# Per- and Polyfluoroalkyl Substances (PFASs) in Drinking Water Using Solid-Phase Extraction and LC-MS/MS

## UCT Part Numbers

**ECWAX126-P**
ENVIRO-CLEAN® WAX
200 mg, 6 mL cartridge, PE frits

**VMF016GL**
16 position glass block manifold

**VMFSTFR06-PFC**
Large volume sample transfer tubes (PTFE free)

**SLC-18100ID21-3UM**
Selectra® C18 HPLC column
(100 × 2.1 mm, 3 µm)

**SLC-18GDC20-3UM**
Selectra® C18 guard cartridge
(10 × 2.1 mm, 3 µm)

**SLGRDHLDR**
Guard cartridge holder

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## Summary:

This application note outlines a simple SPE procedure for the extraction of 26 diverse per- and polyfluoroalkyl substances (PFASs) in drinking water using UCT's polymeric weak-anion exchange SPE cartridges (ENVIRO-CLEAN® WAX). Instrumental analysis was carried out by LC-MS/MS in less than 10 minutes using a Selectra® C18 HPLC column. Overall, excellent recovery and reproducibility were obtained at the low concentrations tested.


## Introduction:

Per- and polyfluoroalkyl substances (PFASs) are a diverse group of synthetic organofluorine compounds that have been widely used in industrial applications and consumer products such as non-stick cookware, food packaging, fire-fighting foams, carpeting, apparels and metal plating. PFASs are persistent in the environment and are extremely resistant to degradation due to heat, acids or bases. They are also bioaccumulative in wildlife and humans and are known to cause reproductive and developmental toxicity in laboratory animals and wildlife.

The United States Environmental Protection Agency (USEPA) has issued drinking water health advisories for two PFASs, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) at 70 ng/L [1]. Several US states also have public health guidelines for PFASs ranging from 20–7,000 ng/L in drinking water.

This study describes a method for the sensitive quantification of 26 PFASs in drinking water, including the 14 covered in the US EPA Method 537 [2]. In addition, fluorochemicals are present in polytetrafluoroethylene (PTFE) materials, so excluding the use of any PTFE labware throughout the sampling and analytical processes (including HPLC solvent inlet tubing) is essential for accurate analysis of PFASs. UCT's large volume sample transfer tubes allow for simplified sample preparation and are PTFE free, preventing any further introduction of contaminates to the samples.

## Sample Pretreatment:

To avoid any potential contamination with PFASs, water samples should not be collected in a fluorinated plastic container. Glass should also be avoided as it has been reported that certain PFASs can be retained on its surface. A polypropylene or polyethylene container is the most suitable option.

- Check the pH of the water sample to ensure that it is in the range of pH 6-8. If necessary, adjust pH using a small amount of 0.1M HCl or 0.1M NaOH (other suitable acids/bases can also be used).
- Spike sample with appropriate concentrations of surrogate standard and mix thoroughly (add target analytes for fortified samples).

## SPE Procedure:

1. **SPE Conditioning**
   a) Rinse cartridge with 3mL methanol.
   b) Rinse the cartridge with 5 mL of pH 7 buffer (0.1M acetate, formate or phosphate buffer), leaving approximately 2 mL of water on the top of the frit.

2. **Sample Extraction**
   a) Attach a large volume sample transfer tube (**VMFSTFR06-PFC**) to the top of each SPE cartridge and place the stainless-steel end of the transfer tube directly into the sample bottle.
   b) Adjust the vacuum so that the flow rate is approximately 5 mL/min.
   c) After the sample is applied to the SPE cartridge, dry the cartridge under high vacuum (10-15 inHg) for 5 minutes to remove any residual water.

3. **Elution**
   a) Insert a 15 mL polypropylene tube into the extraction manifold.
   b) Add 6 mL of methanol containing 1% $NH_4OH$ to the sample container.
      - Note: due to the volatility of $NH_4OH$, it is highly recommended to use fresh elution solvent.
   c) Cap the sample container and thoroughly rinse the sides with the elution solvent.
      - Note: rinsing the sides of the container is important for obtaining good recovery of the long-chain hydrophobic PFASs.
   d) Apply a low vacuum to draw the elution solvent through the large volume sample transfer tubes and onto the SPE sorbent. Continue to elute the PFASs in a fast dropwise fashion.
   e) After the solvent has passed through, apply full vacuum for 30 seconds so that all the elution solvent is collected.

4. **Concentration**
   a) For samples spiked at 100 ppt (0.1 µg/L), the extract was evaporated to 5 mL.
   b) For sample spiked at 10 ppt (0.01 µg/L), the extract was evaporated down to 1 mL.
      - Note: the extract can be evaporated to 0.5 mL in order to achieve better sensitivity (lower MDL); however, longer evaporation may result in lower recovery for some of the volatile analytes.
   c) Add IS.
   d) Vortex the samples and transfer 500 µL to a propylene HPLC vial (PTFE free).

## LC-MS/MS Parameters:

PFASs are ubiquitous in the laboratory environment, mainly through the widespread use of Teflon™ components in analytical equipment, including HPLC. In order to avoid high background in LC-MS/MS analysis, the Teflon™ solvent lines should be replaced with PEEK tubing. However, PFAS contamination is difficult to completely eliminate and depending on the analytical conditions used, any PFAS present in the mobile phase, solvent lines and online degasser can become concentrated in the analytical column and be detected at the same time as the injected sample analyte. To overcome this, a short C18 "delay column" is commonly installed after the solvent mixer and before the sample injector to separate the contaminant peak from any PFAS present in the sample. Alterations to existing HPLC systems can be readily performed, although it is recommended to check with your HPLC's vendor before proceeding [3].

| Instrumentation | |
|---|---|
| **MS/MS system** | Shimadzu LCMS-8050 |
| **Ionization mode** | $ESI^-$ |
| **HPLC system** | Shimadzu Nexara X2 |
| **Delay column** | UCT Selectra® C18, 50 × 4.6 mm, 5 µm (p/n: SLC-1850ID46-5UM) |
| **HPLC column** | UCT Selectra® C18, 100 × 2.1 mm, 3 µm (p/n: SLC-18100ID21-3UM) |
| **Guard column** | UCT Selectra® C18, 10 × 2.0 mm, 3 µm (p/n: SLC-18GDC20-3UM) |
| **Guard column holder** | p/n: SLGRDHLDR |
| **Column temperature** | 45°C |
| **Flow rate** | 300 µL/min |
| **Injection volume** | 5 µL |

| Time (min) | Mobile Phase A (%)<br>10 mM Ammonium Formate | Mobile Phase B (%)<br>Acetonitrile |
|---|---|---|
| 0.0 | 90 | 10 |
| 0.5 | 65 | 35 |
| 5.0 | 5 | 9 5 |
| 6.0 | 5 | 95 |
| 6.1 | 90 | 10 |
| 10.0 | 90 | 10 |

# Retention Times and MRM Transitions

| # | Analyte | Acronym | Precursor Ion | Fragment Ion 1 | Fragment Ion 2 |
|---|---|---|---|---|---|
| | ***Perfluoroalkylcarboxylic acids (PFCAs)*** | | | | |
| 1 | Perfluorobutanoic acid | PFBA | 213.0 | 169.1 | N/A |
| 2 | Perfluoropentanoic acid | PFPeA | 263.0 | 219.0 | 141.1 |
| 3 | Perfluorohexanoic acid | PFHxA | 313.0 | 269.1 | 118.9 |
| 4 | Perlfuoroheptanoic acid | PFHpA | 362.8 | 319.1 | 169.1 |
| 5 | Perfluorooctanoic acid | PFOA | 412.8 | 369.1 | 169.2 |
| 6 | Perfluorononanoic acid | PFNA | 463.1 | 419.0 | 219.2 |
| 7 | Perfluorodecanoic acid | PFDA | 513.1 | 468.9 | 219.1 |
| 8 | Perfluoroundecanoic acid | PFUdA | 563.1 | 518.9 | 268.8 |
| 9 | Perfluorododecanoic acid | PFDoA | 612.9 | 569.0 | 319.1 |
| 10 | Perfluorotridecanoic acid | PFTrDA | 662.9 | 618.9 | 169.2 |
| 11 | Perfluorotetradecanoic acid | PFTeDA | 713.0 | 668.9 | 169.1 |
| | ***Perfluoroalkanesulfonates (PFASs)*** | | | | |
| 12 | Potassium perfluoro-1-butanesulfonate | PFBS | 299.0 | 79.9 | 99.0 |
| 13 | Sodium perfluoro-1-pentanesulfonate | PFPeS | 349.0 | 80.0 | 99.1 |
| 14 | Potassium perfluorohexanesulfonate | PFHxS | 399.0 | 80.0 | 99.0 |
| 15 | Sodium perfluoro-1-heptanesulfonate | PFHpS | 449.1 | 80.0 | 99.1 |
| 16 | Potassium perfluorooctanesulfonate | PFOS | 499.1 | 80.0 | 99.0 |
| 17 | Sodium perfluoro-1-nonanesulfonate | PFNS | 548.9 | 80.0 | 99.1 |
| 18 | Sodium perfluoro-1-decanesulfonate | PFDS | 598.9 | 80.0 | 99.1 |
| | ***Perfluorooctanesulfonamides (FOSAs)*** | | | | |
| 19 | Perfluorooctane sulfonamide | FOSA | 498.1 | 78.0 | 477.9 |
| | ***Fluorotelomer sulfonates (FTSs)*** | | | | |
| 20 | Sodium 1H,1H,2H,2H-perfluoro-1-hexanesulfonate | 4:2 FTS | 327.0 | 307.1 | 81.0 |
| 21 | Sodium 1H,1H,2H,2H-perfluoro-1-octanesulfonate | 6:2 FTS | 427.1 | 407.0 | 81.0 |
| 22 | Sodium 1H,1H,2H,2H-perfluoro-1-decanesulfonate | 8:2 FTS | 527.1 | 506.8 | 81.0 |
| | ***Perfluorooctanesulfonamidoacetic acids (FOSAAs)*** | | | | |
| 23 | Perfluorooctanesulfonamidoacetic acid | FOSAA | 556.1 | 497.9 | 419.0 |
| 24 | N-methyl perfluorooctanesulfonamidoacetic acid | N-MeFOSAA | 569.7 | 418.9 | 482.9 |
| 25 | N-ethyl perfluorooctanesulfonamidoacetic acid | N-EtFOSAA | 584.1 | 419.1 | 526.1 |
| | ***Perfluoroalkylphosphonic acids (PFPAs)*** | | | | |
| 26 | Perfluorohexane phosphonic acid | PFHxPA | 399.0 | 79.0 | 339.2 |
| | | | | | |
| | **Internal Standards** | | | | |
| IS 1 | Perfluoro-n-[2,3,4-$^{13}C_3$]butanoic acid | M3PFBA | 216.1 | 172.1 | N/A |
| IS 2 | Perfluoro-n-[2,3,4-$^{13}C_3$]hexanoic acid | MPFHxA | 315.0 | 270.1 | 120.1 |
| IS 3 | Perfluoro-n-[1,2-$^{13}C_2$]octanoic acid | M2PFOA | 415.0 | 370.0 | 169.1 |
| IS 4 | Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid | MPFDA | 514.9 | 469.9 | 220.1 |
| IS 5 | N-ethyl-$d_5$-perfluoro-1-octanesulfonamidoacetic acid | d5-NEtFOSAA | 588.9 | 419.0 | 530.9 |
| IS 6 | Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate | MPFOS | 502.9 | 80.0 | 99.0 |

**Note:** All standards were purchased in liquid form from Wellington Laboratories LLC. (Overland Park, KS, U.S.A.)

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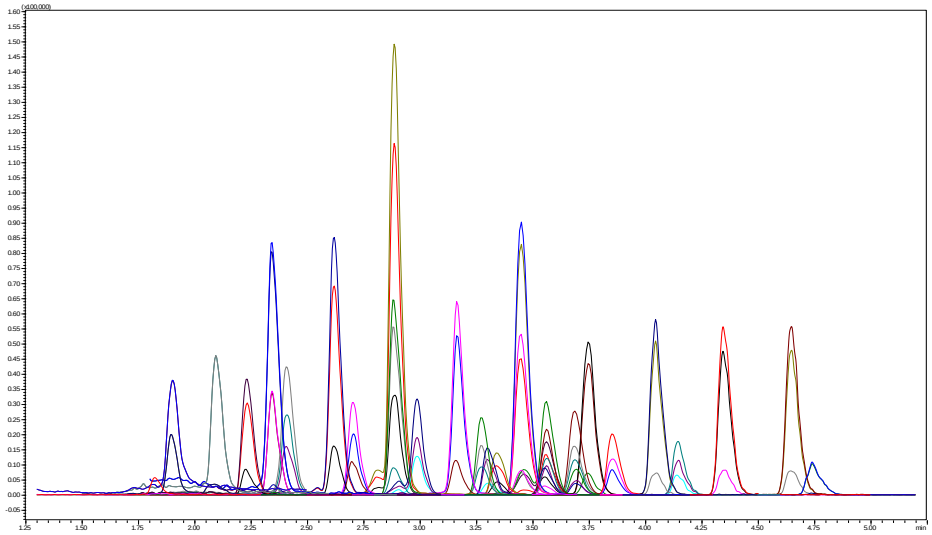

**Figure 1**: PFASs fortified at 10 ppt (0.01 µg/L) in tap water.

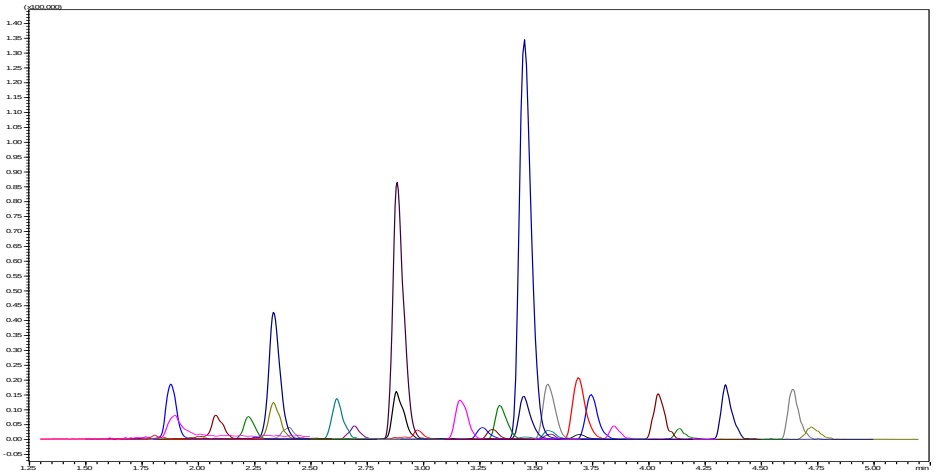

**Figure 2**: PFASs 0.5 ppb standard

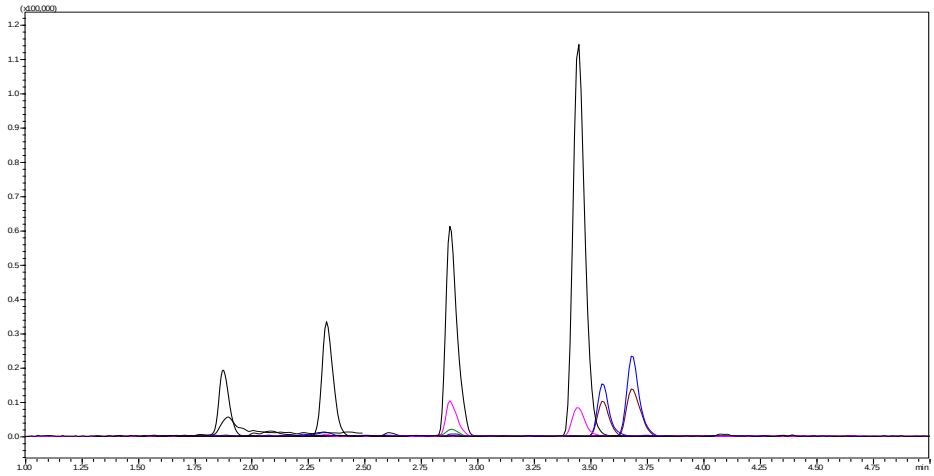

**Figure 3**: Laboratory reagent blank (LRB) with IS.

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| Analyte | RT (min) | IS Group | Calibration Curve Range (µg/L) | $R^2$ |
|---|---|---|---|---|
| PFBA | 1.89 | 1 | 0.5 - 10 | 0.9867 |
| PFPeA | 2.08 | 1 | 0.5 - 10 | 0.9995 |
| PFHxPA | 1.80 | 1 | 0.5 - 10 | 0.9960 |
| PFBS | 2.40 | 6 | 0.5 - 10 | 0.9998 |
| 4:2 FTS | 2.23 | 6 | 0.5 - 10 | 0.9990 |
| PFHxA | 2.33 | 2 | 0.5 - 10 | 0.9994 |
| PFPeS | 2.69 | 6 | 0.5 - 10 | 0.9976 |
| PFHpA | 2.61 | 2 | 0.5 - 10 | 0.9981 |
| PFHxS | 2.97 | 6 | 0.5 - 10 | 0.9992 |
| PFOA | 2.88 | 3 | 0.5 - 10 | 0.9974 |
| 6:2 FTS | 2.76 | 6 | 0.5 - 10 | 0.9952 |
| PFHpS | 3.30 | 6 | 0.5 - 10 | 0.9991 |
| PFNA | 3.18 | 3 | 0.5 - 10 | 0.9968 |
| PFOS | 3.58 | 6 | 0.5 - 10 | 0.9995 |
| PFNS | 3.96 | 6 | 0.5 - 10 | 0.9989 |
| PFDA | 3.46 | 4 | 0.5 - 10 | 0.9998 |
| 8:2 FTS | 3.33 | 6 | 0.5 - 10 | 0.9992 |
| FOSAA | 3.36 | 5 | 0.5 - 10 | 0.9937 |
| PFDS | 4.16 | 6 | 0.5 - 10 | 0.9978 |
| PFUdA | 3.76 | 4 | 0.5 - 10 | 0.9994 |
| N-MeFOSAA | 3.57 | 5 | 0.5 - 10 | 0.9947 |
| N-EtFOSAA | 3.72 | 5 | 0.5 - 10 | 0.9962 |
| PFDoA | 4.07 | 4 | 0.5 - 10 | 0.9979 |
| PFTrDA | 4.37 | 4 | 0.5 - 10 | 0.9981 |
| FOSA | 4.77 | 5 | 0.5 - 10 | 0.9999 |
| PFTeDA | 4.66 | 4 | 0.5 - 10 | 0.9999 |
| **Internal Standards** | | | | |
| M3PFBA | 1.86 | IS 1 | 4 | N/A |
| MPFHxA | 2.33 | IS 2 | 2 | N/A |
| M2PFOA | 2.90 | IS 3 | 4 | N/A |
| MPFDA | 3.48 | IS 4 | 6 | N/A |
| d5-NEtFOSAA | 3.72 | IS 5 | 8 | N/A |
| MPFOS | 3.57 | IS 6 | 4 | N/A |

Note: Calibration curve concentrations = 0.5, 1, 2, 5 and 10 µg/L.

## SPE Results:

| Analyte | Deionized Water (n=4) | | | | Tap Water (n=4) | | | |
|---|---|---|---|---|---|---|---|---|
| | Fortified conc = 10 ppt (0.01 µg/L) | | Fortified conc = 100 ppt (0.1 µg/L) | | Fortified conc = 10 ppt (0.01 µg/L) | | Fortified conc = 100 ppt (0.1 µg/L) | |
| | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| PFBA | 121 | 10.6 | 123 | 7.5 | 105 | 16.6 | 107 | 11.9 |
| PFPeA | 112 | 2.9 | 110 | 5.6 | 102 | 14.5 | 92 | 9.8 |
| PFHxA | 103 | 5.8 | 103 | 6.5 | 109 | 7.6 | 105 | 7.9 |
| PFHpA | 101 | 5.9 | 105 | 4.9 | 99 | 5.1 | 99 | 6.1 |
| PFOA | 99 | 6.9 | 98 | 5.0 | 114 | 9.3 | 99 | 8.7 |
| PFNA | 104 | 5.4 | 108 | 8.1 | 100 | 9.8 | 94 | 10.7 |
| PFDA | 104 | 6.3 | 98 | 3.4 | 107 | 1.9 | 96 | 5.6 |
| PFUdA | 101 | 7.5 | 87 | 4.3 | 95 | 5.3 | 89 | 6.5 |
| PFDoA | 97 | 6.2 | 99 | 3.3 | 104 | 9.4 | 93 | 4.1 |
| PFTrDA | 105 | 5.7 | 102 | 5.7 | 100 | 12.6 | 93 | 7.8 |
| PFTeDA | 95 | 2.1 | 100 | 6.2 | 98 | 13.9 | 92 | 7.2 |
| PFBS | 108 | 5.2 | 110 | 5.0 | 99 | 8.3 | 93 | 5.1 |
| PFPeS | 99 | 8.3 | 100 | 4.1 | 96 | 9.6 | 93 | 5.7 |
| PFHxS | 91 | 12.6 | 98 | 5.9 | 105 | 14.4 | 100 | 7.8 |
| PFHpS | 91 | 8.3 | 90 | 4.7 | 86 | 8.9 | 84 | 6.4 |
| PFOS | 91 | 14.5 | 90 | 2.8 | 90 | 10.6 | 87 | 2.2 |
| PFNS | 96 | 5.9 | 97 | 5.2 | 84 | 6.7 | 86 | 5.5 |
| PFDS | 97 | 3.9 | 93 | 3.4 | 94 | 10.1 | 84 | 6.7 |
| FOSA | 90 | 4.4 | 96 | 7.1 | 82 | 6.3 | 84 | 12.6 |
| N-MeFOSAA | 105 | 6.5 | 96 | 10.8 | 90 | 7.7 | 97 | 9.0 |
| N-EtFOSAA | 100 | 8.6 | 87 | 19.8 | 99 | 6.1 | 101 | 3.8 |
| FOSAA* | 64 | 13.5 | 58 | 0.9 | 30 | 33.6 | 30 | 23.7 |
| PFHxPA | 102 | 10.4 | 106 | 7.3 | 87 | 10.2 | 91 | 8.9 |
| 4:2 FTS | 111 | 5.4 | 108 | 5.7 | 83 | 6.1 | 85 | 6.2 |
| 6:2 FTS** | 158 | 65.1 | 211 | 66.0 | 214 | 107.9 | 77 | 5.4 |
| 8:2 FTS | 113 | 4.5 | 105 | 9.9 | 102 | 6.8 | 84 | 5.3 |

**Note:**

- A 250 mL water sample fortified at 10 ppt (0.01 µg/L) gives a final concentration (after SPE and evaporation to 1 mL) of 2.5 µg/L.
- A 250 mL water sample fortified at 100 ppt (0.1 µg/L) gives a final concentration (after SPE and evaporation to 5 mL) of 5 µg/L.

* Low Recovery of FOSAA due to potential loss during evaporation.

** High Recovery of 6:2 FTS due to potential exogenous contamination.

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## Conclusion:

This application note outlines a simple SPE method for the determination of a wide range of PFASs in drinking water using LC-MS/MS with a Selectra® C18 column. The results indicate that UCT's polymeric weak-anion exchange SPE cartridges (ENVIRO-CLEAN® WAX) can be used to simultaneously extract 26 PFASs in drinking water at environmentally relevant concentrations. All PFASs were quantified with good linearity of calibration using 6 mass-labelled internal standards. The recovery and RSD values obtained were within the acceptance criteria of the EPA method 537. The method can also be used as a starting point for a custom method that is tailored to a specific matrix or additional compounds. In this case, further optimization of the method can be carried out to optimize results.

## References:


1. Unregulated Contaminant Monitoring Rule 3 (UCMR3), accessed online November 2017, http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/
2. EPA Method 537: Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), version 1.1, September 2009, EPA/600/R-08/092.
3. Shimadzu's Parts Compatibility Guide for LCMS Analysis of PFC's; accessed from Shimadzu website on November 2017; http://www.ssi.shimadzu.com/products/literature/lcms/085_Shimadzu%E2%80%99s%20Guide%20to%20US%20DOD_DOE%20Analysis%20of%20PFCs%20using%20the%20LCMS-8060.pdf.





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