Clinical Research

Quantitation of a Panel of Per- and Polyfluoroalkyl Substances (PFAS) in Human Serum

Kristine Van Natta[1], Kirstin Faoro[2], Cynthia Grim[1], Kevin McHale[2], Kerry Hassel[2]
Thermo Fisher Scientific, [1]San Jose, CA, [2]Sommerset, NJ USA

Abstract

Purpose: Develop an LC-MS analytical method for quantitation of PFAS compound in human serum.

Methods: Human serum samples were spiked with 37 PFAS compounds followed by protein precipitation with 4x volume of methanol. The supernatant was analyzed on an LC-MS system equipped with low-PSAF tubing and a delay column to minimize background interferences.

Results: LOQs from 0.025 to 2.5 ng/mL were reached for all compounds.

Introduction

Per- and polyfluoroalkyl substances have been monitored in environmental and industrial samples for some time. Wellness panels are becoming more frequent and quantitating PFAS in biological samples are a part of these panels. Due to the evolving regulations and discoveries of potential health effects with these compounds it important to measure a wide range of PFAS compounds.

Materials and methods

Materials

Human serum, 2x charcoal-stripped, was obtained from BioIVT®. PFAS reference standards were obtained from Wellington Laboratories, Inc. Thermo Scientific™ UHPLC-MS-grade water (W8-1), Thermo Scientific™ UHPLC-MS grade methanol (A458), Fisher Chemical™ Optima™ LC/MS acetic acid in 1 mL glass ampoules (A113), and Fisher Chemical Optima LC/MS Ammonium Acetate (A114-50) were all obtained from Fisher Scientific™.

Other materials include Thermo Scientific SureSTART™ Polypropylene Screw Top Microvials (6ESV9-04PP), Thermo Scientific clear polypropylene vial caps (C5000-50), and Fisherbrand™ round-bottom polypropylene test tubes (FB1495916) all, again, obtained from Fisher Scientific.

Sample Preparation

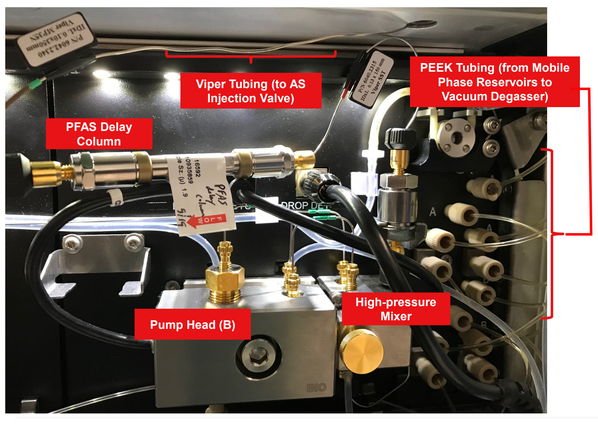
Aliquots of serum were spiked with PFAS compounds in the range of 0.025 to 50 ng/mL. Following addition of ^{13}C- and D-labeled internal standards, four volumes of methanol were added to precipitate the serum proteins. Samples were vortex mixed and centrifuged, and the supernatant was transferred to a polypropylene HPLC vial and sealed with a polypropylene cap.

Instrumentation

Samples were analyzed on a Thermo Scientific Vanquish™ Flex UHPLC coupled to a Thermo Scientific TSQ Altis™ Plus triple quadrupole mass spectrometer.

The Vanquish Flex was equipped with a PFAS retrofit kit (PN 80100-62144) (Figure 1) that replaced solvent line tubing with PPEK tubing that was free of PFAS in the lining.

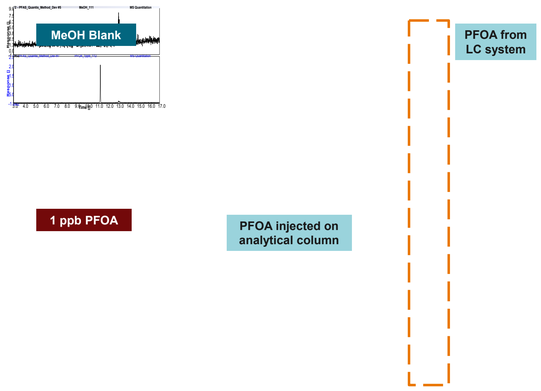
Figure 1. PFAS Kit Retrofit, and delay column set up used to minimize systemic background interferences.



Instrumentation (cont.)

The PFAS kit also implements a delay column to differentiate between PFAS background I the mobile phase from analytes in the injected sample (Figure 2).

Figure 2. Using PFAS Delay Column allows differentiation of PFAS contribution from the solvent system or the sample.



Chromatographic Method

Mobiles phase A is water containing 2 mM ammonium acetate, 2% methanol of 0.1% acetic acid. Mobile phase B is methanol containing 2 mM ammonium acetate, 2% water and o.1% acetic acid. The analytical column used is a Thermo Scientific Accucore™ C18, 2.6 µm particle, 2.1 x 100 mm (PN 17126-102130) maintained at 45ºC. Of special note, that autosampler compartment was kept at 25ºC to reduce adherence of the longer chain PFAS compounds to the walls of the HPLC vial. The chromatographic gradient is shown in Table 1.

Table 1. Chromatographic gradient used to separate PFAS compounds.

Time (min)	%B	Flow (µL/min)
0	5	500
1	30	500
6.5	85	500
7.8	85	500
7.9	5	500
10	5	500

Mass Spectrometric Method

The TSQ Altis Plus was equipped with at heated electrospray ionization (HESI) sprayer. One to three SRM transitions were monitored for each analyte and internal standard. All transitions are in the negative ionization mode. Source and scan properties are listed in Table 2.

Data Analysis

Data was acquired and processed using Thermo Scientific TraceFinder software version 5.2.

Table 2. Ion Source and SRM Properties for PFAS analysis on the TSQ Altis Plus.

Ion Source Property	Value
Ion Source Type	H-ESI
Spray Voltage Neg (V)	500
Sheath Gas (Arb)	55
Aux Gas (Arb)	10
Sweep Gas (Arb)	1
Ion Transfer Tube (ºC)	225
Vaporizer Temp (ºC)	275

SRM Property	Value
Q1 Resolution (FWHM)	0.7
Q3 Resolution (FWHM)	0.7
CID Gas	2.5
Source Fragmentation	0

Results

Calibration range in serum was 0.025-50 ng/mL with a 25 uL injection. The inter- and intra-day precision measurements showed that for all analyte quantitation, the %RSD was below 25%, and the %Diff of the calibration curve was below 25%, which indicated that the developed method was robust and reproducible see Table 3.

Figure 3 shows a composite chromatogram of all the PFAS compounds except the early eluting PFBA.

Figure 4 shows for PFOS: a calibration curve, chromatogram at LOQ of 0.025 ng/mL and internal standard M8PFOS. The %RSD for M8PFOS is 7.96 over the calibration curve replicates (data not shown).

Figure 5 shows the same information for late-eluting PFHxDA.

Table 3. LOQs for PFAS compounds based on linear R^2 >0.99, %diff <30% and %RSD of triplicate injections <30%.

Compound	LOQ (ng/mL)	Compound	LOQ (ng/mL)
10:2FTS	0.25	PFDA	0.025
11Cl-PF2OUdS	0.025	PFDoA	0.025
4:2FTS	0.05	PFDS	0.05
6:2FTCA	0.025	PFECHS	0.05
6:2FTS	0.1	PFHpA	0.05
6:2FTUCA	0.05	PFHpS	0.025
8:2FTCA	0.05	PFHxA	0.05
8:2FTS	0.05	PFHxDA	0.025
8:2FTUCA	0.025	PFHxS	0.05
9Cl-PF3ONS	0.05	PFNA	0.05
ADONA	0.05	PFNS	0.025
FBSA	0.05	PFOA	0.05
FHxSA	0.05	PFOS	0.025
FOSA	0.05	PFPeA	0.05
HFPO-DA-CO2	0.05	PFPeS	0.05
N-EtFOSAA	0.05	PFTeDA	0.025
N-MeFOSAA	0.05	PFTrDA	0.05
PFBA	0.5	PFUdA	0.05
PFBS	0.05		

Figure 3. Composite chromatogram of PFAS compounds on TSQ Altis Plus (PFBA (2.35 min) not shown).

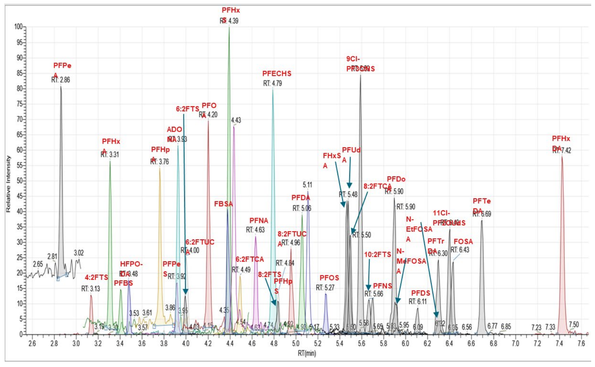
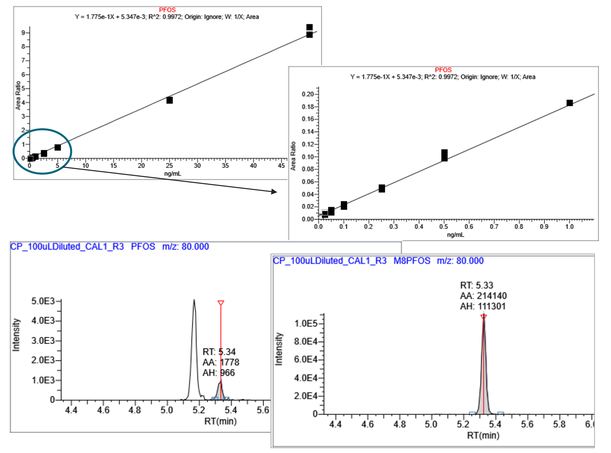


Figure 4. PFOS Calibration curve, chromatogram at LOQ of 0.025 ng/mL and its internal standard M8PFOS. The %RSD for M8PFOS is 7.96 over the calibration curve replicates (data not shown).



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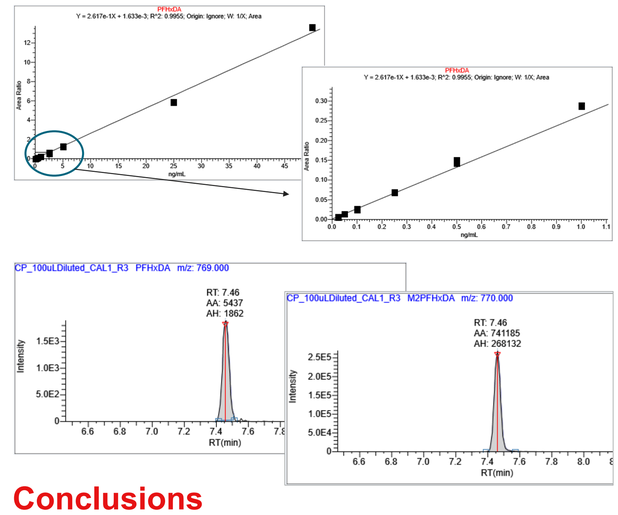

Conclusions

- 10-minute method for 37 PFAS compounds on the Vanquish UHPLC and TSQ Altis Plus was developed. The method showed good accuracy and precision measurements.
- While this method used simple protein precipitation for sample processing, future work includes developing an SPE method to enhance detection limits.

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