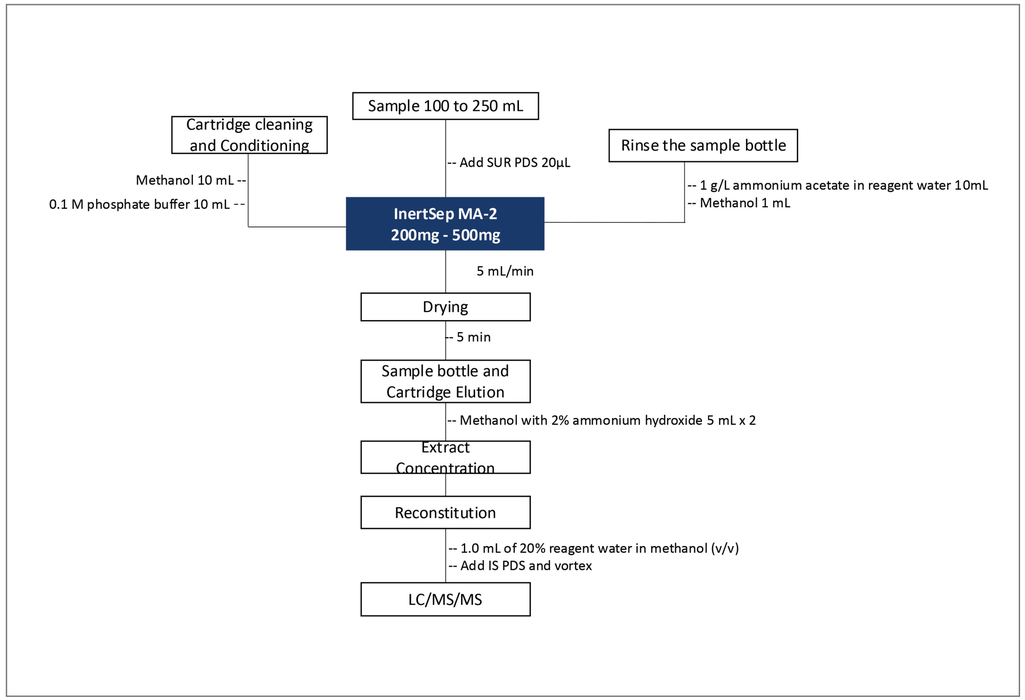
SPE Process for PFAS Analysis (EPA Method 533)

The solid-phase extraction (SPE) procedure for PFAS compounds in EPA Method 533 typically begins with the preparation of a 100–250 mL water sample. The SPE cartridge is conditioned with 10 mL of methanol followed by 10 mL of 0.1 M phosphate buffer. The sample is passed through the cartridge at a flow rate of 5 mL/min to allow interaction between the PFAS compounds and the sorbent material.

After drying the cartridge to remove residual water, PFAS compounds are eluted using two portions of 5 mL methanol containing 2% ammonium hydroxide. The eluate is concentrated and reconstituted in 1.0 mL of a solution containing 20% reagent water in methanol. IS and SUR are added to ensure precision. The sample is then processed with LC-MS/MS.

Advantages of Using InertSep MA-2

- The InertSep MA-2 cartridge, composed of methacrylate polymer, offers specific advantages in the anion exchange SPE process for PFAS analysis
- Enhanced Retention of Anionic PFAS: The methacrylate polymer base of InertSep MA-2, which lacks a reversed-phase mode, effectively retains only anionic PFAS compounds.
  It enables cleanup of hydrophobic contaminants even in complex matrices.
- High Compatibility with EPA Method 533: Offers reliable recovery rates for a wide range of PFAS compounds.



Advanced Techniques for Complex Matrices (EPA Method 1633)

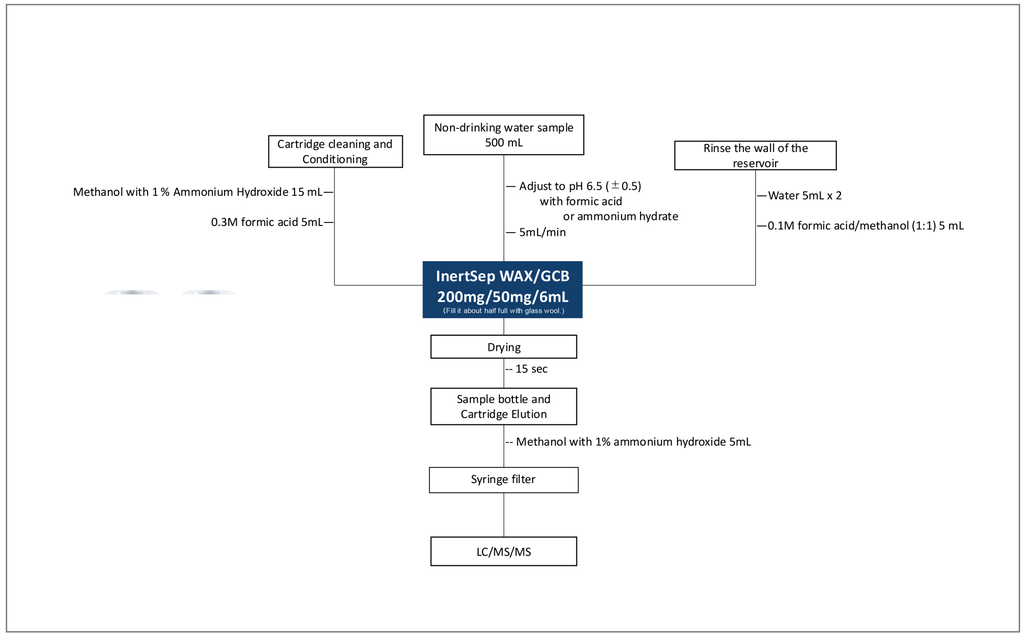
EPA Method 1633 targets diverse sample types, including aqueous, solid, biosolid, and tissue samples. The procedure involves using a dual-layer SPE cartridge, such as InertSep WAX/GCB, for efficient PFAS extraction.

Key steps:

- Sample Preparation: Prepare 500 mL non-potable water samples.
- Cartridge Conditioning: Activate the dual-layer SPE cartridge with methanol containing 1% ammonium hydroxide.
- Sample Loading: Pass the sample at a flow rate of 5 mL/min.
- Cartridge Washing: Use water and a methanol/formic acid solution to remove impurities.
- Elution: Recover PFAS compounds with methanol containing 1% ammonium hydroxide.
- pH Adjustment: Adjust the eluate to pH 6.5 (±0.5) before LC-MS/MS analysis.

InertSep WAX/GCB Dual-Layer Cartridge Benefits

- Wide PFAS Retention: Combines weak anion exchange and graphitized carbon black to target a broader range of PFAS.
- Minimal Contamination: Efficient washing and elution reduce contamination risk.
- Enhanced Accuracy: Provides clean extracts and reliable LC-MS/MS results.



SPE Elution Profile

The solid-phase extraction (SPE) procedure using the InertSep MA-2 cartridge for PFAS analysis in water samples.

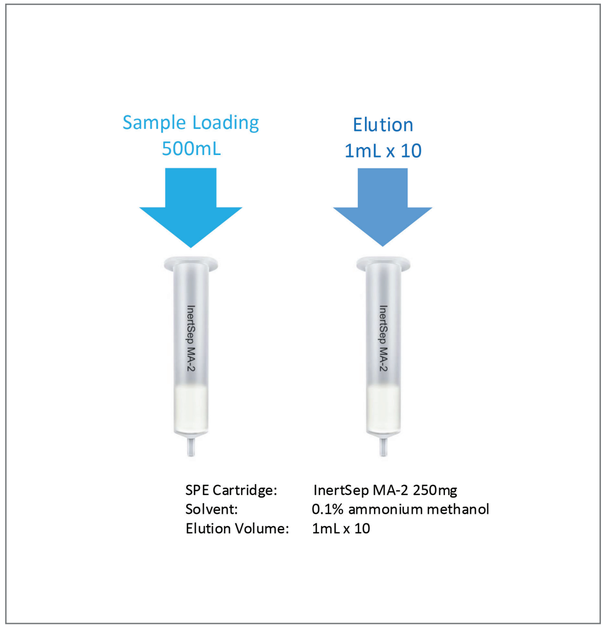
The process involves loading a 500 mL water sample, containing 21 different PFAS compounds, onto the MA-2 cartridge.

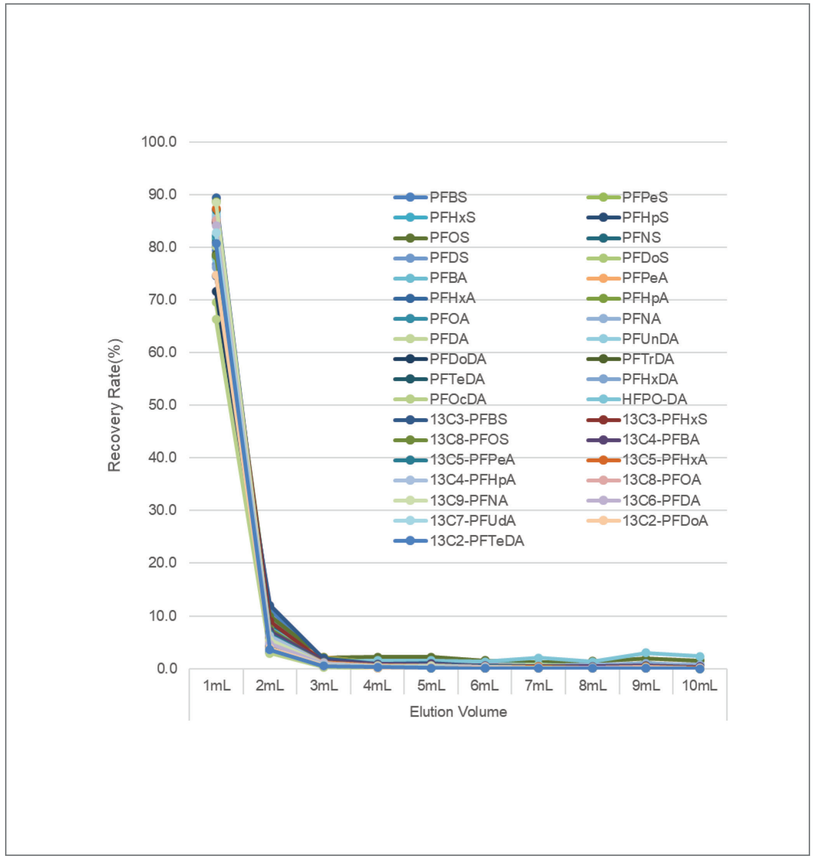
Elution is carried out using 1 mL aliquots of 0.1% ammonium methanol, with a total of 10 elution steps.

The recovery rate for each elution volume is measured to assess the efficiency of the extraction process.

One of the key advantages of the InertSep MA-2 cartridge is its pure ion-exchange mode, which excludes any reversed-phase behavior.

This allows for efficient and effective elution of PFAS compounds, even those with high carbon content, such as PFOxDA (C18), using a small amount of solvent.





SPE Procedure Comparison

The rapid SPE procedure for PFAS analysis involves processing a 30 mL water sample.

The process starts with conditioning the InertSep MA-2 cartridge (150 mg/3 mL) at a flow rate of 5 mL/min. The PFAS compounds are then eluted using 0.1% ammonium hydroxide (NH4OH) in methanol.

The procedure does not require any pH adjustment. After elution, the sample is analyzed using LC-MS/MS.

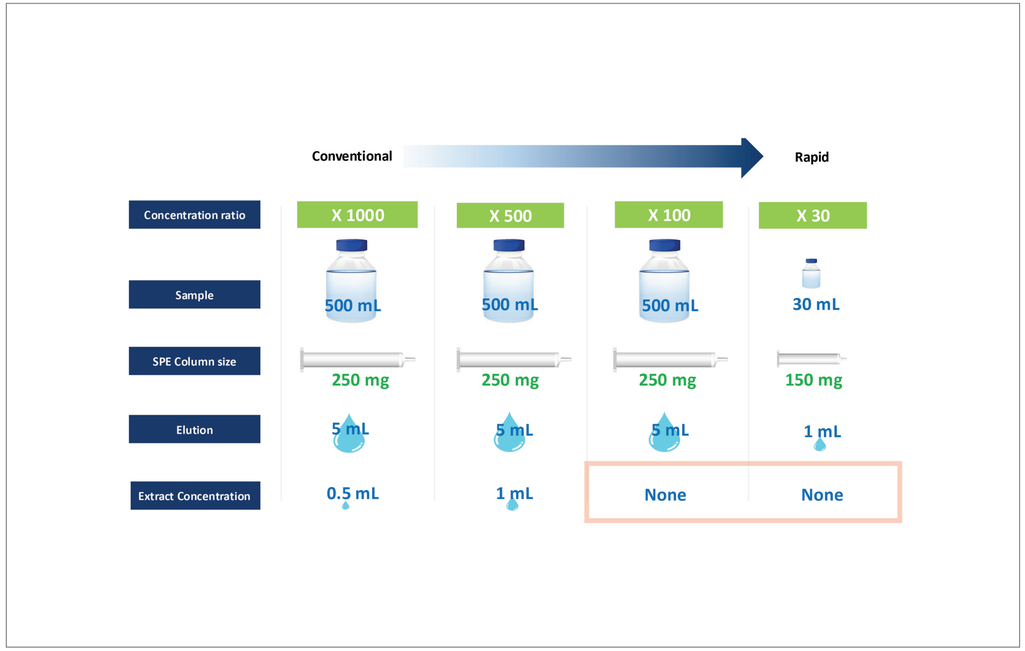
The InertSep MA-2 cartridge offers several key benefits in this SPE process:

Effective Elution of PFAS: The use of 0.1% NH4OH in methanol ensures efficient elution of a wide range of PFAS compounds, including long-chain and high-carbon PFAS species.

No pH Adjustment Required: This simplifies the process, making it faster and easier to implement.

High Recovery Rates: The cartridge delivers high recovery rates for various PFAS compounds, ensuring accurate and reliable results, as shown by recovery percentages ranging from 72% to 114% across different PFAS.

These factors make the InertSep MA-2 an optimal choice for rapid and efficient PFAS extraction in water samples.



Rapid SPE Procedure

The rapid SPE procedure for PFAS analysis involves processing a 30 mL water sample.

The process starts with conditioning the InertSep MA-2 cartridge (150 mg/3 mL) at a flow rate of 5 mL/min. The PFAS compounds are then eluted using 0.1% ammonium hydroxide (NH4OH) in methanol.

The procedure does not require any pH adjustment. After elution, the sample is analyzed using LC-MS/MS.

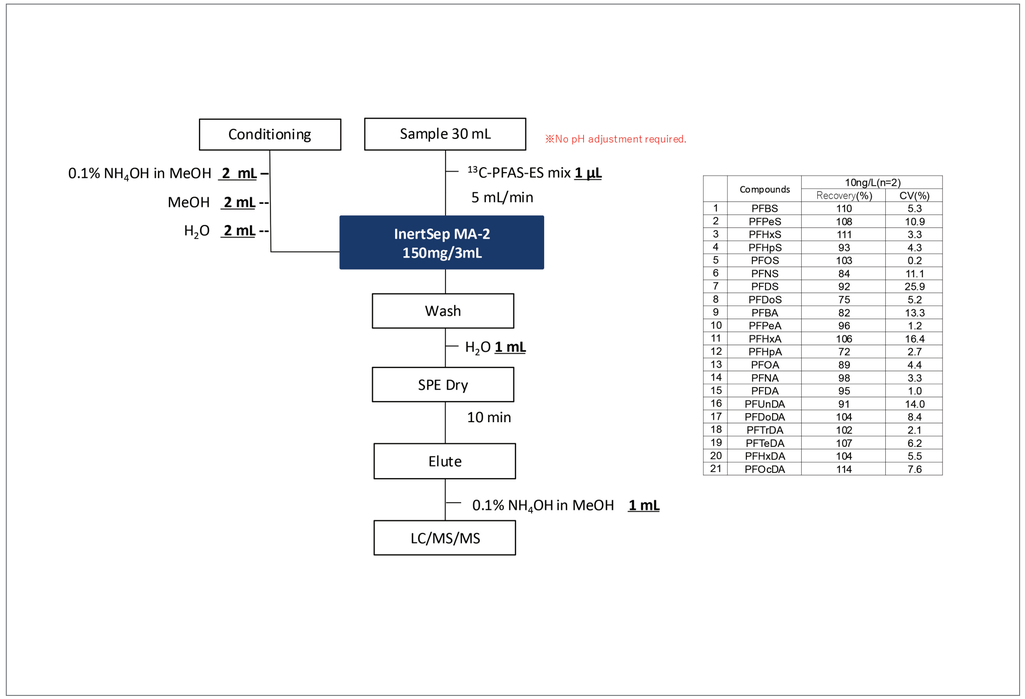
The InertSep MA-2 cartridge offers several key benefits in this SPE process:

Effective Elution of PFAS: The use of 0.1% NH4OH in methanol ensures efficient elution of a wide range of PFAS compounds, including long-chain and high-carbon PFAS species.

No pH Adjustment Required: This simplifies the process, making it faster and easier to implement.

High Recovery Rates: The cartridge delivers high recovery rates for various PFAS compounds, ensuring accurate and reliable results, as shown by recovery percentages ranging from 72% to 114% across different PFAS.

These factors make the InertSep MA-2 an optimal choice for rapid and efficient PFAS extraction in water samples.



	Compounds	10ng/L(n=2)	
		Recovery(%)	CV(%)
1	PFBS	110	5.3
2	PFPeS	108	10.9
3	PFHxS	111	3.3
4	PFHpS	93	4.3
5	PFOS	103	0.2
6	PFNS	84	11.1
7	PFDS	92	25.9
8	PFDoS	75	5.2
9	PFBA	82	13.3
10	PFPeA	96	1.2
11	PFHxA	106	16.4
12	PFHpA	72	2.7
13	PFOA	89	4.4
14	PFNA	98	3.3
15	PFDA	95	1.0
16	PFUnDA	91	14.0
17	PFDoDA	104	8.4
18	PFTrDA	102	2.1
19	PFTeDA	107	6.2
20	PFHxDA	104	5.5
21	PFOcDA	114	7.6

LC-MS/MS Conditions

In PFAS analysis using LC-MS/MS, contamination from the HPLC system and mobile phase often poses a challenge. This process improves analysis by utilizing a PFAS-specific delay column, which minimizes interference by delaying the elution position of blanks originating from the HPLC system and mobile phase.

Role of InertSustain AQ-C18

The InertSustain AQ-C18 column exhibits excellent retention for highly polar compounds. As shown in the chromatogram, it demonstrates strong retention and superior peak shapes for polar compounds such as PFPrA. This indicates that even when methanol coexists in the sample after SPE extraction, the peaks remain stable upon injection into the LC system.
Delay Column for PFAS

The delay column (3.0 x 30 mm) is strategically positioned to prevent background PFAS contamination that could interfere with the detection of target analytes.

It effectively traps trace PFAS present in the LC system, ensuring that these do not contaminate the main analytical column. This addition significantly improves the accuracy of PFAS measurements, reducing potential false positives from system-derived contamination.

HPLC Condition

System	Nexera UHPLC System(Shimadzu)
Column	InertSustain C18 HP 3 µm, 2.1 x 150 mm Delay column for PFAS 3.0 x 30 mm
Elution	A) 10 mmol/L Ammonium acetate B) Acetonitrile
Gradient	Time A% B% 0.0 80 20 2.0 80 20 15.0 0 100 17.0 0 100 17.1 80 20 23.0 80 20
Flow rate	0.3 mL/min
Col. Temp.	40°C
Injection Vol.	1 µL

MS/MS Condition

System	4000 QTRAP(SCIEX)
Mode	ESI, Negative, MRM CUR CAD IS TEM GS1 GS2 ihe 20 12 -3700 400 30 30 on

Transition

This PFAS analysis focuses on the detection and quantification of PFAS compounds in drinking water samples, leveraging an advanced LC-MS/MS setup that incorporates both an InertSustain AQ-C18 column and a Delay column for PFAS. The C18 column enables effective separation of PFAS compounds, while the delay column minimizes background contamination, enhancing accuracy.

Results indicate precise retention times and quantification for various PFAS analytes, confirming the setup's capability to handle low-concentration PFAS in complex matrices.

The consistent recoveries and low variance across samples validate the method's reliability for sensitive PFAS detection in environmental water samples.

Compounds		Q1/Q3	DP	EP	CE	CXP
C4 Perfluorobutanoic acid	PFBA	213/169	-45	-10	-14	-9
C5 Perfluoropentanoic acid	PFPeA	263/219	-50	-10	-11	-9
C6 Perfluorohexanoic acid	PFHxA	313/269	-50	-10	-15	-9
C7 Perfluoroheptanoic acid	PFHpA	363/319	-55	-10	-14	-9
C8 Perfluorooctanoic acid	PFOA	413/369	-45	-10	-14	-9
C9 Perfluorononanoic acid	PFNA	463/419	-65	-10	-16	-9
C10 Perfluorodecanoic acid	PFDA	513/469	-65	-10	-14	-9
C11 Perfluoroundecanoic acid	PFUnDA	563/519	-65	-10	-16	-9
C12 Perfluorododecanoic acid	PFDoDA	613/569	-40	-10	-17	-9
C13 Perfluorotridecanoic acid	PFTrDA	663/619	-50	-10	-19	-9
C14 Perfluorotetradecanoic acid	PFTeDA	713/669	-50	-10	-15	-9
C16 Perfluorohexadecanoic acid	PFHxDA	813/769	-65	-10	-17	-9
C18 Perfluorooctadecanoic acid	PFOcDA	913/869	-65	-10	-17	-12
C4 Perfluorobutanesulfonic acid	PFBS	299/80	-80	-10	-62	-3
C5 Perfluoropentanesulfonic acid	PFPeS	349/80	-100	-10	-70	-13
C6 Perfluorohexanesulfonic acid	PFHxS	399/80	-80	-10	-80	-3
C7 Perfluoroheptanesulfonic acid	PFHpS	449/80	-100	-10	-104	-15
C8 Perfluorooctanesulfonic acid	PFOS	499/80	-90	-10	-95	-3
C9 Perfluorononanonesulfonic acid	PFNS	549/80	-105	-10	-116	-13
C10 Perfluorodecanesulfonic acid	PFDS	599/80	-80	-10	-80	-3
C12 Perfluorododecanesulfonic acid	PFDoS	699/80	-115	-10	-126	-13

Compounds	Q1/Q3	DP	EP	CE	CXP
$^{13}C_4$-PFBA	217/172	-30	-10	-14	-31
$^{13}C_5$-PFPeA	268/223	-25	-10	-12	-11
$^{13}C_5$-PFHxA	318/273	-30	-10	-14	-47
$^{13}C_4$-PFHpA	367/322	-30	-10	-14	-19
$^{13}C_8$-PFOA	421/376	-30	-10	-14	-9
$^{13}C_9$-PFNA	472/427	-30	-10	-14	-11
$^{13}C^6$-PFDA	519/474	-40	-10	-16	-13
$^{13}C_7$-PFUdA	570/525	-60	-10	-16	-7
$^{13}C_2$-PFDoA	615/570	-40	-10	-18	-15
$^{13}C_2$-PFTeDA	715/670	-45	-10	-18	-17
$^{13}C_3$-PFBS	302/80	-75	-10	-70	-13
$^{13}C_3$-PFHxS	402/80	-75	-10	-84	-13
$^{13}C_8$-PFOS	507/80	-110	-10	-90	-13
$^{13}C^3$-PFBA	216/172	-30	-10	-14	-19
$^{13}C_2$-PFOA	415/370	-30	-10	-14	-9
$^{13}C_2$-PFDA	515/470	-35	-10	-16	-35
$^{13}C_4$-PFOS	503/80	-105	-10	-120	-13

PFAS 21 Chromatogram

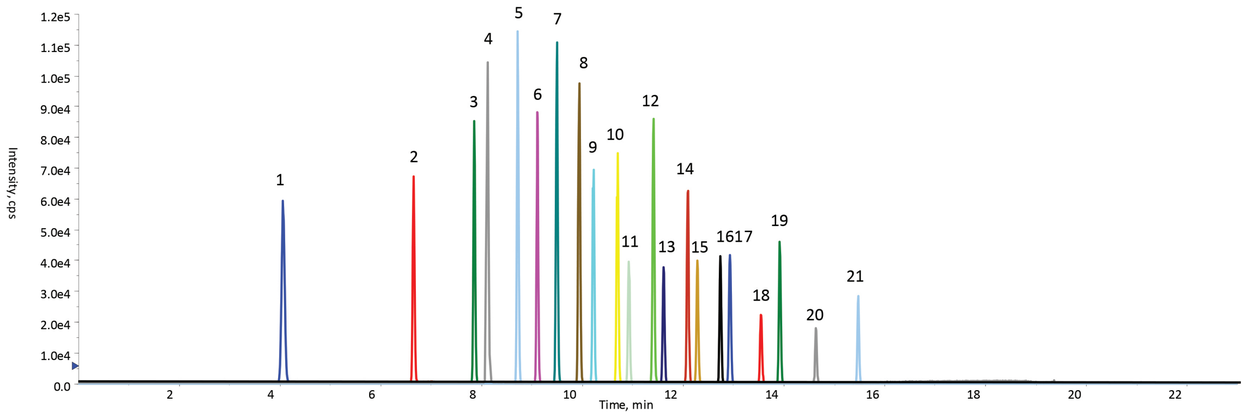
This PFAS analysis evaluated drinking water samples, focusing on the performance of the InertSustain AQ-C18 column paired with a PFAS-specific delay column within an LC-MS/MS system.

The C18 HP column successfully facilitated PFAS compound separation, while the delay column minimized background interference, reducing potential contamination from the analytical setup.

The results show consistent retention times and high recovery rates across various PFAS analytes, confirming the efficacy of the two-column configuration.

The use of ammonium acetate with acetonitrile in gradient elution proved effective, providing low variability and reliable sensitivity for detecting low-concentration PFAS.

These outcomes suggest that the method is well-suited for routine analysis of PFAS in drinking water, offering high sensitivity and minimal interference for accurate quantification.



1.	C4 Perfluorobutanoic acid	PFBA	8.	C6 Perfluorohexanesulfonic acid	PFHxS	15.	C12 Perfluorododecanoic acid	PFDoDA
2.	C5 Perfluoropentanoic acid	PFPeA	9.	C9 Perfluorononanoic acid	PFNA	16.	C10 Perfluorodecanesulfonic acid	PFDS
3.	C6 Perfluorohexanoic acid	PFHxA	10.	C7 Perfluoroheptanesulfonic acid	PFHpS	17.	C13 Perfluorotridecanoic acid	PFTrDA
4.	C4 Perfluorobutanesulfonic acid	PFBS	11.	C10 Perfluorodecanoic acid	PFDA	18.	C14 Perfluorotetradecanoic acid	PFTeDA
5.	C7 Perfluoroheptanoic acid	PFHpA	12.	C8 Perfluorooctanesulfonic acid	PFOS	19.	C12 Perfluorododecanesulfonic acid	PFDoS
6.	C5 Perfluoropentanesulfonic acid	PFPeS	13.	C11 Perfluoroundecanoic acid	PFUnDA	20.	C16 Perfluorohexadecanoic acid	PFHxDA
7.	C8 Perfluorooctanoic acid	PFOA	14.	C9 Perfluorononanonesulfonic acid	PFNS	21.	C18 Perfluorooctadecanoic acid	PFOcDA

Delay Column for PFAS

The Delay Column for PFAS by GL Sciences is specifically engineered to reduce background contamination from PFAS compounds present within the LC-MS/MS system.

In particular, care must be taken to minimize the effects of PFAS background and contamination eluting from fluorinated resins such as polytetrafluoroethylene (PTFE), which are commonly used as components in LC systems.

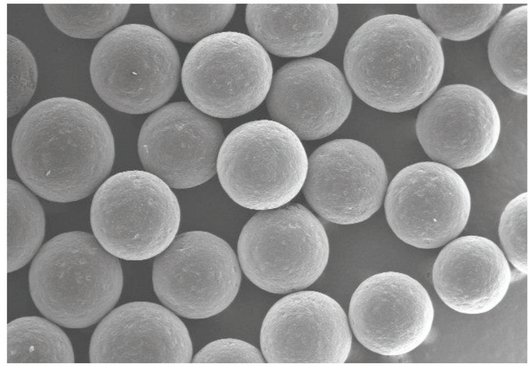
Key characteristics include:

Selective Retention: The delay column is designed to selectively retain background PFAS without affecting the target analytes, preserving the integrity of the sample analysis.

Improved Sensitivity: By removing background PFAS, the column enables lower detection limits, enhancing the method's sensitivity.

Enhanced Accuracy and Reproducibility: This column reduces contamination-related variability, which improves both accuracy and reproducibility of PFAS quantification in low-concentration samples.

These features make the GL Sciences Delay Column for PFAS an essential component for laboratories aiming for precise and reliable PFAS analysis.



Delay Column for PFAS Installation Position

The solid-phase extraction-LC/MS/MS method has been used for the analysis of PFAS in drinking-water under EPA Methods 537.1 and 533.

Because some countries and regions have low targets, it can be difficult to achieve stable, sensitive and accurate measurements that meet the required levels.

Care must be taken to minimize the effects of blanks eluting from fluorinated resins such as PTFE, which are commonly used as components in LC systems.

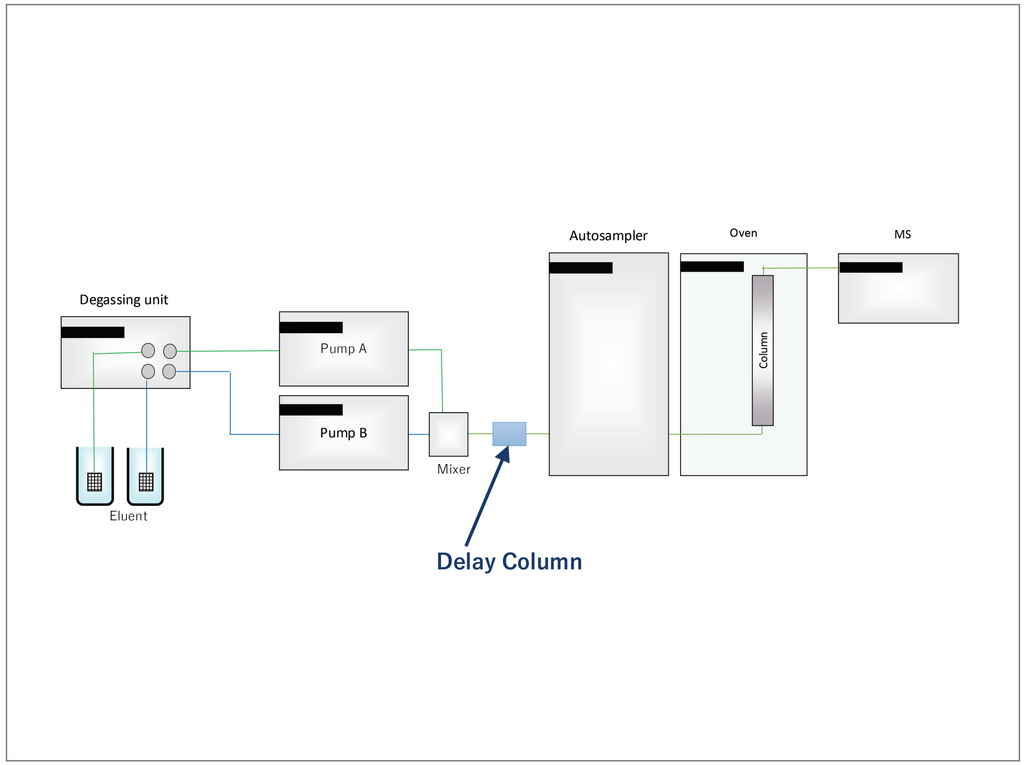
A known countermeasure, is to delay the elution time of the blank peak by connecting a Delay column packed with a C18 material before the autosampler, and to shift the retention time from the peak derived from the sample.

However, it is difficult to sufficiently increase the difference between the two retention times with a conventional C18 column.

Due to the relationship between the rise in pressure and the gradient delay time, column sizes are limited.

Therefore, in order to obtain a stable PFAS analysis, we have developed a new Delay column.
Our Delay column is packed with high-purity activated carbon beads.

To speed up solid phase extraction, you can also scale down the amount of sample water, the size of the SPE cartridge and the amount of the elution solvent.



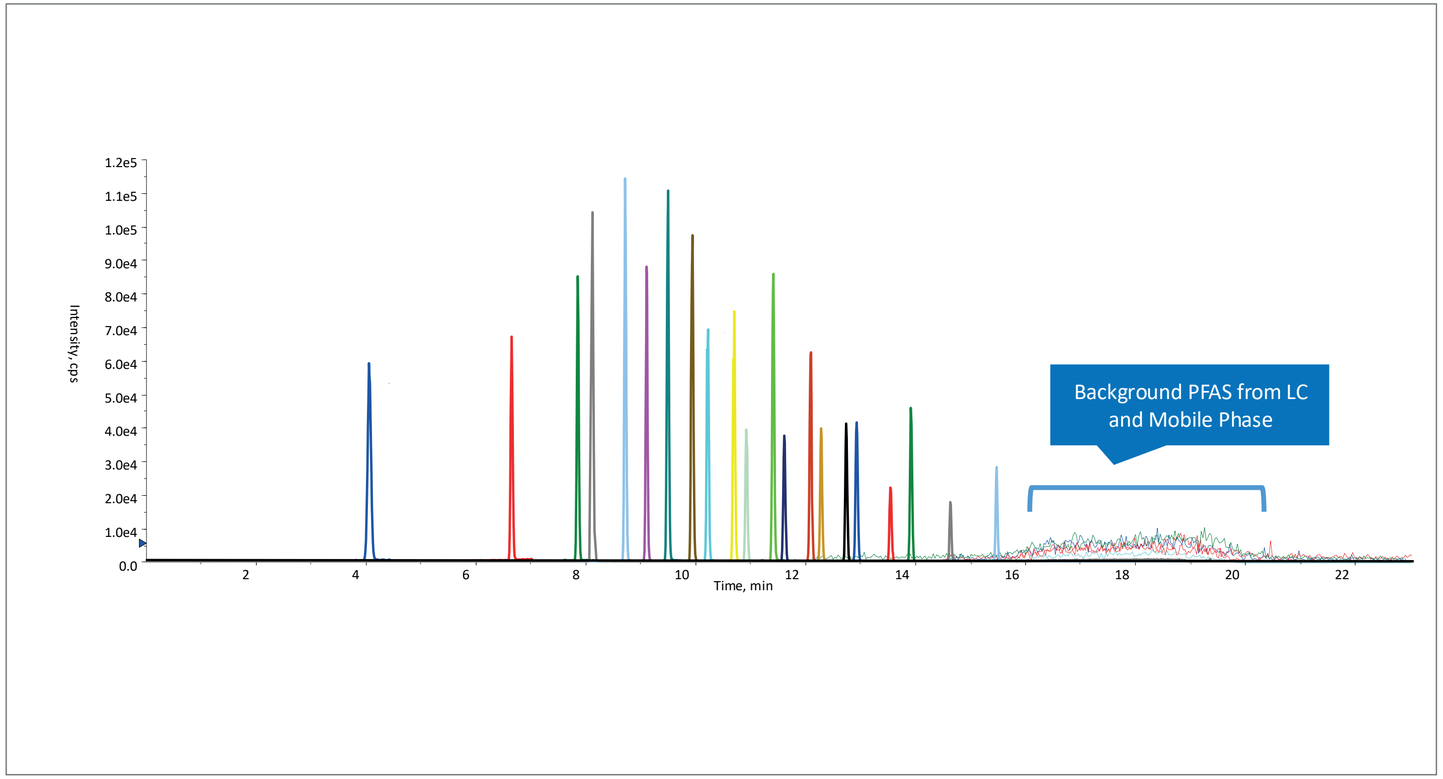
Chromatogram Using Delay Column for PFAS

The use of GL Sciences' Delay Column for PFAS demonstrated significant improvements in reducing system-related contamination, leading to clearer and more accurate detection of target PFAS compounds in drinking water samples.

The column effectively retained background PFAS that might otherwise interfere with the analytes, allowing for precise identification and quantification.

Data from the analysis showed consistent retention times and reduced baseline noise, which confirmed that the Delay Column minimized contamination from the LC-MS/MS system itself.

This enhancement resulted in improved sensitivity and reliability of the PFAS measurements, particularly beneficial for detecting low-level PFAS concentrations with greater confidence.



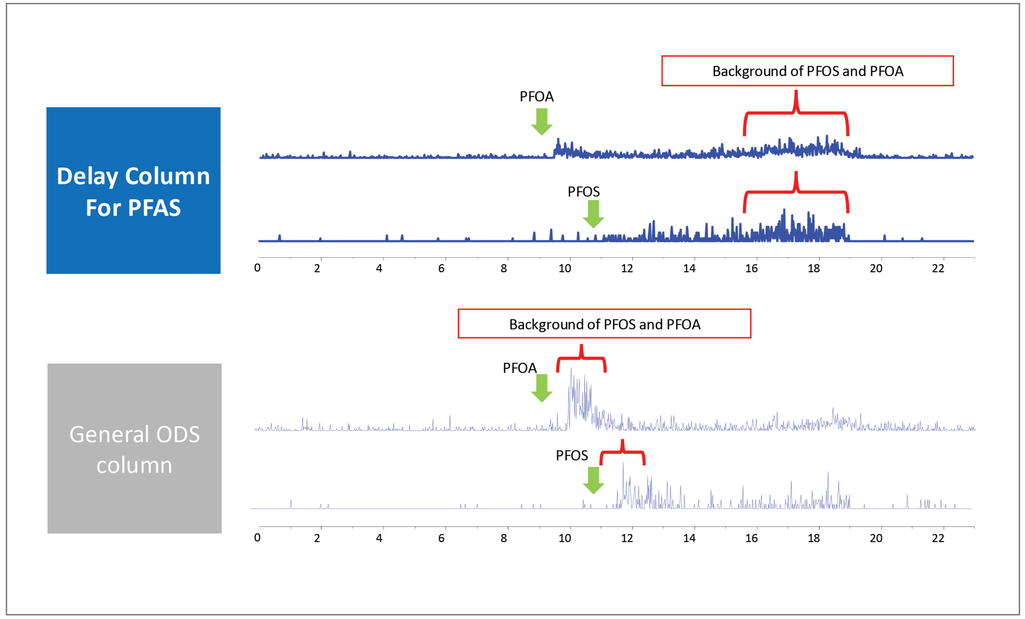
“Delay Column for PFAS” Effect

A comparison of the Delay column for PFAS with a general ODS column, which is commonly used as the Delay column. In contrast to the general ODS column that shows only a small delay and insufficient isolation, the Delay column for PFAS offers a better separation.

GL Sciences’ Delay Column offers superior retention for PFAS, allowing for effective separation between system-derived PFAS and those present in samples. This capability is critical for achieving accurate and interference-free PFAS measurements, significantly reducing baseline noise.

In contrast, when a general ODS column is used as a delay column, its lower retention for PFAS results in only a slight delay of system-derived PFAS. Consequently, this increases background interference caused by system-derived PFAS and reduces the reliability of quantification at low PFAS concentrations.

Overall, GL Sciences’ Delay Column provides superior reliability for PFAS detection compared to a general ODS column used as a delay column, enhancing sensitivity and accuracy in trace-level analysis.



Pressure Comparison

Based on the provided content, here is an explanation highlighting the unique feature of the Delay Column for PFAS, specifically its ability to maintain stable pressure:

The Delay Column for PFAS is designed to trap background PFAS contamination effectively without causing an increase in system pressure.

This feature is particularly advantageous in LC-MS/MS applications, as it allows for the reliable removal of system-derived PFAS contaminants while maintaining a stable and low-pressure environment.
The column's low backpressure reduces strain on the system, enabling prolonged operation and minimizing maintenance needs, which is beneficial for labs conducting continuous or high-throughput PFAS analyses.

This stable pressure performance differentiates the Delay Column for PFAS from other columns that may increase pressure and lead to operational inefficiencies.

Delay Column	Analytical Column	System Pressure
None	InertSustain C18 (2.1 x 150 mm 3 µm-HP)	**19.8 MPa**
Delay Column for PFAS (3.0 x 30 mm)		19.8 MPa
General ODS column (2.1 x 50 mm 3 µm)		23 MPa

※System: LC(Nexera, Shimadzu), MS/MS(4000 QTRAP, SCIEX)

Delay Column for PFAS does not increase the LC system pressure

Analysis of Ultra-Short Chain PFAS (DFA and TFA) Using an Anion-Exchange Mixed-Mode Reversed-Phase Column

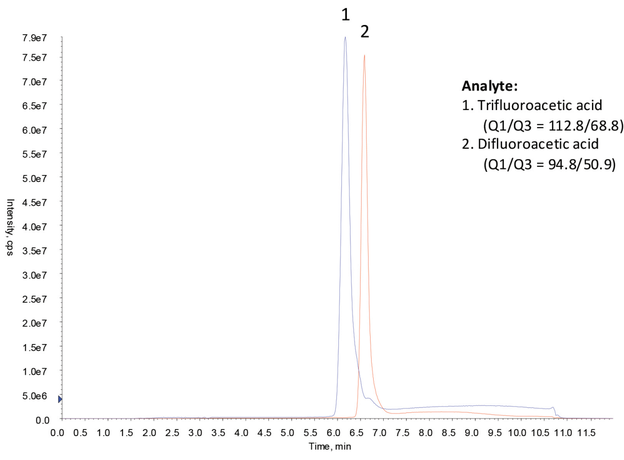
Ultra-short chain perfluoroalkyl substances (PFAS), such as Difluoroacetic acid (DFA) and trifluoroacetic acid (TFA), are emerging contaminants with significant environmental and health concerns.

Their low molecular weight and high mobility make them particularly difficult to analyze.

This study introduces a reliable method for analyzing DFA and TFA in water using a mixed mode reverse phase and anion exchange separation column (InertSustain AX-C18).

The method demonstrated clear retention and separation of DFA and TFA.
Notably, the use of PEEK column hardware effectively avoided metal interactions, which can often distort peak shapes.
The results show that the column is well-suited for ultra-short chain PFAS analysis, providing accurate and consistent separation under optimized conditions.

This approach offers a practical solution for water quality monitoring and can contribute to a better understanding of these emerging contaminants.



Analyte:
1. Trifluoroacetic acid 5 µg/mL
   (Q1/Q3 = 112.8/68.8)
2. Difluoroacetic acid 1 µg/mL
   (Q1/Q3 = 94.8/50.9)

Conditions

System : ExionLC AD (SCIEX)
Column : InertSustain AX-C18 (GL Sciences Inc.)
(3 µm, 150 x 2.1 mm I.D., Metal-free hardware)
Column Cat. No. : 5020-91059
Eluent : A) 10 mM CH_3COONH_4
with 0.1 % CH_3COOH in H_2O
B) CH_3CN

Time (min)	A (vol %)	B (vol %)
0	30	70
5.0	2	98
8.0	2	98
8.1	30	70
12.0	30	70

Flow Rate : 0.2 mL/min
Col. Temp. : 40 °C
Detection : MS/MS (ESI, Negative)

CUR	CAD	IS	TEM	GS1	GS2
35	10	-4500	400	30	30

Injection Vol. : 2 µL