EPA
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Agency

USEPA (NERL/RTP) sample collection and analysis protocols for perfluorinated compounds in surface and well water

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Presentation Outline

- Summary of USEPA (NERL/RTP) surface and well water collection protocols
- Based on two draft protocols
- Discussion of some important fine points
- Further discussion of method application tomorrow in follow up presentation

Disclaimers and Caveats

- Not "Official" EPA methods – just what worked for our labaratory
- Strong emphasis on basic quality assurance principles
- Basic methods used in three assessments:

Nakayama, S.; Strynar, M. J.; Helfant, L.; Egeghy, P.; Ye, X.; Lindstrom, A. B. Perfluorinated compounds in the Cape Fear Drainage Basin in North Carolina. *Environ. Sci. Technol.* **2007**, *41*, 5271–5276.

(20) Nakayama, S. F.; Strynar, M. J.; Reiner, J. L.; Delinsky, A. D.; Lindstrom, A. B. Determination of Perfluorinated Compounds in the Upper Mississippi River Basin. *Environ. Sci. Technol.* **2010**, 4103–4109.

(24) Lindstrom, A. B.; Strynar, M. J.; Delinsky, A. D.; Nakayama, S. F.; McMillan, L.; Libelo, E. L.; Neill, M.; Thomas, L. Application of WWTP biosolids and resulting perfluorinated compound contamination of surface and well water in Decatur, Alabama, USA. *Environ. Sci. Technol.* **2011**, *45*, 8015−8021.

EPA Environmental News

March 2009, ES&T

EPA finds record PFOS, PFOA levels in Alabama grazing fields

Because of very high levels of perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and other perfluorochemicals found in agricultural soils near Decatur, Ala., scientists with the U.S. EPA, the U.S. Department of Agriculture (USDA), and the U.S. Food and Drug Administration (FDA) are investigating whether perfluorinated chemicals have entered the human food chain and contaminated meat.

The source of PFOA and PFOS, both of which occur at low part-per-million levels, is treated municipal sewage sludge, or biosolids, that were applied to some 5000 acres of agricultural land, according to Gail Mitchell, EPA Region 4's deputy director of water management. EPA is still investigating how the chemicals got into the sludge, adds Cathy Fehrenbacher, chief of EPA's exposure assessment branch, which is tasked with investigating the fate and transfor grazing beef cattle for 12 years, according to Mitchell.

If the chemicals are found to have contaminated meat, the results would mark the first time that perfluorochemicals have been traced from sludge to commercially produced food. In 2006, perfluorochemical contamination of two German rivers was traced to fields treated with sludge (*Environ. Sci. Technol.*

USDA AGRICULTURAL RESEARCH SERVICE

Cattle may have picked up PFOA from sludge that was spread on fields where they graze.

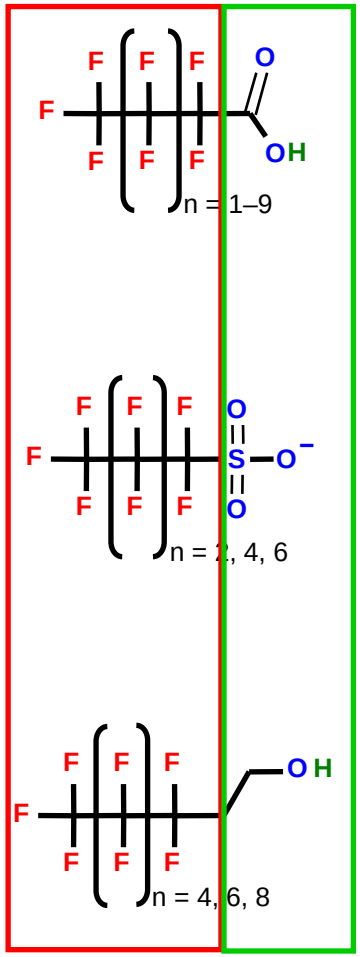
sampling private drinking-water wells located much closer to the fields. These wells serve fewer than 100 people, Mitchell estimates.

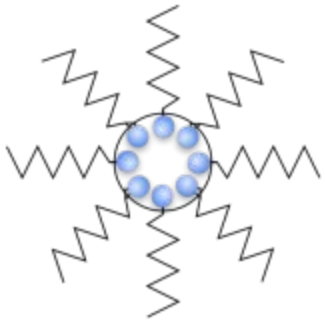
EPA officials notified both USDA and FDA about the high levels of perfluorinated chemicals because the land was used for grazing cattle, Mitchell says. USDA is responsible for inspecting raw meat such as beef or chicken for potential contamination, and FDA oversees processed foods. But neither USDA nor FDA has analyzed any samples.

The high concentrations of perfluorochemicals in the Decatursludge could be a rare situation, or a common one—published data on the concentrations of perfluorinated chemicals in sludge are minimal, and almost nothing is known about concentrations in soils, says Christopher Higgins of the Colorado School of Mines. "Based on published reports, the levels in the soil are high compared

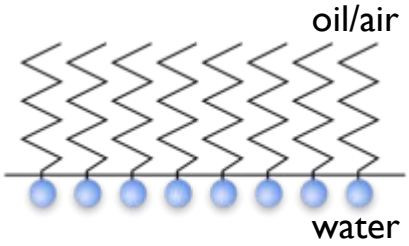
Properties of fluorochemicals

Oleo/Hydro-phobic Hydro-philic

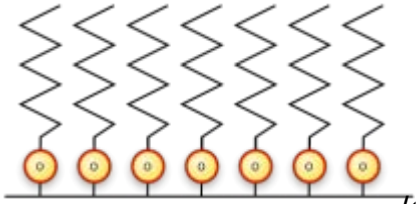




Micelles



Micro-layer



Polymer

Sample collection methods

United States Environmental Protection Agency

SS Kemmerer sampler

Lab-made dip sampler

Sample Collection

2.0 Summary of Method

Surface or well water samples are collected in pre-cleaned 1L high density polyethylene (HDPE Nalgene) bottles. Dilute nitric acid is added at the time of collection for sample preservation. Samples are maintained at ambient temperatures post sampling through laboratory analysis.

3.0 Materials

3.1 Nalgene HDPE bottles, pre cleaned, 1 L size, (EP Scientific Products, Miami, OK)
3.2 Methanol (B&J High Purity, Muskegon, MI)
3.3 Nitric acid, 5 mL ampoules of 35 % , (EP Scientific Products, Miami, OK)
3.4 PFCA-MXA PFC target compound mixture, 5µg/mL (Wellington Laboratories, www.well-labs.com)

Quality Control

6.0. Preparation of Quality Control Samples

6.1 Quality Control Samples will consist of field blanks, duplicates, and trip spikes (or field controls) that will be included at a rate of 10% of the total number of field samples collected. (Note that trip spikes (field controls) consist of a set of two bottles spiked at different concentrations.)

6.2 Field blanks will prepared in the RTP PFC laboratory by filling pre-cleaned 1 L collection bottles with deionized laboratory grade water, previously determined to be PFC-free. The samples will be preserved with the addition of 5 mL of 35% nitric acid, supplied in premeasured ampoules, with an orange EP HNO_3 sticker placed on the collection bottle to indicate that the preservation agent has been added. The sample will then be tightly capped and mixed well. These bottles will be clearly marked with red tape on the caps indicating that they should not be removed from the shipping container or opened during sampling.

Quality Control

6.3 Trip spikes (or field controls) will be prepared at low (200 ng/L) and high (400 ng/L) levels of all of the compounds on the target list. Preparation will be as follows:

6.3.1 PFCA–MXA standard containing all of the target compounds at 5 µg/mL (= 5 ng/µL) will be purchased from Wellington Laboratories (http://www.well-labs.com/).

6.3.2 To prepare low level QC spikes, 40 µL of this standard will be added to 1 L of deionized laboratory grade water (5 ng/µL x 40 µL = 200 ng/L) and capped and mixed well. The samples will be preserved with the addition of 5 mL of 35% nitric acid, supplied in premeasured ampoules, with an orange EP HNO_3 sticker placed on the collection bottle to indicate that the preservation agent has been added. The sample will then be tightly capped and mixed well.

6.3.3 To prepare high level QC spikes, 80 µL of this standard will be added to 1 L of deionized laboratory grade water (5 ng/µL x 80 µL = 400 ng/L) and capped and mixed well. The samples will be preserved with the addition of 5 mL of 35% nitric acid, supplied in premeasured ampoules, with an orange EP HNO_3 sticker placed on the collection bottle to indicate that the preservation agent has been added. The sample will then be tightly capped and mixed well.

Sample Collection

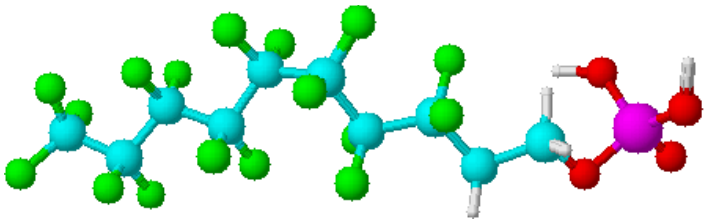
Sample Preservation

7.6 Add 5 mL of 35% nitric acid, supplied in the premeasured ampoules, into the sample, cap tightly, place an orange EP HNO_3 sticker onto the water collection bottle to indicate that the preservation agent has been added, and mix well.

7.7 Return sample bottles to the original shipping container (coolers) and maintain at ambient temperature. Do not cool.

Quality Assurance – Quality Control

Trip spikes (200 and 400 ng/L)
Trip blanks (DI)
Lab QC spikes (200 and 400 ng/L)
Duplicate samples (>10%)
Occasionally standard addition



Target Compounds
C_4-C_{12} carboxylic acids
PFBS, PFHS, PFOS, PFDS
Internal Standards
$^{13}C_2$-PFHxA, $^{18}O_2$-PFHxS,
$^{13}C_2$-PFOA, $^{18}O_2$-PFOS,
$^{13}C_2$-PFUnA

Sample Analysis

2. Summary of Method

Oasis WAX solid phase extraction (SPE) cartridges are preconditioned for the collection and retention of PFCs. The target compounds are eluted with solvents and the resulting eluent is concentrated to 0.5 mL. The final concentrate is then diluted with buffer solution and analyzed by UPLC-MS/MS, operated in negative electro spray ionization (ESI) mode. Quantitation is completed using a multipoint calibration curve and internal standard calculation. The Limit of Quantitation (LOQ) of the method is 10.0 ng/L (10 ppt).

Note: For samples that are suspected to contain high concentrations a pre-screening may be performed prior to actual sample analysis. This step may aid in the determination of sample extraction volumes.

Table 1. Perfluorinated analytes, abbreviations, internal standards, LC/MS/MS transitions, confirmation ions, and ion ratios monitored in analysis

Analyte	Quantitation transition	Confirmation transition	IS	ion ratio* (mean)	ion ratio (SD)	Instrumental LOD (ng/L)†	LOQ (ng/L)‡
Perfluorobutanoic acid (C4)	212.80 → 168.75	NA	$^{13}C_2$-C6	NA	NA	0.05	0.5
Perfluoropentanoic acid (C5)	262.85 → 218.75	NA				0.05	0.5
Perfluorohexanoic acid (C6)	312.70 →268.70	312.70 →118.70		17.67	2.72	0.05	0.5
perfluoroheptanoic acid (C7)	362.65 → 318.70	362.65 → 168.65		5.33	0.41	0.05	0.5
perfluorooctanoic acid (C8)	412.60 → 368.65	412.60 → 168.70	$^{13}C_8$-C8	4.57	0.58	0.05	0.5
perfluorononanoic acid (C9)	462.60 → 418.60	462.60 → 218.75		4.05	0.34	0.05	0.5
perfluorodecanoic acid (C10)	512.60 → 468.55	512.60 → 468.55	$^{13}C_2$-C11	6.81	1.10	0.05	0.5
perfluorobutane sulfonate (PFBS)	298.70 → 79.90	298.70 → 98.80	$^{18}O_2$-PFHS	1.58	0.14	0.05	0.5
perfluorohexane sulfonate (PFHS)	398.65 → 79.90	398.65 → 98.80		0.86	0.08	0.05	0.5
perfluorooctane sulfonate (PFOS)	498.65 → 79.90	498.65 → 98.80	$^{18}O_2$-PFOS	0.63	0.05	0.05	0.5
1,2-$^{13}C_2$- perfluorohexanoic acid ($^{13}C_2$-C6)	314.75 → 269.75	-	Internal Standards (IS)			-	-
$^{18}O_2$-Sodium perfluorohexanesulfonate ($^{18}O_2$-PFHS)	402.65 → 83.90	-				-	-
1,2,3,4,5,6,7,8-$^{13}C_2$-perfluorooctanoic ($^{13}C_8$-C8)	429.65 → 375.75	-				-	-
$^{18}O_2$-Ammonium perfluorooctanesulfonate ($^{18}O_2$-PFOS)	502.60 → 83.90	-				-	-
perfluoroundecanoic acid ($^{13}C_2$-C11)	564.60 → 519.65	-				-	-

10.3 Sample Pretreatment and Filtration

10.3.1 Volume Measurement

Pour whole water sample into a methanol rinsed 1-L HDPE graduated cylinder and record the volume.

10.3.2 Methanol Rinse of the Sample Container

Add 10 mL of methanol into the emptied bottle, cap, and shake well (Do not discard the rinse). Pour the sample back into the bottle and mix with the methanol rinse.

Note: This step must be performed immediately prior to the filtration steps that follow (10.3.4).

10.3.3 Internal Standard Addition

Add 50 µL of working internal standard solution (containing enough mass of the IS to match the midpoint of the standard curve, 500 ng/L) to each sample and mix well.

10.3.4 Filtration

Filter the whole sample with GF/A glass fiber filter under gentle vacuum. If any particulate matter remains in the original sample bottle, rinse it clean by adding 20 mL of with DI water, mixing thoroughly, and discarding this rinsate to waste. Then pour the entire sample back into the original bottle for the analysis.

Method Summary – Sample Processing

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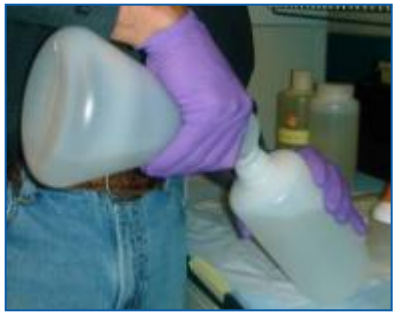
1 L HDPE bottles
5 mL HNO_3 (35%)
Shipped ambient

Measure volume in 1-L HDPE graduated cylinder

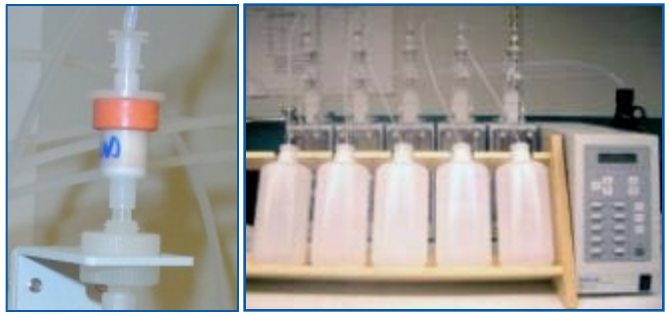
MeOH rinse
Bottle (10 mL)
HDPE bottle



Add measured water back to original container containing MeOH rinse

Add Internal Standard

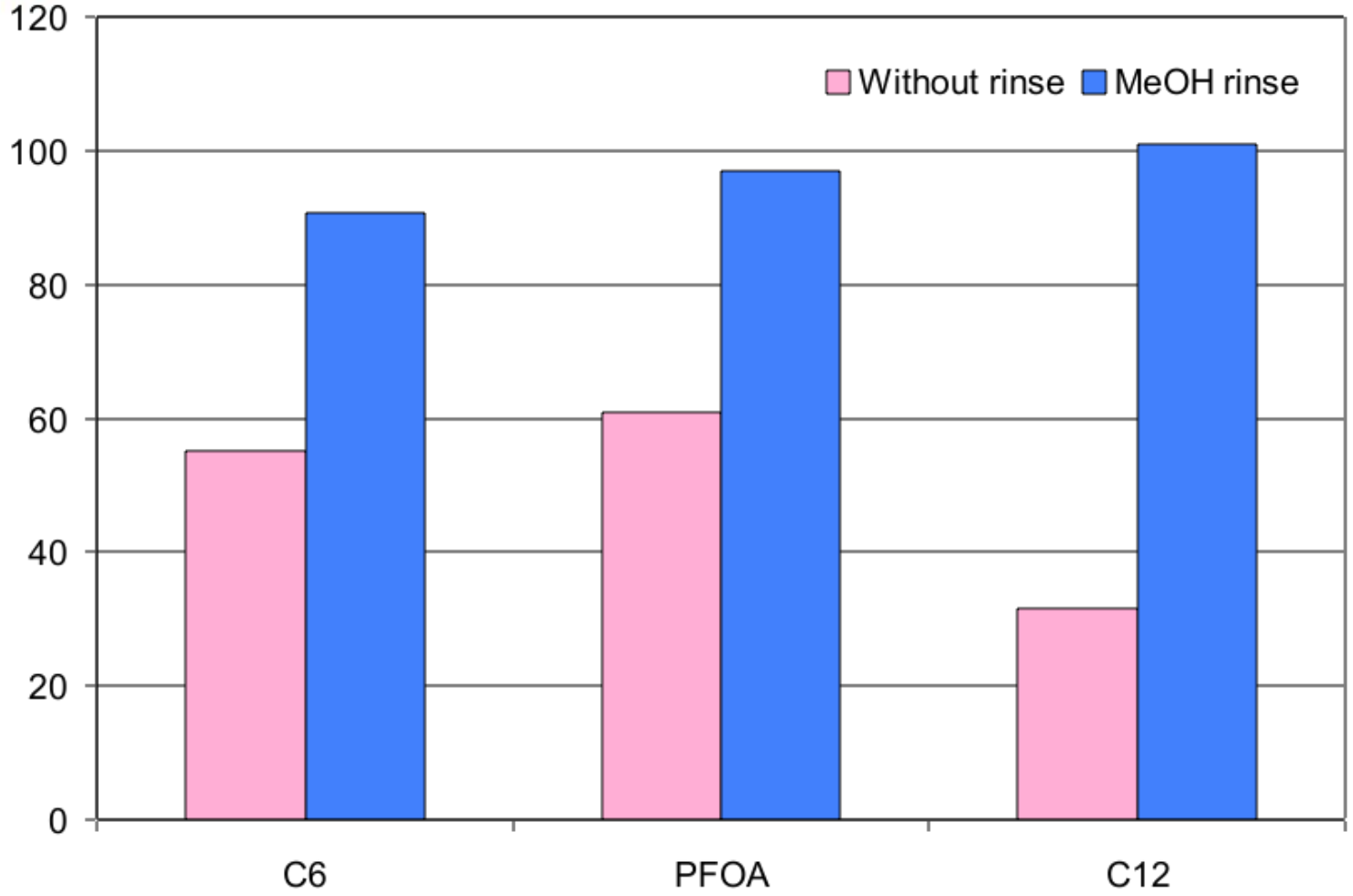
Filter contents
Whatman GF/A 1.7 um



Load onto SPE tube Waters
Plus style WAX/HLB

Nakayama et al., 2007 Environ. Sci. Technol. 2007, 41, 5271-5276

MeOH rinse of collection bottle



DI + 50 ng/L, stored at room temp for 1 week

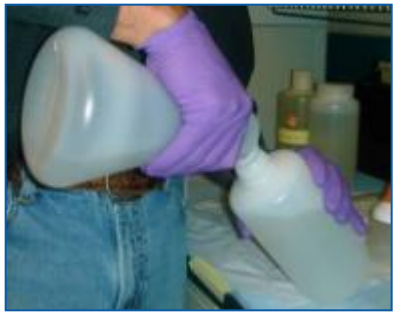
Method Summary – Sample Processing

EPA United States Environmental Protection Agency

1 L HDPE bottles
5 mL HNO_3 (35%)
Shipped ambient

Measure volume in 1-L HDPE graduated cylinder

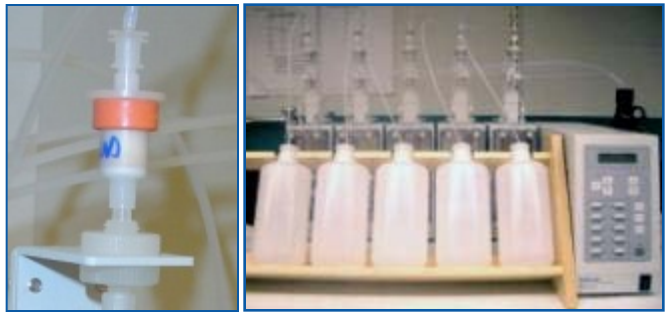
MeOH rinse
Bottle (10 mL)
HDPE bottle



Add measured water back to original container containing MeOH rinse

Add Internal Standard

Filter contents
Whatman GF/A 1.7 um



Load onto SPE tube Waters
Plus style WAX/HLB

Nakayama et al., 2007 Environ. Sci. Technol. 2007, 41, 5271-5276

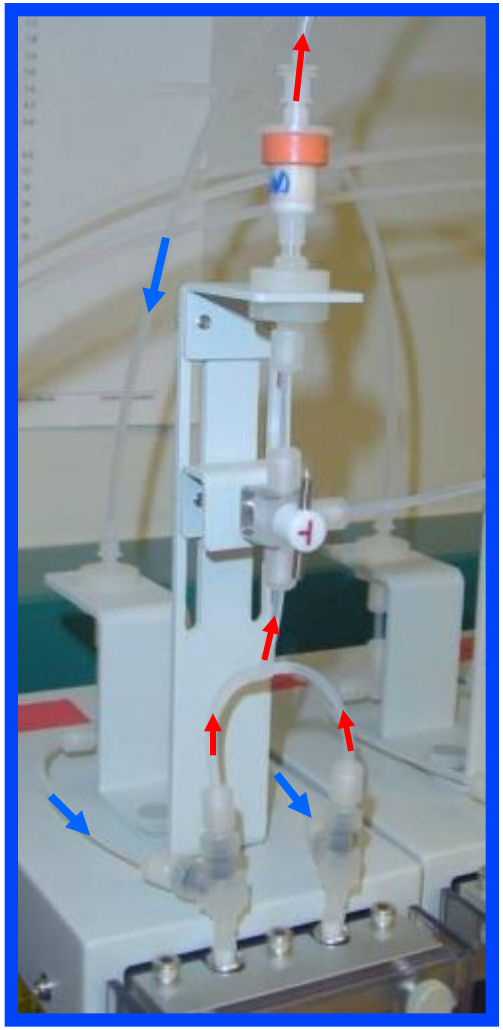
Sample Preparation

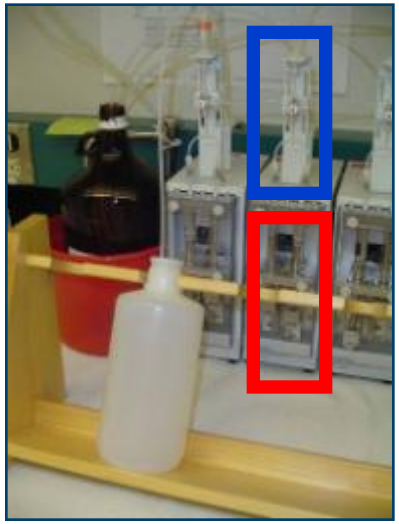
10.4.3 Loading Samples onto Cartridge

Pass 500 mL of sample water containing the IS through the cartridge at a steady flow rate of 10 mL/min using the positive pressure pump.

Note: If more sensitivity is required, the volume of sample loaded onto the SPE cartridge can be increased. However, the sample volume must be known and recorded for later calculation of the concentration (wt/volume). It is also important to note that increasing the sample volume will also increase the potential for matrix interference.

Positive Displacement SPE Loading: 10 mL/ minute





11. Determination of Analyte Concentration

11.1 UPLC

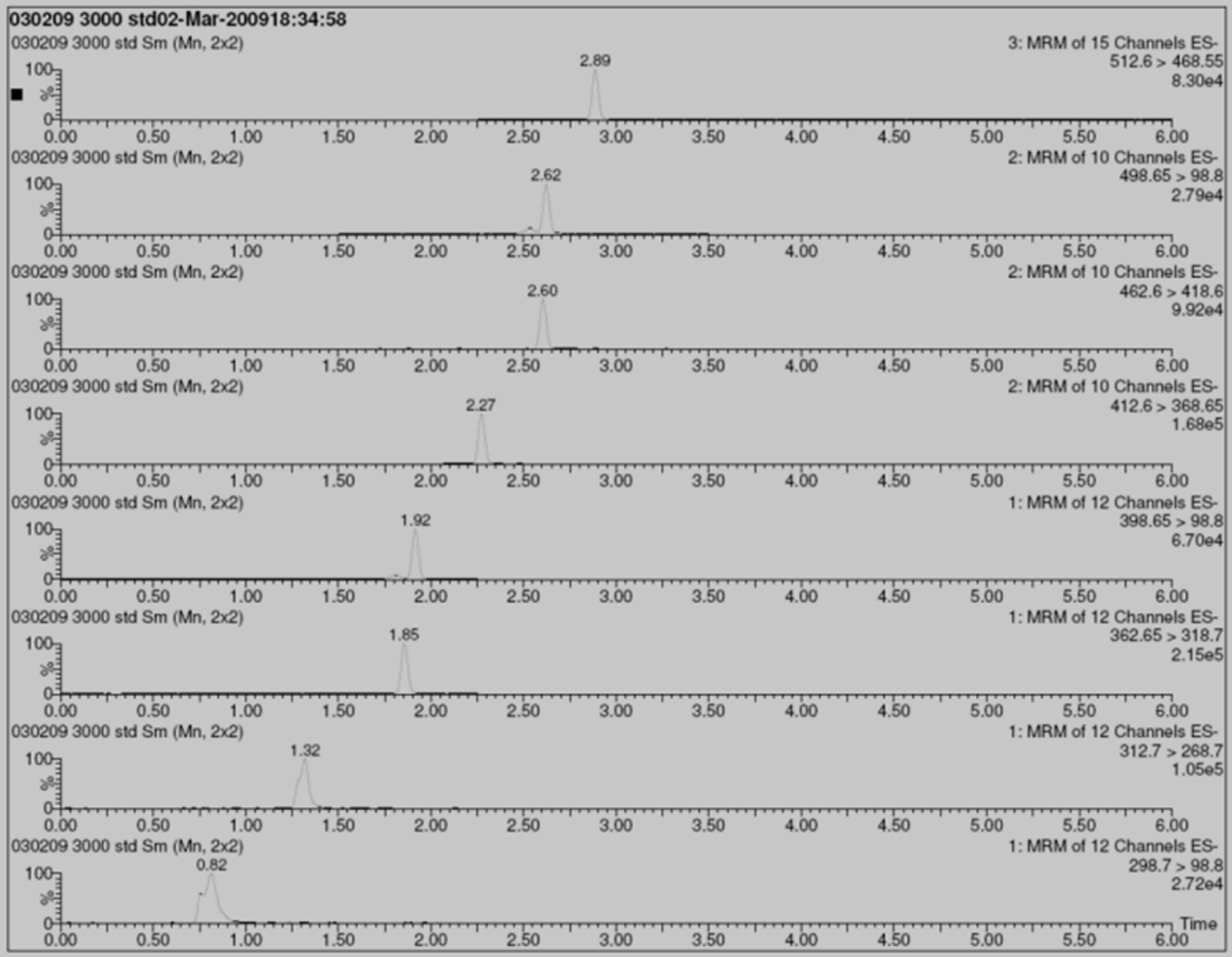
Using the instrument system and column outlined in Section 8.1.1, set up the method conditions using the following parameters:

Reservoirs: A: 2 mM ammonium acetate in DI water with 5% methanol, B: 2 mM ammonium acetate in 100% methanol
Column: BEH C18 reverse phase, 2.1×50 mm, 1.7 µm particle size
Flow rate: 500 µL/min
Column temperature: 50°C
Injection Volume: 20 µL
Gradient mobile phase program:

Time	A	B	curve
0.00	60	40	initial
0.50	60	40	6
3.50	10	90	6
3.60	0	100	6
4.50	0	100	6
4.60	60	40	6
6.00	60	40	6

Note: The gradient program should be adjusted, if necessary, for the complete separation of all analytes from any matrix interferences.

Example Calibration Sample. Plots are of primary MRMs for each analyte starting from the bottom as PFBS, C6, C7, PFHS, C8, C9, PFOS, and C10 respectively.



Take Home Messages

- Don't trust any data until you get comfortable with the method and the quality of analysis
- For every batch, check blanks are blank; spikes show good recovery; any chance of loss or contamination from sampling though analysis; matrix effects; analyte really exits
- Report and review all information related with method performance along with actual data
- Always be aware of fallibility of your method and yourself