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Quantification of fluorotelomer-based chemicals in mammalian matrices by monitoring perfluoroalkyl chain fragments with GC/MS☆

W. Matthew Henderson[a,b,*], Eric J. Weber[a], Stephen E. Duirk[a], John W. Washington[a], Mary Alice Smith[b]

[a] National Exposure Research Laboratory, U.S. Environmental Protection Agency, Athens, GA 30605, USA
[b] Interdisciplinary Toxicology Program, College of Public Health, University of Georgia, Athens, GA 30602, USA

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Abstract

Perfluorocarboxylic acids (PFCAs), namely perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), have been identified as persistent, bioaccumulative and potentially toxic compounds. The structural analog, 8-2 fluorotelomer alcohol (8-2 fTOH) is considered the probable precursor of these stable metabolites. Because simultaneous quantification is needed for volatile and non-volatile perfluorinated chemicals (PFCs) in complex matrices, a GC/MS method was developed and tested based on selected ion monitoring of perfluorinated alkyl parent chain fragment ions. Although the method requires a derivatization step, combined GC/MS analysis of PFCA-me's and FTOHs increases analytical efficiency and decreases sample analysis time. The method instrument detection limits are between 7.1 and 24.5 ng/mL extract (MTBE), and the method quantification limits are below 50 ng/mL serum or ng/g liver for all PFCs investigated. Recoveries from mouse serum and liver homogenates, which were spiked with FTOHs and PFCAs at levels of 25 and 200 ng/mL or ng/g, ranged from 81 to 101%. Finally, the utility of the method was demonstrated by dosing male CD-1 mice with 30 mg/kg-BW of 8-2 fTOH and quantifying PFCs 6 h post-treatment. The advantages of this method are (1) the simultaneous detection of both volatile and non-volatile fluorotelomer-based chemicals in complex matrices, such as mammalian tissues, (2) as a confirmatory method to LC–MS/MS, and (3) as an alternative method of analysis for laboratories without access to LC–MS/MS.

Keywords: Perfluorinated chemicals; Fluorotelomer alcohols; PFOA; Method validation; Biological tissue analysis; GC/MS

1. Introduction

The concern over perfluorinated chemicals (PFCs) has been propagated by their widespread use in consumer products coupled with their persistence in the environment [1]. Of particular concern is the environmental and mammalian bioavailability of perfluorooctanoic acid (PFOA) which has been shown to be globally distributed [2], bioaccumulative [3,4], persistent and potentially toxic [5,6].

Sources for the release of PFOA into the environment include the thermal breakdown of perfluorinated polymers in cookware and internal combustion engines, losses from production and application facilities [7], and losses from treated consumer articles [1,8]. Neither of these source terms, however, seems likely to account for the global distribution of PFOA currently being reported. Some have hypothesized that the volatile perfluoroalkyl telomers released during preparation of telomer-based products, are readily transported via atmospheric processes and subsequently transformed into PFOA [8,9]. Of interest among the polyfluorinated telomers are the fluorotelomer alcohols (fTOH), in particular 8-2 fTOH. The FTOHs have been shown to be present in the North American troposphere at concentrations ranging from 11 to 165 pg/m^3 [8], and it is estimated that the global production in 2002 exceeded 6.5 million kg/year [7].

The β-oxidation of 8-2 fTOH, resulting in the formation of PFOA, has been shown to occur in the mammalian liver via liver peroxisomes for which the reactions mimic that of fatty-acid

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* Corresponding author at: National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Athens, GA 30605, USA. Tel.: +1 706 254 3117; fax: +1 706 355 8202.
E-mail address: Henderson.matt@epa.gov (W.M. Henderson).

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oxidation [10,11]. 8-2 fTOH has been shown to be the metabolized *in vitro* into PFOA by both rat and human hepatocytes [12,13]. Notably, no evidence has been found for subsequent metabolism of the terminal acids, PFOA or PFNA [11–15].

Liquid chromatography, tandem mass spectrometry (LC–MS/MS) is the predominant method utilized for the low-level, compound-specific quantification of PFCAs [16–18] while gas chromatography, mass spectrometry (GC/MS) methodology has been traditionally utilized for determination of FTOHs [19,20]. Although GC(NCI)/MS has proven useful for the detection of volatile fluorotelomer-based chemicals, resolution of intermediate and terminal acids resulted in significantly greater detection limits [19,21]. Because of the inter-relationship among these compounds, there is a great need to measure both of these types of analytes by a single technique, particularly in small biological samples [22].

Appropriate analytical methodology, sampling, sample preparation procedures, as well as applicable measurement techniques are being established by many researchers for PFCs that are amenable to analysis by LC–MS/MS, most notably PFOA [17,23]. However, use of LC–MS/MS is often problematic due to instrumental components that contain many of these fluorotelomer-based chemicals as well as their metabolic products. A few publications have reported the analysis and quantification of fluorotelomer alcohols (FTOHs) in mammalian matrices with detection limits comparable to this study [12,20]. An analysis of the 8-2 fTOH in biological matrices (rat serum, liver and kidney tissue) has been established, but did not focus on the simultaneous analysis of both volatile and non-volatile metabolites [20].

We report a method for the simultaneous analysis of C6–C12 PFCs (FTOHs and methyl esters of PFCAs) in both serum and liver. Following ion-pair extraction of PFCAs and derivatization with diazomethane, extracts that also contain the FTOHs were analyzed with GC/MS using selected ion monitoring (SIM) of the perfluoroalkyl parent chain fragments produced by electron ionization (EI). The mass fragments produced by the FTOHs and PFCA-me's are unlikely to be inherently present in biological samples as evidenced by blank matrix analysis of both serum and liver. Monitoring the ratios of three fragment ions determined in standards (relative to a quantification ion) coupled with retention times of the standards allows PFC analysis and quantification.

2. Experimental materials and methods

2.1. Chemicals

All chemical reagents used in this study were obtained at the highest purity, greater than 98% as determined by the supplier. Perfluorooctanoic acid (PFOA; CAS Number 335-67-1), perfluorononanoic acid (PFNA; CAS Number 375-95-1), 8-2 fluorotelomer alcohol (8-2 fTOH; CAS Number 678-39-7), methyl perfluorooctanoate (PFOA-me; CAS Number 376-27-2), methyl perfluorononanoate (PFNA-me; CAS Number 51502-45-5), methyl perfluoroheptanoate (PFHA-me; CAS Number 14312-89-1), and 3-(perfluorooctyl) propanol (3-PFOP; CAS Number 1651-41-8), were purchased from Oakwood Research Chemicals (West Columbia, SC) as were all other PFCs used for comparative analysis. Both 8-2 fluorotelomer acid (8-2 fTCA; CAS Number 27854-31-5) and 8-2 fluorotelomer unsaturated acid (8-2 fTUCA; CAS Number 70887-84-2) were purchased from TerraChem, Incorporated (Shawnee Mission, KS). Tetrabutylammonium hydrogen sulfate (TBAHS) and sodium carbonate, were purchased from Aldrich Chemical (Milwaukee WI), as were Diazald®, carbitol, and potassium hydroxide.

2.2. Mammalian matrices

Male CD-1 mice were purchased from Charles River Laboratory (Raleigh, NC). Before initiating the study, animals were allowed to acclimate for 7 days in micro-isolator cages (five animals per cage). The cages were housed in temperature and humidity controlled rooms maintained at 21–26 °C and 30–70% RH with a 12 h light/dark cycle. Animals were fed Lab Diet Certified Rodent Chow with both food and water provided *ad libitum*. Mice were gavaged with 8-2 fTOH in a 1:1 mixture of deionized water: propylene glycol at a dose ratio of 30 mg/kg body weight (BW). Animals were sacrificed 6 h post-treatment by carbon-dioxide asphyxiation and blood and liver samples were collected for PFC analysis. Tissues for spike-recovery experiments were similarly obtained from animals receiving vehicle controls. All tissues were flash frozen with a combination of liquid nitrogen and dry ice, and stored at −80 °C until analyses were complete.

2.3. Calibration standard preparation and matrix spiking

Concentrated solutions (5 and 40 μg/mL) of each standard were prepared in MTBE and diluted as required. Recovery experiments in thawed mammalian matrices were conducted by spiking 1 mL of serum or 1 g of liver with 5 μL concentrated standard solutions in methanol to achieve final PFC levels of 25 or 200 ng/mL or ng/g, respectively.

2.4. Sample extraction and derivatization

With slight modifications, PFCAs and FTCAs in serum and liver homogenates were extracted using a previously published ion-pair extraction method [22]. The method enabled concurrent liquid–liquid extraction of the FTOHs and PFCAs. Briefly, 1 mL of serum was added to 1 mL of 0.5 M TBAHS solution (adjusted to pH 10 with sodium hydroxide), 2 mL of 0.25 M sodium carbonate buffer and 10 μL of internal standard (20 μg/mL PFHA-me and 100 μg/mL 3-PFOP) in a 15 mL polypropylene tube. After thorough mixing, 2 mL MTBE was added to the solution and the solution vortexed for 15 min. The mixture was centrifuged for 10 min and 1.75 mL of the organic layer was removed for GC/MS analysis of PFCs. Sequential MTBE washes did not dramatically increase analyte recovery and concentration of the MTBE extracts under nitrogen flow resulted in significant 8-2 fTOH losses (>40%). Because these steps did not improve extraction efficiency and method performance, additional MTBE washes and concentration steps were not included in the final methods (data not shown). The derivatization of

non-volatile metabolites (PFCAs and FTCAs) was performed by reacting the carboxylic acids with diazomethane to form the more volatile methyl esters. Diazomethane was generated with a Wheaton micro-generator (Aldrich Chemical Company, Milwaukee, WI) by reacting Diazald® with potassium hydroxide (KOH) as described by Ngan and Toofan [24]. Samples were derivatized by adding 0.75 mL of MTBE from the extraction step to 0.25 mL of ether-trapped diazomethane for 30 min.

Similarly, liver extractions were also performed using the combined liquid–liquid and ion-pair extraction technique. For the extraction of liver samples, a liver homogenate of 1.0 g of liver diluted in 1.0 mL of Milli-Q water was prepared using an electric homogenizer. The homogenate was transferred to a new polypropylene tube containing the same reagents as the serum extraction (discussed above).

2.5. GS/MS conditions

Samples were analyzed using a HP 6890 Series Gas Chromatograph equipped with a 5973 Mass Selective Detector and a HP 6890 Series Injector (Palo Alto, CA). The GC oven was equipped with a Restek (Bellefonte, PA) RTX200MS (30 m × 0.25 mm i.d., 0.5 μm d.f.) trifluoropropylmethylpolysiloxane-phase column. Samples were introduced (2 μL) into the capillary inlet operated in splitless mode (injector temperature 225 °C). The oven temperature was held for 1 min at 60 °C, ramped 5 °C/min to 150 °C, increased 50 °C/min to 250°C and then held for 4 min (total run time equals 25 min) under constant pressure (1.02 psi) with an approximate flow of 0.5 mL/min. The mass spectrometer was operated in selected ion monitoring (SIM) mode with scanning at *m/z* values 131, 169, 231, and 331 with a dwell time of 100 ms. The carrier gas was helium and the transfer line maintained at 280 °C. Furthermore, the MS quad and source were set at 150 °C and 230 °C, respectively, with an electron energy of −70 ev and 1294 EM volts. All silicone and Teflon®-based analytical products were avoided during sample preparation and GC/MS analysis.

3. Results and discussion

The majority of current analytical techniques rely on LC–MS/MS for the detection and quantification perfluorocarboxylic acids (PFCAs) and GC/MS for the detection of volatile, precursor compounds. Our effort has focused on the development of analytical methodology for telomer alcohols (namely 8-2 fTOH), as well as the metabolic products of 8-2 fTOH, predominately PFOA and PFNA. In addition, the analytical method allows monitoring of the intermediate, non-volatile FTCAs, 8-2 fTCA and 8-2 fTUCA which are known to be transient metabolites of 8-2 fTOH [12–14]. Based on the articles by Martin et al. [19] and Ellis and Mabury [21], initial trials in our laboratory utilized negative chemical ionization (NCI) GC/MS for the detection of fluorotelomer-based chemicals. While NCI allowed more sensitive detection limits for the 8-2 fTOH (7 ppb as reported by Martin et al. [19] and approximately 4 ppb in our efforts), chromatography could not resolve the perfluorinated acids and/or their methyl ester counterparts using chemical ionization. Using GC(NCI)/MS for the analysis of both PFOA-me and PFNA-me resulted in detection limits greater than those established for the current EI method (data not shown).

Although this method results in IDLs and MDLs higher than literature reported values of non-volatile PFCs, it has the advantage of simultaneously quantifying both volatile and non-volatile fluorotelomer-based chemicals. In addition, monitoring perfluoroalkyl parent chain ions with GC/MS also has the ability to identify unknown metabolites by coupling other derivatization techniques. Disadvantages include higher IDLs and MDLs for non-volatile PFCs and extended sample preparation (approximately 45 min/8 samples). However, this method can also be used as a confirmatory method to LC–MS/MS, and offers a reliable alternative method of fluorotelomer-based chemical analysis by small-scale laboratories.

3.1. Method development and characterization (GC/MS analysis)

Initially, electron ionization (EI) spectra of the PFCs were generated over the range of 40–500 atomic mass units (amu), for 8-2 fTOH, the methyl esters of PFOA and PFNA, the methyl esters of the intermediate compounds (8-2 fTCA and 8-2 fTUCA), and the internal standards (PFHA-Me and 3-PFOP). Using the chromatographic conditions outlined in the methods section, fragment ions suitable for detection and quantification with GC/MS were selected. Each of the spectra contained the deprotonated molecular ion, but in low abundance, and therefore, quantification based on these ions would result in higher limits of detection. Upon investigating the fragmentation patterns, the majority of ions were less than 150 *m/z* as reported by Szostek and Pricket [20]. However, quantification was based on ions determined to be common among PFCs and included ions of *m/z* 131, 169, 231, 331 (Fig. 1). Chemical structures associated with these ions are C_3F_5, C_3F_7, C_5F_9 and C_7F_{13}, respectively, and are expected to include unsaturated-secondary and saturated-primary perfluorinated carbonium ions. These ions were formed by fragmentation of the perfluoroalkyl tail associated with the compounds of interest in 8-2 fTOH metabolism and degradation. Furthermore, it is also hypothesized that monitoring for these common ions allows identification of currently unknown structurally related, volatile fluorotelomer-based chemicals.

The method was tested with FTOHs ranging from 8 to 12 carbons (6-2 fTOH to 10-2 fTOH) at 500 ng/mL and PFCA-me's ranging from 6 to 12 carbons at 250 ng/mL (data not shown), all with fragmentation patterns containing the perfluoroalkyl chain ions. The range of PFCA-me's tested, and commercially available FTOHs, all contained the desired mass fragments except the shorter chained perfluorohexanoic acid (PFHxA) and 4–2 fTOH. These two chemicals, due to smaller molecular weight, are absent of mass fragment 331 *m/z* and were quantified based on ions 131, 169, and 231 *m/z*. Because of PFHxA's increased volatility as a short-chain methyl ester, the initial hold time (1 min at 60 °C) had to be increased to 3 min to prevent PFHxA from eluting with the solvent front. Instrumental response, based on area under the curve, increases for both homolog series of

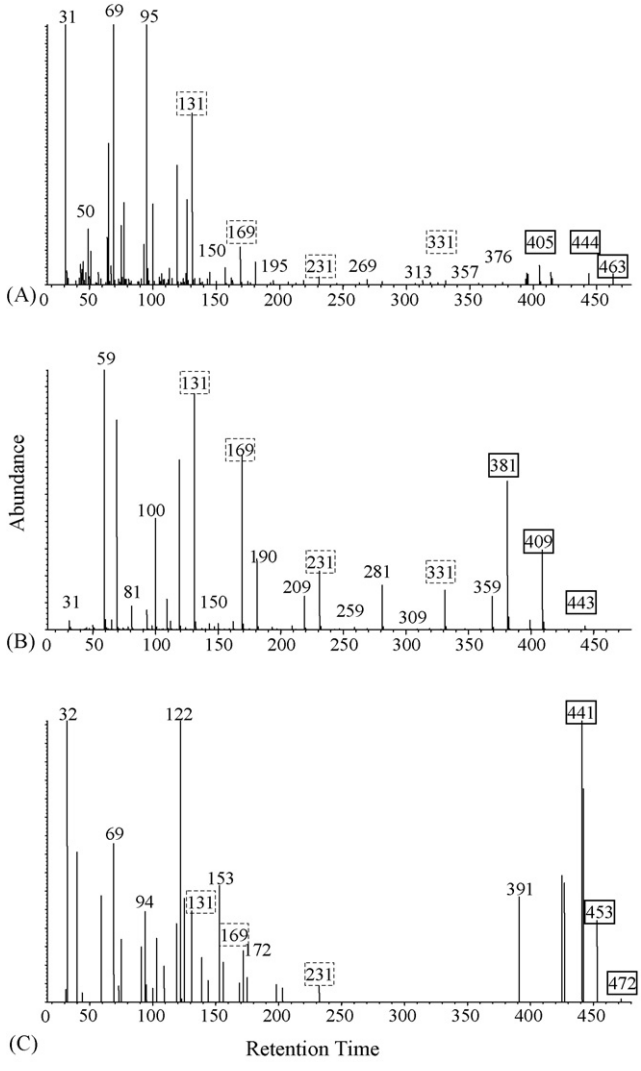

Fig. 1. Representative mass spectra of 8-2 fTOH (A), and the derivatized metabolites PFOA-me (B), and 8-2 fTUCA-me (C). Chemical-specific ions, structurally related to each compound, are denoted by solid boxes and those common to the group of PFCs investigated in this study are illustrated by dashed squares.

PFCA-me's and FTOHs, reaching maximum values when 10 carbons are present and plateaus for longer chain compounds. Peak shouldering occurred early in the method run (PFHxA-me and PFOA-me) and can be attributed to the 5 °C/min ramp. This

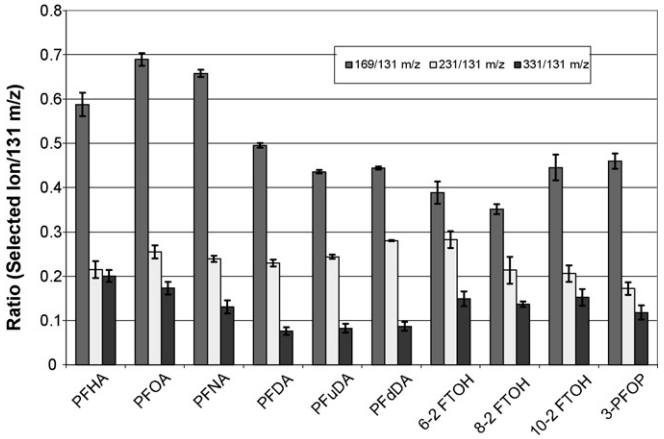

Fig. 2. Ratio of average abundance of ions 169, 231, and 331 *m/z* normalized to 131 *m/z* for PFHA-me (C7 acid methyl ester) through PFdDA-me (C12 acid methyl ester) and the 6-2, 8-2, and 10-2 FTOHs in MTBE. 3-PFOP and PFHA-me are used as internal standards for the FTOHs and FTCA-me's, respectively.

effect was experimentally corrected by incrementally increasing this rate; however, peak separation for compounds eluting later in the chromatography was compromised. Therefore, integration was conducted including the peak shoulders and correlation coefficients (r^2) exceeded 0.99 (Table 1).

When examining the relative abundance of the ions monitored, 131 and 231 *m/z* increase with increasing chain length in PFCAs, while ion 331 *m/z* abundance decreases (Fig. 2). An increasing trend was apparent with ion 169 *m/z* in the group of FTOHs examined while the abundance of 231 and 331 *m/z* remained constant. The EI spectra of 6-2 fTOH and 8-2 fTOH have been previously investigated by Napoli et al. [25]. In agreement with their research, similarly dominant, structurally-specific ions were noted for the FTOHs. The developed method was compared with GC/MS conditions relying on the chemical-specific, parent ions (Fig. 1). Based on area under the curve, the method monitoring for mass fragments 131, 169, 231, and 331 *m/z* allowed for greater sensitivity and lower detection limits than previously established analytical techniques. Thus, quantification of each analyte was calculated using the area under the curve for all ions monitored. Furthermore, quantification based on the ion with the largest abundance (*m/z* 131 for all PFCs) was investigated. When all

Table 1
Fragmentation patterns, retention times, and method validation parameters for PFCs implicated in 8-2 fTOH metabolism

Compound	Molecular weight (amu)	*RT* (min)[a]	Calibration range (ng/mL); r^2[b]	Estimated IDL[c] (ng/mL)
8-2 fTOH	464.12	11.11	5–500; 0.9908	5.6
8-2 fTCA-me	478.11	12.08	5–200; 0.9916	24.5
8-2 fTUCA-me	458.10	12.83	5–200; 0.9959	7.1
PFNA-me	478.11	7.90	5–500; 0.9977	4.5
PFOA-me	428.10	6.28	5–500; 0.9924	5.5
PFHA-me[d]	378.09	4.90	10–250; 0.9912	7.6
3-PFOP[d]	478.14	14.91	10–250; 0.9935	11.2

[a] Retention time of chemical under chromatographic conditions outlined above.
[b] r^2 – Correlation coefficient for regression analysis over calibration range.
[c] Instrumental detection limit – concentration equivalent to three times the standard deviation of replicates ($n \geq 7$) in reagent blank (MTBE).
[d] PFHA-me and 3-PFOP were used as internal standards for the PFCA-me's and FTOHs, respectively.

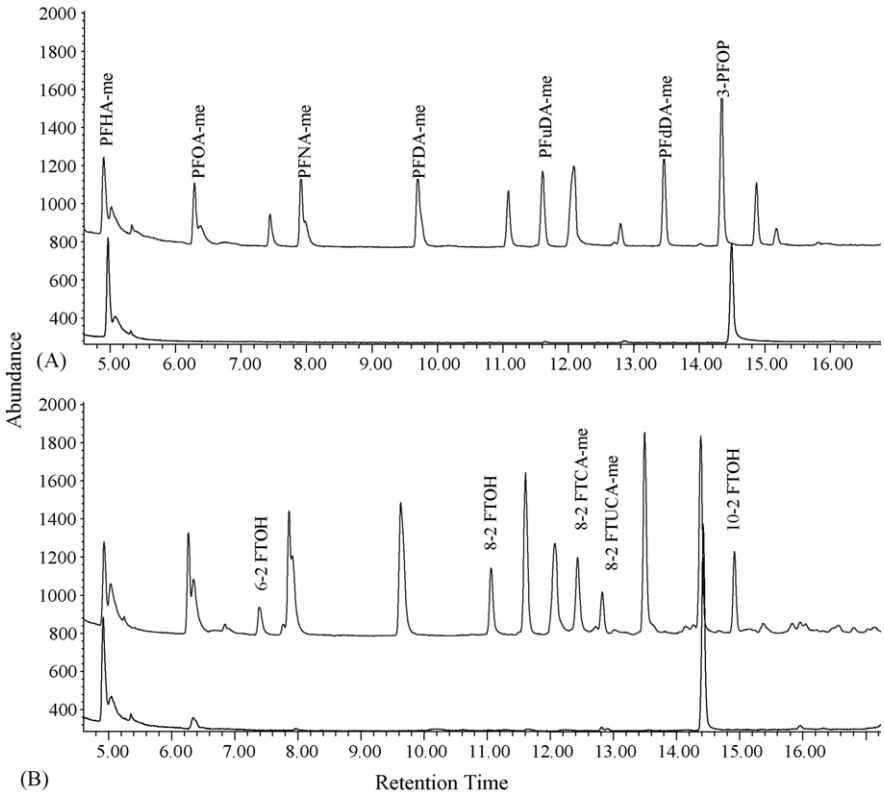

Fig. 3. Representative chromatograms of serum (A) and liver (B) spike-recovery experiments, including matrix blanks (bottom plot), using the developed method for monitoring fragments 131, 169, 231, and 331 *m/z*. Internal standards and PFCA-me's are labeled in (A) and FTOHs and intermediate 8-2 fTCA-me's identified in (B). Chromatographic plots have been offset by a factor of +500 units to aid in visualization of peak height and area under the curve differences.

ions were normalized to mass fragment 131 *m/z*, similar trends were noted and quantification and confirmation can be based on two qualifying ions (*m/z* 169 and 231) in ratio to the quantifying ion (*m/z* 131) coupled with standard retention times in each matrix (Fig. 2). Specificity can be added by monitoring for ions 59 or 95 *m/z* that are characteristic for methyl esters and alcohols and to further confirm compound classes (Fig. 3).

3.2. Detection limits and calibration

Based on United States Environmental Protection Agency (U.S. EPA) guidelines (40 CFR part 136, Appendix B.), IDLs were defined as the concentration (ng/mL) equivalent to three times the standard deviation of replicate instrumental measurements of the target analyte in MTBE. To establish the method quantification limit, a minimum of seven samples of each matrix (serum and liver), at both the low and high spike levels, were processed through the entire preparation and analytical method; including ion-pair extraction and derivatization with diazomethane. The standard deviation (σ) of the replicates was computed, and the MQL was determined by multiplying σ times a Student's *t*-value (STV) appropriate for a 99% confidence level (i.e., $n = 7$, STV = 3.143). Instrument detection limits for all of the target analytes were determined to be in the low ng/mL range (Table 1). Average IDLs for fTOH target analytes in MTBE ranged from 5.6 to 9.0 ng/mL, the PFCA-me analytes in MTBE ranged from 4.5 to 7.6 ng/mL, and the FTCAs ranged from 7.1 to 24.5 ng/mL in MTBE (Table 1). Calibration data were then obtained on a linear range from 5 to 500 ng/mL depending on the analyte, using at least 5 standards for each calibration. All analytes had an r^2 value greater than 0.99. Method quantification limits for each analyte, calculated at both 25 and 200 ng/mL serum or ng/g liver, are reported in Table 2.

Within-day precision was tested by injecting 10 replicate samples (25 ng/mL or ng/g) processed through extraction and derivatization, to compare repetitive instrumental response and to further validate % extraction and derivatization. Between-day precision of GC/MS analysis was determined by repeatedly analyzing the samples from above over a 7 day period ($n = 5$ replicates/day for days 1, 5 and 7). The coefficient of variation for between-day precision was slightly greater than within-day most notably when comparing values for 8-2 fTOH, 8-2 fTCA and 8-2 fTUCA (Table 2). This is expected due to the stability of the intermediate acid metabolites and the volatility of 8-2 fTOH [26].

Standard solutions were also made in methanol, MTBE and acetonitrile to determine which solvent worked best for the developed chromatographic conditions. As published by Szostek and Prickett [20], solvent selection impacted the response, baseline stability, and signals to noise (S/N) ratios obtained. MTBE resulted in minimal background/solvent interference, sharp analyte peaks and reproducibility compared to the other solvents (data not shown). MTBE was also the preferred solvent due to its use in the ion-pair extraction method

Table 2
Method performance parameters for volatile and non-volatile PFCs extracted from serum or liver samples

Compound	Estimated MQL[a]		Within-day precision (COV)[b]	Between-day precision (COV)[c]
	Low[d]	High[e]	Low	Low
8-2 fTOH	15.6	22.8	2.7	13.8
8-2 fTCA	ND[f]	49.5	5.6	8.6
8-2 fTUCA	ND[f]	25.4	9.6	11.3
PFNA	13.9	17.3	2.9	8.0
PFOA	9.3	11.9	3.3	8.0
PFHA[g]	18.1	21.3	5.0	5.8
3-PFOP[g]	17.3	16.8	7.2	9.0

[a] Method quantification limit – concentration equivalent to three times the standard deviation of replicates processed through the entire method ($n \geq 7$) appropriate for a 99% confidence interval. MQL presented is the average from both serum and liver samples.
[b] COV – Coefficient of variation for within-day accuracy ($n \geq 7$) calculated as the average of serum and liver values at the low spike level.
[c] COV – Coefficient of variation for between-day accuracy over a period of 7 days ($n = 5$ replicates/day for days 1, 5 and 7) calculated as the average of serum and liver values at the low spike level.
[d] Low = Target spike level of 25 ng/mL serum or ng/g liver.
[e] High = Target spike level of 200 ng/mL serum or ng/g liver.
[f] ND = Not determined because the MQL's were above 25 ng/mL (ng/g) spike-level and were therefore calculated based on 200 ng/mL (ng/g) concentration.
[g] PFHA-me and 3-PFOP were used as internal standards for the PFCA-me's and FTOHs, respectively.

modified by Hansen et al. [22] and as a diazomethane solvent in the derivatization step.

3.3. Method performance (extraction and derivatization)

Percent derivatization experiments were conducted using MTBE as the ethereal trap for diazomethane in solvent blank, and in serum and liver homogenates. To determine percent derivatization the following were processed with diazomethane as described above: PFHA, PFOA, PFNA, 8-2 fTCA and 8-2 fTUCA. PFHA, PFOA and PFNA methyl ester standards were used in calculating derivatization for the two perfluorocarboxylates. Standard solutions of the carboxylic acids and other PFCAs of interest were prepared (see Section 2), derivatized and the area under the curve compared to that of their respective methyl ester (me) standards (Table 3). Percent derivatization increased as the carbon chain decreased for the series of PFCAs investigated. Because PFHA-me was the shortest chain PFCA tested and coupled with the improved performance of the modified derivatization technique employed, PFHA-me was used as an internal standard to more accurately reflect concentrations of PFOA and PFNA and the telomer acids in our samples.

Table 3
Validation parameters for volatile and non-volatile PFCs extracted from both serum and liver samples

Compound	% Derivatization[a]	% Recovery (Serum)[b]	% Recovery (Liver)[b]
8-2 fTOH	91 ± 3.5[c]	88 ± 6.7	84 ± 6.3
8-2 fTCA	ND[d]	91 ± 4.9	89 ± 2.6
8-2 fTUCA	ND[d]	93 ± 1.9	81 ± 5.9
PFNA	92 ± 3.6	98 ± 2.4	89 ± 8.3
PFOA	98 ± 4.1	101 ± 2.1	90 ± 3.7
PFHA[e]	114 ± 9.2	84 ± 5.6	81 ± 4.8
3-PFOP[e]	99 ± 6.4[c]	92 ± 3.7	90 ± 6.1

[a] Average % derivatization for $n = 10$ replicates ± standard deviation.
[b] Average % recovery for $n = 10$ spiked replicates for each matrix sample ± standard deviation at the 25 ng/mL spike level.
[c] % derivatization for 8-2 fTOH and 3-PFOP is considered amount surviving derivatization conditions.
[d] ND = Not determined because standards are not currently commercially available.
[e] PFHA-me and 3-PFOP were used as internal standards for the PFCA-me's and FTOHs, respectively.

As expected (due to minimal matrix interference), percent derivatization was greatest in MTBE blanks. The complexity of the tissue (liver more complex than serum), aids in explaining the decrease in % derivatization in the liver. Increasing the derivatization time for the liver extracts (≥45 min) increased derivatization performance.

Several methodologies for the production of diazomethane were evaluated (data not shown). The formation of diazomethane by reacting *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) with sodium hydroxide (NaOH) resulted in significantly lower percent derivatization than did the method utilizing Diazald® [24]. Derivatization for all compounds exceeded 90%, which is in agreement with those published by Ngan and Toofan [24]. Average percent derivatization for each compound is listed in Table 2. Previously mentioned temporal modifications decreased standard error across matrices to within 5% (data not shown). Furthermore, percent derivatization (Table 3) for 8-2 fTOH and 3-PFOP is considered the amount remaining after derivatization when compared to standard solutions.

The methyl esters of the intermediate metabolites are not commercially available; therefore percent derivatization was not evaluated for these compounds. Extraction and recovery were estimated for 8-2 fTCA and 8-2 fTUCA from standard curves constructed from derivatized (assumed 100%) standards. Formation of the FTUCAs from their corresponding FTCAs was shown to occur in methanol and water by Loewen et al. [26]; therefore, consideration must be given when quantifying these compounds to avoid possible confounding effects due to experimental conversion. It was assumed that heat generated by the reaction with diazomethane resulted in conversion of 8-2 fTCA to 8-2 fTUCA (approximately 8%); however, heat generated did not significantly reduce the yield of the other analytes.

3.4. Mammalian matrices spike-recovery experiments

Extraction efficiencies for each matrix are presented in Table 3 as are the data obtained from method validation in mouse serum and liver homogenates. Recovery in mammalian matrices exceeded 81% for all PFCs investigated. Hansen et al. [22] first reported utilization of ion-pair extraction for PFOA determination in mammalian matrices, for both serum and liver. For PFOA, compiled extraction efficiencies for serum and liver averaged 101 and 87%, respectively [22]. In agreement with the results of Hansen et al. [22], extraction efficiencies for perfluorocarboxylates in this study were greater in serum than liver, and percent extraction decreased with increasing perfluoroalkyl chain length.

3.5. Quantitative analysis of 8-2 fTOH metabolism

Following the procedure outlined above and based on the proposed metabolism pathway of the 8-2 fTOH, GC/MS analysis was conducted for 8-2 fTOH, 8-2 fTCA, 8-2 fTUCA, PFNA, and PFOA. Concentrations of 8-2 fTOH, 6 h after treatment in both serum and liver were 97 ± 26 ng/mL and 134 ± 42 ng/g, respectively. Several studies have illustrated that the metabolism of 8-2 fTOH is rapid and most of the parent compound is biotransformed at 6 h post-treatment *in vivo* and between 1-3 h *in vitro* [11,12]. Martin and co-workers determined that at 4 h post-treatment *in vitro*, 78% of the parent fTOH had been metabolized; however, metabolites only accounted for approximately 8.5% total molar mass [12]. Concentrations of metabolites present exhibited the following trend; 8-2 fTUCA > 8-2 fTCA > PFOA > PFNA [12]. In serum, the concentration of PFOA was 972 ± 44 ng/mL and 277 ± 29 ng/g in liver while PFNA concentrations were 65 ± 15 ng/mL and 60 ± 22 ng/g, respectively. All data was validated with LC/MS–MS using the method of Powley et al. [18] with less than 20% variation.

3.6. Method significance

The present study describes a method for the simultaneous determination of several FTOHs and PFCA-me's by a GC/MS analysis in SIM mode. A selective and sensitive method for analysis of perfluorinated chemicals (PFCs) in both environmental and mammalian tissue matrices included monitoring for mass fragments 131, 169, 231, and 331 *m/z*, which are all common to the perfluorinated alkyl chains of this class of compounds. The IDL of the method ranges from 4.5 to 24.5 ng/mL, depending on the analyte. The average IDL for FTOHs and terminal PFCA-me's averaged 5.1 ng/mL (MTBE) while the MQL for all analytes was less than 50.0 ng/mL (ng/g). This study demonstrates that this method can be used to measure perfluorinated compounds in environmental samples and mammalian matrices. Although average IDLs and MQLs are higher than currently reported methods, this method has the advantage of simultaneously detecting both volatile and non-volatile fluorotelomer-based chemicals in complex matrices.

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References

[1] M.J. Dinglasan-Panlilio, S.A. Mabury, Environ. Sci. Technol. 40 (2006) 1447.
[2] J.P. Giesy, K. Kannan, P.D. Jones, Sci. World. 1 (2001) 627.
[3] J.W. Martin, D.M. Whittle, D.C. Muir, S.A. Mabury, Environ. Sci. Technol. 38 (2004) 5379.
[4] K. Kannan, L. Tao, E. Sinclair, S.D. Pastva, D.J. Jude, J.P. Giesy, Arch. Environ. Contam. Toxicol. 48 (2005) 559.
[5] G.L. Kennedy Jr., J.L. Butenhoff, G.W. Olsen, J.C. O'Connor, A.M. Seacat, R.G. Perkins, L.B. Biegel, S.R. Murphy, D.G. Farrar, Crit. Rev. Toxicol. 34 (2004) 351.
[6] C. Lau, J.L. Butenhoff, J.M. Rogers, Toxicol. Appl. Pharmacol. 198 (2004) 231.
[7] K. Prevedouros, I.T. Cousins, R.C. Buck, S.H. Korzeniowski, Environ. Sci. Technol. 40 (2006) 32.
[8] N.L. Stock, F.K. Lau, D.A. Ellis, J.W. Martin, D.C. Muir, S.A. Mabury, Environ Sci Technol 38 (2004) 991.
[9] D.A. Ellis, J.W. Martin, A.O. De Silva, S.A. Mabury, M.D. Hurley, M.P. Sulbaek Andersen, T.J. Wallington, Environ. Sci. Technol. 38 (2004) 3316.
[10] N. Kudo, N. Bandai, E. Suzuki, M. Katakura, Y. Kawashima, Chem. Biol. Interact. 124 (2000) 119.
[11] N. Kudo, Y. Iwase, H. Okayachi, Y. Yamakawa, Y. Kawashima, Toxicol. Sci. 86 (2005) 231.
[12] J.W. Martin, S.A. Mabury, P.J. O'Brien, Chem. Biol. Interact. 155 (2005) 165.
[13] M.J. Dinglasan, Y. Ye, E.A. Edwards, S.A. Mabury, Environ. Sci. Technol. 38 (2004) 2857.
[14] W.J. Fasano, S.C. Carpenter, S.A. Gannon, T.A. Snow, J.C. Stadler, G.L. Kennedy, R.C. Buck, S.H. Korzeniowski, P.M. Hinderliter, R.A. Kemper, Toxicol. Sci. 91 (2006) 341.
[15] N. Wang, B. Szostek, R.C. Buck, P.W. Folsom, L.M. Sulecki, V. Capka, W.R. Berti, J.T. Gannon, Environ. Sci. Technol. 39 (2005) 7516.
[16] C. Sottani, C. Minoia, Rapid Commun. Mass. Spectrom. 16 (2002) 650.
[17] J.M. Flaherty, P.D. Connolly, E.R. Decker, S.M. Kennedy, M.E. Ellefson, W.K. Reagen, B. Szostek, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 819 (2005) 329.
[18] C.R. Powley, S.W. George, T.W. Ryan, R.C. Buck, Anal. Chem. 77 (2005) 6353.
[19] J.W. Martin, D.C. Muir, C.A. Moody, D.A. Ellis, W.C. Kwan, K.R. Solomon, S.A. Mabury, Anal. Chem. 74 (2002) 584.
[20] B. Szostek, K.B. Prickett, J. Chromatogr. B 813 (2004) 313.
[21] D.A. Ellis, S.A. Mabury, J. Am. Soc. Mass Spectrom. 14 (2003) 1177.
[22] K.J. Hansen, L.A. Clemen, M.E. Ellefson, H.O. Johnson, Environ. Sci. Technol. 35 (2001) 766.
[23] M. Stadalius, P. Connolly, K. L'Empereur, J.M. Flaherty, T. Isemura, M.A. Kaiser, W. Knaup, M. Noguchi, J Chromatogr. A (2006).
[24] F. Ngan, M. Toofan, J. Chromatogr. Sci. 29 (1991) 8.
[25] M. Napoli, A. Scipioni, L. Conte, E. Legnaro, L.N. Krotz, J. Fluorine Chem. 66 (1994) 249.
[26] M. Loewen, T. Halldorson, F. Wang, G. Tomy, Environ. Sci. Technol. 39 (2005) 2944.