Solid-phase microextraction of perfluorinated compounds in biological matrices

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Abstract

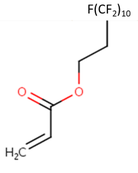
Measurements of non-ionic perfluorinated compounds (PFCs) in biological matrices, including plasma, are needed for accurate determination of internal dose. Traditional sample preparation techniques for extracting these target compounds from plasma are time consuming and could result in lower recoveries for the more volatile PFCs. For comparison and possible performance improvements, liquid-liquid extraction (LLE) will be compared to solid-phase microextraction (SPME)-gas chromatography (GC)/mass spectrometry (MS) techniques for extraction of target compounds from plasma. Other non-ionic PFCs have been successfully extracted from water and sediment samples using headspace-SPME resulting in reduced sample preparation time and matrix interferences during quantitation compared to traditional techniques. Extending high throughput SPME techniques to biological matrices for PFCs could result in similar improvements and decreased detection limits. Initially, both headspace and direct-immersion SPME will be assessed to determine which technique yields the highest extraction efficiency for the target compounds. After selection of the specific SPME technique, SPME parameters such as fiber coating, phase volume, extraction temperature, extraction time, addition of organic additives, and GC desorption temperature and time will be optimized using standards spiked into blank plasma matrix. A final side-by-side comparison of LLE and the optimized SPME technique for the target compounds will be conducted with spiked plasma matrix for at least 3 concentrations and 7 replicates. Results from this study will include accuracy, precision, detection limits, and signal to noise ratios for each compound and method. If the SPME technique shows promise in these proof of concept studies, additional extension of SPME techniques to biological matrices other than plasma could be investigated.

Materials & Methods

Analytes of Interest:

1) 10:2 fluorotelomer acrylate (10:2 FTACr)
- DTXSID9037743
- Molecular Weight: 618.185 g/mol
- LogK_{ow}: 7.46

2) Internal standard: 2-perfluorodecyl-[1,1-2H2- 1,2-13C2]-ethanol (10:2 FTOH 13C2)
- Wellington Laboratories (MFDET)



10:2 FTACr

Materials & Methods Continued

Testing of Sample Preparation Techniques:

1) Supported Liquid Extraction (SLE)
- SLE is analogous to LLE- uses an inert support material instead of 2 immiscible solvents
- Biotage ISOLUTE 200 µL SLE plate
- Buffers: 1% formic acid (FA), water, and 0.5 M ammonium hydroxide
- Extraction solvents: methyl tert-butyl ether (MTBE), dichloromethane (DCM), and ethyl acetate (EA)
- Method: 1) loaded 100 µL spiked plasma: 100 µL buffer; 2) applied vacuum to initiate loading; 3) waited 5 min; 4) loaded 1 mL extraction solvent; 5) waited 5 min; 6) applied vacuum to complete elution; 7) evaporated under nitrogen and reconstituted in 1 mL EA

2) Liquid liquid extraction (LLE)
- Extraction solvents: MTBE and EA
- Compared with and without evaporation under nitrogen
- Method: 1) added 500 µL extraction solvent to 50 µL spiked plasma; 2) vortexed for 2 min at 1800 rpm; 3) centrifuged for 5 min at 15,000 rpm at 4°C; 4) removed extraction solvent; 5) repeated steps 2-4 2x; each replication extraction analyzed separately to determine total needed for complete recovery 6) for half of the samples, evaporated under nitrogen and reconstituted in 1 mL EA

3) Headspace-SPME
- Parameters optimized: fiber coating, fiber desorption temperature and time, extraction time, and salt addition
- Agitator: 50 °C with agitation of 10 sec on/2 sec off
- 50 µL plasma in 20 mL headspace vial

GC/MS/MS Analysis:
- Thermo Trace 1300 GC/TSQ 8000 with TriPlus RSH Autosampler
- Column: Restek Rtx-1701 (30m × 0.25mm I.D. × 0.25 µm film thickness) with 10 m deactivated Integra-Guard guard column; column flow 1 mL/min helium
- Oven temperature program: 60 °C (10 min); 5 °C/min to 75 °C; 20 °C/min to 185 °C; 50 °C/min to 260 °C (2 min)
- Transfer line: 275 °C; Ion source temperature: 250 °C
- MS operated in electron ionization (EI) selected reaction monitoring (SRM) mode
  - 10:2 FTACr: 618.1>99.1 (Quantitation, Quant); 99.1>57.0 (Qualifier, Qual,1); 131.0>69.0 (Qual2)
  - 10:2 FTOH 13C2: 96.0>69.0 (Quant)

Results & Discussion

SLE & LLE

LLE Extraction Solvent	No Evaporation-Extracts 1 & 2	Evaporation-Extracts 1 & 2
MTBE		
Replicate 1	90.7	0.0
Replicate 2	91.3	0.0
Replicate 3	90.1	0.0
EA		
Replicate 1	98.0	20.7
Replicate 2	104.0	101.4
Replicate 3	121.5	0.0

Table 1. Percent recovery of spiked 10:2 FTACr plasma with LLE.

SLE:
- 0% recovery for all replicates and treatments (data not shown)
- Likely a result of evaporation to dryness under N_2

LLE:
- Both MTBE & EA acceptable extraction solvents
- Extraction needs to be repeated twice (2 x 500 µL)
- Low recovery with evaporation- possible rate of N_2 too high or result of evaporation to dryness

Headspace-SPME

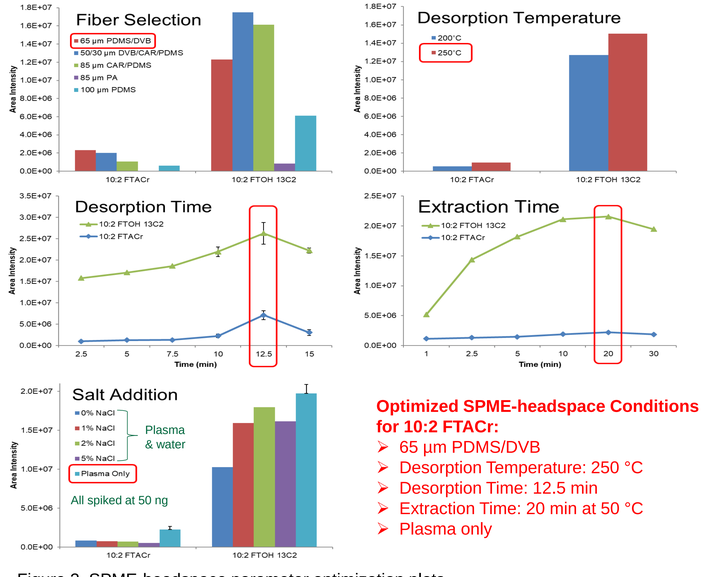


Figure 2. SPME-headspace parameter optimization plots.

Optimized SPME-headspace Conditions for 10:2 FTACr:
- 65 µm PDMS/DVB
- Desorption Temperature: 250 °C
- Desorption Time: 12.5 min
- Extraction Time: 20 min at 50 °C
- Plasma only

Results & Discussion Continued

Headspace-SPME

- Optimized method ~ 45 min/sample
- Optimized method for 10:2 FTACr, not the IS (10:2 FTOH 13C2)
- Higher response for 10:2 FTOH 13C2 vs. 10:2 FTACr
  - Alcohols more suited to headspace-SPME method
  - 10:2 FTACr breaks apart more with EI than 10:2 FTOH 13C2

GC/MS/MS Analysis

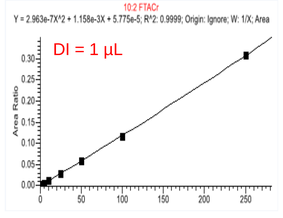


Figure 3. Direct injection (DI) calibration curve for 10:2 FTACr.

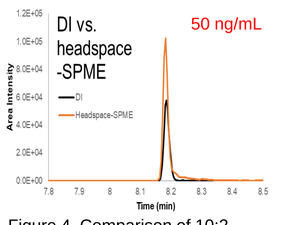


Figure 4. Comparison of 10:2 FTACr area intensity across 50 ng/mL calibration standard.

Figure 3:
- Calibration Curve: 2.5 ng/mL – 250 ng/mL; R^2 = 0.9999
- Quant ion still detected at 1 ng/mL, but Qual ions non-detect

Figure 4:
- Area larger for SPME vs. DI (with final volume = 1 mL) → SPME possibly more sensitive

Conclusions & Future Work

- SPME-headspace vs. LLE:
  - More time consuming, but no hands-on sample preparation needed
  - No possible loss of analytes from solvent evaporation
  - Possibly more sensitive
- A matrix-matched DI and SPME-headspace calibration curve needed to quantify limits of detection
- Determine possible reason for loss of target analytes with solvent evaporation under N_2