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Determination of 8:2 fluorotelomer alcohol in animal plasma and tissues by gas chromatography–mass spectrometry

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Abstract

Fluorotelomer alcohols (FTOHs) constitute an important group of compounds among the perfluoroalkyl substances (PFAS). The PFAS have recently been a focus of many environmental and biological studies. This generated a strong need for analytical methods for analysis of PFAS at trace levels in various environmental and biological matrices. A quantitative analytical method for analysis of 8:2 FTOH in rat plasma and rat liver, kidney, and adipose tissue using GC–MS with electron impact (EI) ionization was developed and validated. Extraction of water-diluted plasma with methyl *tert*-butyl ether (MTBE) was used for rat plasma. The analysis of rat liver or kidney tissues required homogenization of tissue on ice, extraction with hexane, and clean up of the extract by silica (Si) normal-phase solid phase extraction (SPE). Similarly, the adipose tissue was dissolved in *n*-heptane and cleaned up by Si SPE. The methods were validated by performing spike recovery experiments for each type of matrix investigated and tested on authentic samples originating from 8:2 FTOH toxicological studies.
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Keywords: Fluorotelomer alcohols; Biological tissues; GC–MS

1. Introduction

The availability of analytical methodology for determination of perfluoroalkyl substances (PFAS) in various biological and environmental matrices has recently attracted much attention as the environmental and toxicological effects of these compounds are debated [1]. Among various PFAS, the perfluorinated carboxylic acids and sulfonates have been the subject of many toxicological and environmental monitoring studies resulting in a relatively well-established analytical methodology for their measurement [2]. Fluorotelomer alcohols (FTOHs) represent a subgroup of PFAS, named after a production process called telomerization, and are used as intermediates for production of various fluorosurfactants and polymers [3,4]. The FTOHs are six to twelve carbon chain length, linear, partially fluorinated alcohols, their nomenclature derived from the number of fluorinated and hydrogenated carbons in the molecule (e.g. 8:2 FTOH for $CF_3–(CF_2)_7–CH_2–CH_2–OH$). The telomerization process results in fluorinated alcohols of even-number carbon chain lengths, the 6:2, 8:2, and 10:2 FTOH alcohols being the major components of the mixture. The environmental and biological fate of these alcohols, and especially 8:2 FTOH, have recently been a subject of many studies. Ellis et al. [5] studied their atmospheric fate; Stock et al. [6] investigated their occurrence in the North American troposphere. Wang et al. [7] and Dinglasan et al. [8] investigated the biotransformation of 8:2 FTOH in bacterial cultures. Hagen et al. [9] studied the metabolism of 8:2 FTOH in rats. As the environmental and toxicological significance of fluorotelomer alcohols is investigated and informed by new studies, there is a critical need for reliable analytical methodology to measure these compounds in biological and environmental matrices.

There are no published analytical methods for determination of fluorotelomer alcohols in complex biological or environmental matrices reported in literature. A method for determination of fluorotelomer alcohols in air samples (at-

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mospheric monitoring) was reported by Martin et al. [10]. GC–MS detection with negative and positive chemical ionization was used for the analysis of methanol/ethyl acetate extracts of air sampling medium and allowed instrumental detection of 0.8–20 pg fluorotelomer alcohols. Unusual fragmentation patterns observed for the fluorotelomer alcohols with chemical ionization were discussed by Ellis and Mabury [11]. The fragmentation patterns for fluorinated compounds including 6:2 FTOH obtained for electron impact ionization were investigated by Napoli et al. [12].

To the best of our knowledge, this paper represents the first published work describing the development and validation of an analytical method for determination of 8:2 FTOH in biological matrices. The development and validation was focused on rat plasma and rat tissues (adipose, liver, and kidney) and was mainly targeted towards supporting toxicological studies with 8:2 FTOH. However, the methodology and learnings developed should be easily transferable to other fluorotelomer alcohols and other biological matrices.

2. Experimental

2.1. Chemicals and standards

Solvents used were obtained from: methanol (HPLC grade), isopropanol (99.99%), methyl *tert*-butyl ether (MTBE) (99.99%), acetone (HPLC/GC grade) from EM Science, Gibbstown, NJ; Vertrel XF (1,1,1,2,3,4,4,5,5,5-decafluoropentane, 99%, from E.I. DuPont, Wilmington, DE); hexane (B&J GC^2) and ethyl acetate (HPLC/GC grade, >99.9%) from Burdick-Jackson, Muskegon, MI; *n*-heptane (ultra-resi analyzed) and diethyl ether (99.0%) from J.T. Baker, Phillipsburg, NJ. Perchloric acid (70%) was purchased from Aldrich, Milwaukee, WI.

The 1H,1H,2H,2H-perfluorodecan-1-ol (CF_3–$(CF_2)_7$–CH_2–CH_2–OH; 8:2 FTOH, 97.6%), 1H,1H,2H,2H-perfluorododecan-1-ol (CF_3–$(CF_2)_9$–CH_2–CH_2–OH; 10:2 FTOH, 98%), 1H,1H,2H,2H-perfluro-7-methyloctan-1-ol ($(CF_3)_2$–CF–$(CF_2)_4$–CH_2–CH_2–OH, 7:2 FA-iso, 98%), 1H,1H,2H,2H-perfluro-9-methyldecan-1-ol ($(CF_3)_2$–CF–$(CF_2)_6$–CH_2–CH_2–OH, 9:2 FA-iso, 98%), 1H,1H-perfluoro-1-nonanol (CF_3–$(CF_2)_7$–CH_2–OH; 8:1 FA, >95%), 1H,1H-perfluoro-1-decanol (CF_3–$(CF_2)_8$–CH_2–OH; 9:1 FA, >98%) were obtained from Oakwood Products Inc., West Columbia, SC.

2.2. Apparatus

HP 6890 Plus GC (Agilent, Palo Alto, CA) equipped with a HP split/splitless and programmable temperature vaporization (PTV) inlets, HP 5973 Mass Selective Detector (Agilent, Palo Alto, CA), MPS2-MultiPurposeSampler (Gerstal Inc., Baltimore, MD) was used for all data collection. Gas chromatographic separations were performed on a DB-5MS, 30 m × 0.25 mm, 1 μm film thickness column (Agilent, Palo Alto, CA). The GC oven program was: start at 80 °C and held for 2 min, ramped 20 °C/min to 120 °C, and ramped 50 °C/min to 300 °C and held for 3 min. Helium was used as the carrier gas at the flow rate of 1.0 mL/min in the constant flow mode. Typically 2 μL injections with 5:1 split ratio were performed at 250 °C inlet temperature. The MSD transfer line was kept at 280 °C for all experiments. This set of experimental conditions is referred to as system I. A hold time of 10 min at 300 °C and a 300 °C inlet temperature (system II) were used in final methods and during the later stages of method development. Typically, the ions *m/z* 31, 69, 95, and 131 were monitored in a single ion monitoring (SIM) mode and quantitation was performed using peak area of one, selected ion. Limited experiments were done using large volume injections with the PTV inlet. The GC oven program for these experiments was: start at 50 °C and held for 3 min, ramped 40 °C/min to 300 °C and held for 5 min. The PTV injector program was: start 20 °C, held for 0.2 min; ramp 700 °C/min to 300 °C; held for 1 min; vent time: 0.2 min; vent pressure 1 psi, vent flow 15 mL/min; purge flow 25 mL/min; purge time 1.2 min (system III).

All sample preparation was done in 15 mL polypropylene centrifuge tubes (Corning Inc., Corning, NY) for tissues and 1.7 mL conical micro-centrifuge tubes (VWR International, West Chester, PA) for plasma.

The SPE columns: isolute CN (cyanopropyl), isolute FL (florisil), isolute diol (2,3-dihydroxypropoxypropyl), isolute NH2 (aminopropyl); isolute Si (silica), including the 5 g/25 mL isolute Si for cleaning of hexane, and isolute 2.5 g sodium sulfate drying cartridges were purchased from International Sorbent Technology (Hengoed, UK).

2.3. Sample preparation

2.3.1. Stock solutions

Stock solutions of fluorinated alcohols were made by dissolving the alcohols in methanol at approximately 1000 μg/mL. These stocks were used for matrix spiking and preparation of calibration standards. The stock solutions were stored refrigerated and were stable for a period of at least 3 months. Fresh dilutions of the stock solutions in appropriate solvents were made with every set of samples analyzed. Spiking of the methanol stocks of analytes was done using GC syringes.

2.3.2. Extraction procedure for rat plasma

The optimized extraction procedure for determination of 8:2 FTOH in rat plasma involved use of 250 μL of rat plasma sample, spiking an appropriate amount of surrogate methanol stock (7:2 FA-iso), vortexing the sample, adding 250 μL of water and 1 mL of MTBE. The sample was then extracted by vortexing for 15 min and centrifuged for 10 min; then 0.6 mL of the MTBE extract was transferred to a glass GC vial, internal standard added (9:2 FA-iso), and the extract was an-

alyzed by GC–MS with system II conditions. The calibration standards containing a fixed amount of 9:2 FA-iso internal standard and variable amounts of 8:2 FTOH and 7:2 FA-iso were made in MTBE. GC–MS quantitation was based on peak areas ratios obtained for ion *m/z* 95. Calibration curves for analyte and surrogate were established by regression of appropriate peak area ratios (analyte or surrogate versus internal standard) versus the concentrations ratio.

2.3.3. Extraction and clean-up procedure for adipose, liver, and kidney tissue

The optimized procedure that was used for analysis of liver and kidney samples originating from toxicological studies involved mincing of the partially thawed tissue with a stainless steel razor blade and placing 0.5 g of minced tissue in a 15 mL plastic tube. Two milliliters of water was added, the 7:2 FA-iso surrogate was spiked, and 15 μL of concentrated perchloric acid was added. The tissue was homogenized holding the tube on ice and using a glass wand. Use of a Polytron homogenizer should be avoided as it leads to significant losses of analyte. After homogenization, 6 mL of cleaned hexane was added, and the analyte was extracted by vortexing the tube for 15 min. The tube was held for 1 min on dry ice and centrifuged for 15 min. The hexane layer was loaded onto a pre-cleaned (1 mL acetone) and conditioned (2 mL hexane) 500 mg Si column through the Na_2SO_4 drying cartridge. Six milliliters of hexane was used to wash the SPE column, and 1 mL isopropanol was used to elute the analyte. A 0.5 mL aliquot of isopropanol extract was spiked with internal standard (9:2 FA-iso) and analyzed by GC–MS with system II conditions. SIM of masses 69, 95, 131 *m/z* were monitored but quantitation was based on peak areas ratios obtained for ion *m/z* 95, using calculations analogous to these used for rat plasma. The calibration standards containing a fixed amount of 9:2 FA-iso internal standard and variable amounts of 8:2 FTOH and 7:2 FA-iso were made in isopropanol.

Initial tests revealed the need for clean up of the hexane as the impurities present in hexane severely interfered with the 8:2 FTOH analysis. This was done by passing up to 1 L of hexane through a 5 g Si SPE column.

Analysis of rat adipose tissues originating from toxicological studies was done by spiking 7:2 FA-iso surrogate to 0.3 g adipose tissue and dissolving the adipose tissue in 6 mL heptane, aided by glass-wand homogenization. The heptane extract was loaded on a 500 mg Si column that was preconditioned with 1 mL acetone and 2 mL heptane. The column was washed with 6 mL heptane and eluted with 1 mL isopropanol. A 0.5 mL aliquot of isopropanol extract was removed and spiked with 9:2 FA-iso internal standard. SIM of masses 69, 95, 131 *m/z* were acquired (system II conditions), but quantitation was based on peak areas ratios obtained for ion *m/z* 131. The calibration standards containing a fixed amount of 9:2 FA-iso internal standard and variable amounts of 8:2 FTOH and 7:2 FA-iso were made in isopropanol.

3. Results and discussion

3.1. EI spectra of fluorinated alcohols

Electron impact (EI) spectra of fluorinated alcohols listed in Section 2 were examined in order to select ions suitable for quantitation of 8:2 FTOH and potential internal standards. The EI spectra at 70 eV of selected alcohols were obtained by injection of approximately 10 μg/mL MTBE solutions of the alcohols. The spectra were collected using the chromatographic conditions described in Section 2 (system I). Fig. 1 presents the EI spectra obtained for 8:2 FTOH and 9:1 FA alcohols, representative of fluorinated alcohols containing two or one methylene groups in the structure. Generally, a low abundance deprotonated molecular ion was observed for all examined fluorinated alcohols. Several low abundance ions are also present above 300 *m/z*. However, their abundance including the deprotonated molecular ion is not sufficient to be used for quantitation at trace levels, even though monitoring of these ions would be desired from the matrix selectivity and molecule identification perspective. The majority of the signal in the spectrum is found in the ions of *m/z* less than 150. Ions of *m/z*: 31 ($CH_2{=}OH^+$), 69 (CF_3^+), 95 ($C_3F_3H_2^+$), 131 ($C_3F_5^+$) were considered for quantitation using a single ion monitoring mode. The ions of *m/z* 31, 69, and 131 were present in the EI spectra of all examined fluorinated alcohols. The ion *m/z* 95 is characteristic to fluorinated alcohols containing two methylene groups, as in 8:2 FTOH. The choice of ion that was selected for SIM quantitation was considered for each animal matrix and was dictated by the encountered interference problems and the method sensitivity requirements for each matrix.

3.2. Optimization of extraction procedure for rat plasma

3.2.1. Selection of extraction solvent

Several water miscible (methanol, isopropanol) and water immiscible (MTBE, ethyl ether, Vertrel XF, ethyl acetate) solvents were considered for the extraction of 8:2 FTOH from rat plasma. Initially, a mixed standard of four alcohols (8:1 FA, 9:1 FA, 8:2 FTOH, 10:2 FTOH) at 100 ng/mL was prepared in each of the solvents; the mixture and the solvents themselves were injected into the GC–MS system, monitoring ions 31, 69, and 131 *m/z*. The GC–MS parameters were essentially the same as for system I described in Section 2 with the exception that the second ramp of the GC oven program was 50 °C/min to 250 °C with the hold for 2 min. The number of background peaks derived from the solvent, potential of interference with examined alcohols, as well as the magnitude of the response for fluorinated alcohols were examined.

Injections of the fluorinated alcohol standards prepared in different solvents indicated differences between solvents in response obtained for the examined alcohols, baseline stability, and the number of background signals potentially interfering with the analysis. The most favorable results, taking into account the above considerations, were obtained for MTBE.

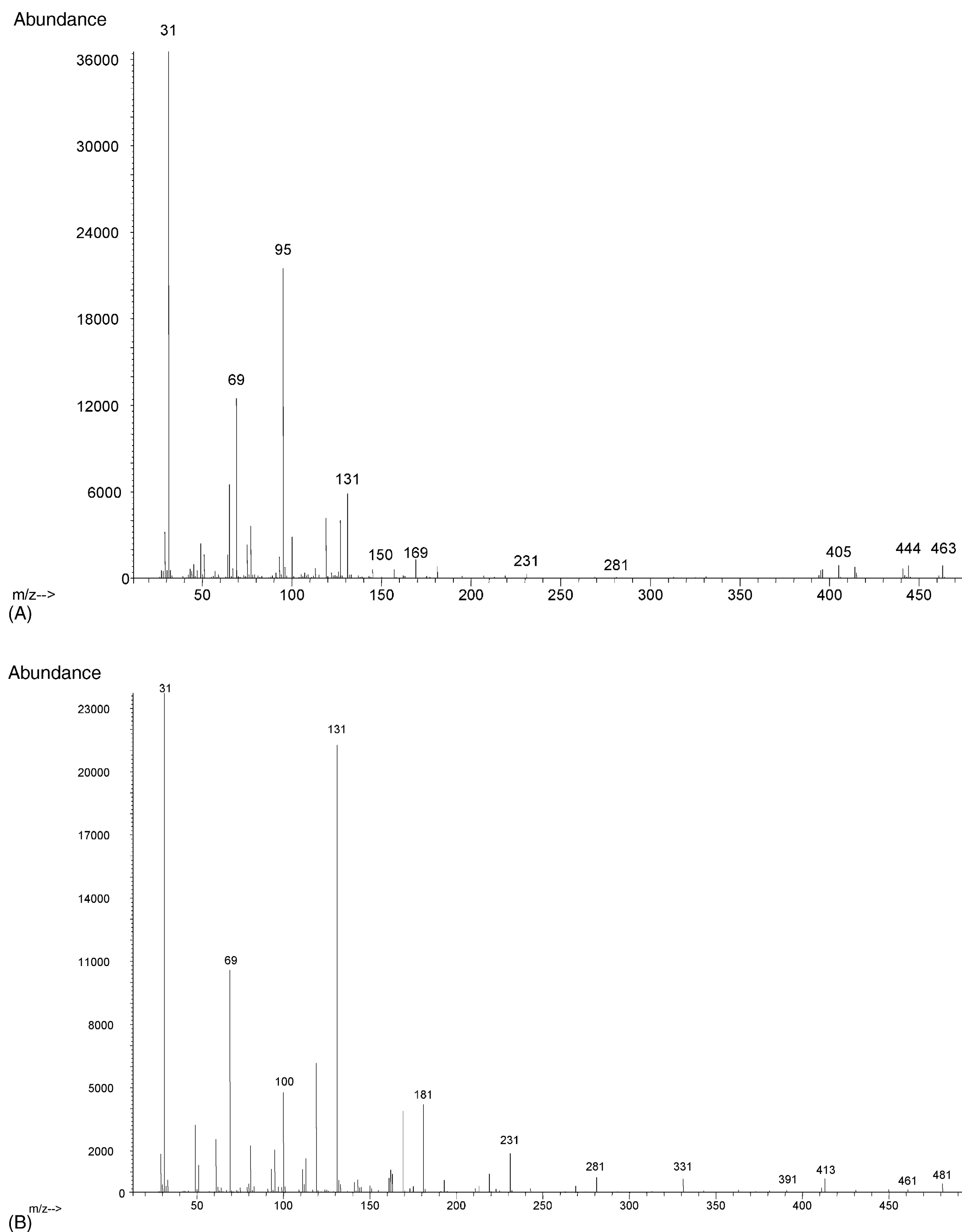

Fig. 1. EI spectra of 8:2 FTOH (A) and 9:1 FA (B).

However, isopropanol and Vertrel XF performed equally well in that respect. Ethyl acetate exhibited a low and flat baseline, but several large peaks were observed around the 3 min retention time, originating from the impurities present in the examined brand of the solvent. Ethyl ether, used as purchased, showed multiple signals originating from the solvent itself and interfered with the signals of the examined fluorinated alcohols. Fig. 2 compares the chromatograms of four examined fluorinated alcohols for the 100 ng/mL standard prepared in methanol and MTBE. The chromatogram for the standard made in methanol shows significantly higher background, sloping baseline, and lower response for the fluoroalcohols. The high background and sloping baseline for the methanol trace is mainly due to ion *m/z*: 31, characteristic for alcohols ($CH_2{=}OH^+$), and represents the tail of the solvent peak. However, the response for the other ions examined (*m/z*: 69

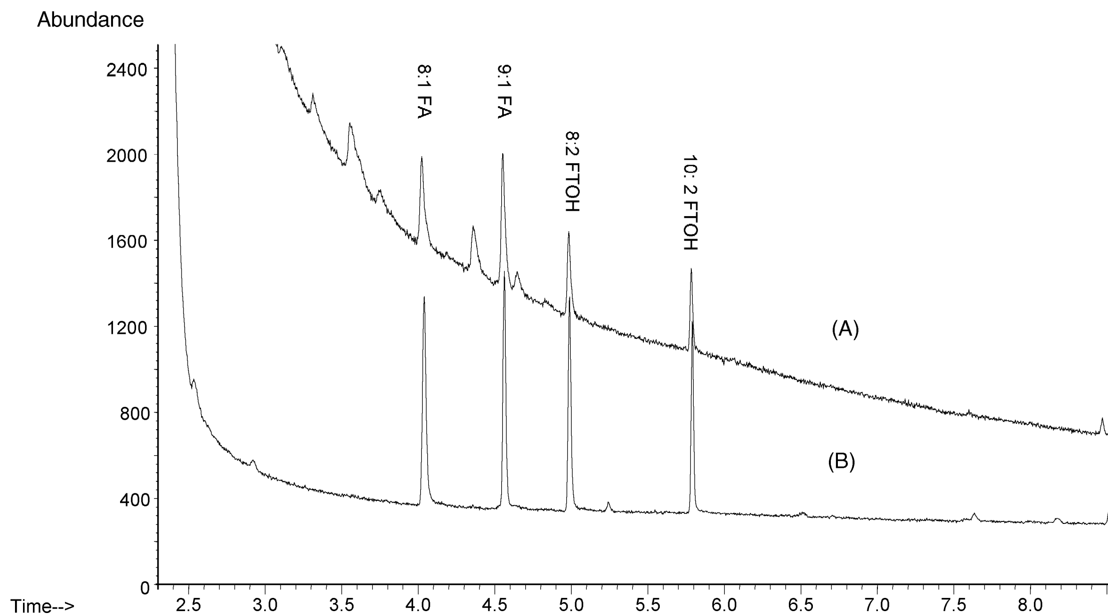

Fig. 2. Total ion chromatogram (ions *m*/*z*: 31, 69, 95, 131) for selected fluorinated alcohols injected in methanol (A) and MTBE (B).

and 131) in the case of methanol was only approximately 50% of that obtained with the MTBE standard, indicating a difference in response for these particular chromatographic conditions.

The second step in selection of appropriate extraction solvent for rat plasma was investigation of plasma extracts obtained for blank rat plasma with the following solvents: Vertrel XF, MTBE, ethyl acetate, methanol, and isopropanol. The extraction using water immiscible solvents was done by placing 100 μL of rat plasma in a 1.7 mL micro-centrifuge tube, adding 1 mL of water, and 0.5 mL of the appropriate solvent. Similarly, 100 μL of rat plasma and 0.5 mL of solvent were added to a 1.7 mL micro-centrifuge tube for the water miscible solvents. The contents of the tube were vortexed on a multi-tube vortexer for 15 min, followed by 5 min of centrifugation. An aliquot of the blank plasma extract was spiked with four alcohols at 100 ng/mL each. The blank and spiked solvent extracts were examined using the GC–MS method described above for pure solvents.

Blank and spiked rat plasma extracts obtained with different solvents were examined in order to select a solvent for further method optimization. The three water immiscible solvents gave similar results. However, Vertrel XF extraction of rat plasma would often result in extracts in a gelled form, which were very difficult to break up and unusable for further analysis. Therefore, Vertrel XF was not considered for further optimization.

MTBE and ethyl acetate were found to be similar in respect to extracting potential interferences from plasma, providing a relatively clean region of chromatogram where the fluorinated alcohols elute. Ethyl acetate by itself contains some impurities with large signals appearing before the 4 min retention time. The chromatograms obtained for methanol- and isopropanol-spiked rat plasma extracts showed that these solvents extract more compounds from rat plasma that directly interfere with the investigated fluorinated alcohols; the 8:2 FTOH signal especially is strongly affected by the presence of the interfering signal at 5 min. Neither of these solvents were pursued further as extraction solvents for plasma. Based on this initial screening and other considerations, discussed below, MTBE was chosen as the extraction solvent for further method optimization.

3.2.2. *Optimization of extraction procedure with MTBE*

The 8:2 FTOH is considered a semi-volatile compound. This property of the analyte significantly limits the options for procedures that can be used for extraction, concentration, solvent exchange, and clean up. The degree of analyte loss was investigated for MTBE solutions of 8:2 FTOH. The analyte is completely lost if the solvent is evaporated to dryness in an evaporator even at room temperature. Significant losses are also observed when the solvent (MTBE) is only partially evaporated in order to concentrate the solution.

Another limitation in the method development for 8:2 FTOH is the strong influence of the cleanness of the extract on the chromatographic performance (peak shape and response) of the GC–MS system. The contamination of the chromatographic column by the non-volatile components of the extract leads to significant peak broadening and signal reduction in consecutive injections. The rate of this performance degradation is dependent on the amount of the extract injected and the purity of the extract. Fig. 3 illustrates this for large volume injections of plasma extracts with system III conditions. The large volume injection using the PTV inlet was developed in order to increase the sensitivity of the analysis. This injection technique allowed detection of 8:2 FTOH in MTBE at less than 0.5 ng/mL with a 10 μL injection. However, relatively quick degradation of peak shape and response with

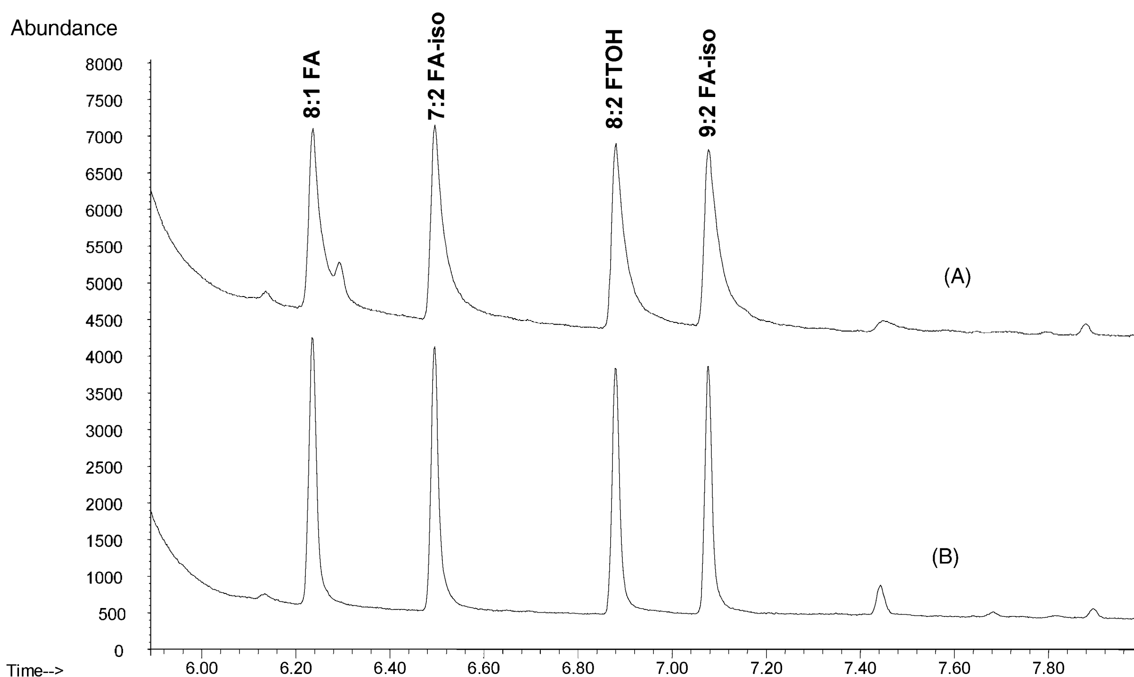

Fig. 3. Response for fluorinated alcohols obtained for the 10-ng/mL MTBE standard with a 10 μL large volume injections: initial (B), after eight injections of MTBE rat plasma extract (A). The baseline of (A) was offset by +4000 units for clear comparison.

limited number of injections made this approach not feasible for MTBE plasma extracts, without further clean up of MTBE extracts. These problems were also present for split injections, but the degradation of chromatographic performance was much slower because less extract was introduced into the column with a single injection. In order to restore the performance of the column, it had to be removed from the GC–MS system and cleaned with solvents: methanol, isopropanol, and hexane (6 mL of each).

The optimization of the extraction procedure for 8:2 FTOH involved finding a fluorinated alcohol that could serve the purpose of surrogate (spiked into a sample before the extraction procedure) and using a separate fluorinated alcohol as the internal standard (spiked into a sample extract before GC–MS analysis) in the method. The use of surrogate and internal standard in the method was intended to distinguish between the recoveries of the sample preparation procedure and changes of the instrumental response (matrix effects, column contamination). Simply, as the surrogate is spiked to each sample before extraction procedure, a recovery value is obtained for each sample preparation, allowing a better control of method performance. Initial experiments were done with 9:1 FA as the surrogate and 8:1 FA as the internal standard. However, the recovery of the 9:1 FA alcohol was much lower than that of 8:2 FTOH, practically eliminating it as the potential surrogate for this procedure. Two other alcohols were examined as potential surrogates, namely 7:2 FA-iso and 9:2 FA-iso.

The optimization of the extraction procedure was done for 100 μL of rat plasma. The amount of water needed to dilute plasma to enhance the extraction with a water immiscible solvent and the amount of MTBE were optimized to obtain a single-step extraction procedure to avoid any need for multiple sample transfers. Best recoveries for 8:2 FTOH were obtained when 0.5 mL of water and 0.5 mL MTBE were used with 100 μL of rat plasma. Using this procedure, recoveries of 8:2 FTOH, 7:2 FA-iso, and 9:2 FA-iso were tested for two different levels of spiking with vortexing time of 30 min. The 15 min vortexing time was also investigated. The potential losses of the 8:2 FTOH due to volatility during sample manipulation were tested by spiking plasma in the microcentrifuge tube, storing the capped tube overnight at room temperature, and subsequently extracting the analyte with MTBE. The results of these experiments are summarized in Table 1. Acceptable recoveries of 8:2 FTOH were obtained for all investigated conditions. The recoveries for the 7:2 FA-iso alcohol closely followed the ones obtained for 8:2 FTOH, indicating that 7:2 FA-iso can be used as the surrogate for 8:2 FTOH in this procedure. Additionally, 15 min of vortexing was sufficient for quantitative extraction. The final procedure that was applied to analysis of rat plasma samples originating from toxicological studies was modified to lower the detection limits. It involved use of 250 μL of rat plasma sample, as described in Section 2.

3.3. *Optimization of SPE clean-up procedure for rat tissue extracts*

The analysis of rat liver tissue was first attempted by injection of MTBE extracts obtained from direct extraction of homogenized liver with addition of water. However, a single injection of such an extract contaminated the GC column

Table 1
Percent recoveries for fluorinated alcohols from rat plasma

Compound	15 min vortex[a]	30 min vortex[a]	Overnight recovery[a]	250 ng/mL spike in rat plasma	50 ng/mL spike in rat plasma
8:2 FTOH	96 ± 7[b]	98 ± 5	96 ± 4	102 ± 18	90 ± 10
7:2 FA-iso	103 ± 4	99 ± 7	112 ± 7	105 ± 12	89 ± 7
9:2 FA-iso	81 ± 5	90 ± 6	89 ± 5	92 ± 29	87 ± 21

[a] 500 ng/mL spike in rat plasma; 8:1 FA used as internal standard.
[b] Percent recovery ± standard deviation.

so severely that the chromatographic peak shape and sensitivity was lost, and the column required extensive solvent cleaning. This experience led to a need to develop a clean-up procedure for tissue extracts. Taking into account the volatility of the analyte, the severe losses of analyte during solvent evaporation procedures and the choice of solvents that work best for GC–MS, the SPE selection was limited to the normal-phase mode. As there are no literature reports of SPE conditions for 8:2 FTOH, a range of loading and elution solvents was tested for a range of normal phase chemistry SPE columns. The selection of a loading and elution solvent was done for each column type by loading 2 mL of 500 ng/mL 8:2 FTOH in appropriate solvents on a SPE column and measuring the percent of 8:2 FTOH that passed through the column. The following solvents were tested: MTBE, hexane, acetone, methanol, isopropanol, Vertrel XF, ethyl acetate, and the following SPE columns were tested: amino (NH2), cyano (CN), diol, florisil (Fl), and silica (Si). Hexane was demonstrated to the best loading solvent of the solvents tested for each of the SPE column chemistry tested. These experiments also showed that MTBE was acceptable neither as a loading nor elution solvent because approximately 30–60% (depending on the column chemistry) of 8:2 FTOH passed through the SPE column. The weakest analyte retention was obtained for isopropanol, with the percent of 8:2 FTOH that passed through the SPE column ranging from 30 to 95% for Si column. Acceptable results were also obtained for acetone as the elution solvent. Therefore, further optimization of SPE clean up was narrowed to the use of hexane as a loading solvent, isopropanol or acetone as the elution solvent, and Si or NH2 as the SPE column chemistry.

Further experiments with spiked rat liver and adipose tissue extracts and SPE clean up using 100 mg NH2 columns showed that isopropanol and acetone gave equivalent analyte recoveries. However, acetone was abandoned as an elution solvent because it gave chromatograms with more interfering signals, and its volatility made sample handling more difficult. Further optimization was done by testing analyte recoveries for analyte spiked to loading solvents (hexane or heptane), hexane liver or adipose tissue extracts, and directly to homogenized liver and adipose tissue. Typically, 500 ng of 8:2 FTOH and 7:2 FA-iso (surrogate) were spiked using a methanol stock solution; 6 mL of solvent was used for the extraction and eluted from the SPE column with 1 mL isopropanol. The 9:2 FA-iso was used as the internal standard and was added to the isopropanol extract. Heptane was tested as the extraction solvent because the adipose tissue dissolved in it much more readily than in hexane.

The analyte and surrogate recoveries for analytes spiked into hexane or heptane loaded on either Si or NH2 column with 100 or 500 mg sorbent were generally 100 ± 10%. However, the recoveries of 80 and 70% were obtained for spiked heptane adipose extracts for 500 mg Si and NH2 columns, respectively. The spiked liver hexane extracts gave recoveries of 50 ± 10% for 100 mg NH2 or Si columns. The recoveries were improved by 10–20% when the liver extracts were loaded onto the SPE column through the Na_2SO_4 drying cartridge. The drying cartridge was removed from the system before analyte elution with isopropanol. Further testing of the method at different level of analyte spiking, using 100 mg NH2 columns and hexane extraction, demonstrated that acceptable recoveries of analyte could be obtained only for higher levels of spiking (250 or 500 ng of analyte). At 100 and 50 ng of analyte spiked into homogenized liver tissue, the recoveries dropped down to an average of 65 and 30%, respectively. However, when a 500 mg Si column was used

Table 2
Summary of method performance results for rat plasma, and adipose, liver, and kidney rat tissues

Matrix	Spike level[a]	8:2 FTOH recovery (%)[b]	7:2 FA-iso recovery (%)[b]	Calibration range[c]	Estimated LOD
Adipose	190 ng/g	63 ± 1	67 ± 10	10–250 ng/mL; 3 μL (r^2: 0.999; 0.999)	12 ± 4 ng/g
	950 ng/g	100 ± 3	95 ± 7	50–750 ng/mL; 1 μL (r^2: 0.999; 0.998)	
Liver	100 ng/g	98 ± 4	97 ± 15	5–100 ng/mL; 4 μL (r^2: 0.999; 0.999)	6.0 ± 4 ng/g
	200 ng/g	80 ± 5	79 ± 9	25–500 ng/mL; 2 μL (r^2: 0.999; 0.998)	
Kidney	100 ng/g	72 ± 5	75 ± 10	5–100 ng/mL; 4 μL (r^2: 1.00; 1.00)	4.0 ± 0.5 ng/g
Plasma	100 ng/mL	86 ± 7	82 ± 15	5–100 ng/mL; 4 μL (r^2: 0.999; 1.00)	5.0 ± 1.6 ng/mL
	200 ng/mL	113 ± 11	104 ± 17	25–200 ng/mL; 2 μL (r^2: 0.993; 0.987)	

[a] Indicates spike level of 8:2 FTOH and 7:2 FA-iso in the investigated matrix.
[b] Average percent recovery for all spike replicates ± standard deviation.
[c] Indicates calibration standards range; injection volume; r^2 for 8:2 FTOH and 7:2 FA-iso.

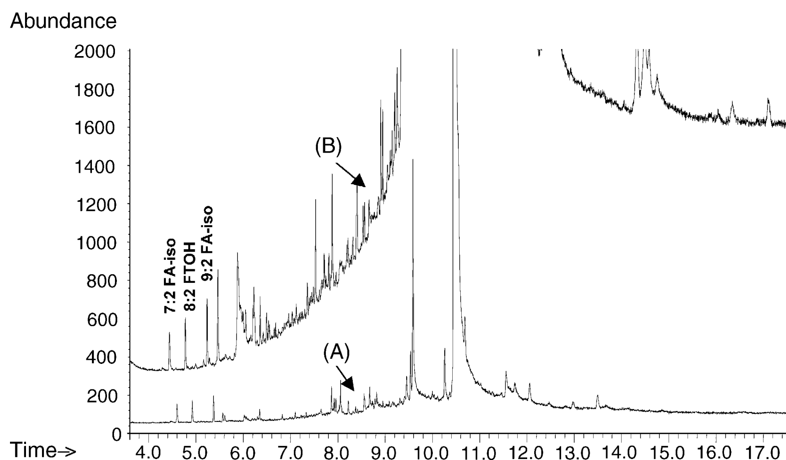

Fig. 4. Chromatogram of ion *m/z* 95 for rat plasma spiked with 100 ng/mL 8:2 FTOH (A) and rat liver spiked with 200 ng/g 8:2 FTOH (B). The true baseline for liver spike starts at about 150 units and was shifted up for display purpose.

the recoveries averaged around 80%. Therefore, a 500 mg Si column eluted with isopropanol and use of Na_2SO_4 drying cartridge were accepted as optimal for analysis of liver, kidney, and adipose tissues.

Potential losses of 8:2 FTOH during the homogenization were investigated by spiking a 2 g portion of rat liver with analyte and homogenizing the tissue using a Polytron homogenizer. Three 0.2 g aliquots of homogenate were analyzed and average recoveries of 30% were obtained, indicating that another means of homogenization needed to be used.

3.4. Sample analysis and method performance

The optimized procedures developed for analysis of 8:2 FTOH in rat plasma, rat liver, kidney, and adipose tissues were applied to samples originating from toxicological studies of 8:2 FTOH using procedures described in Section 2. Each sequence of sample preparations included a set of spiked blank sample matrices to verify the performance of the method. Table 2 summarizes the method performance data obtained for all investigated biological matrices. The recoveries for rat plasma were generally in the range from 80 to 120%.

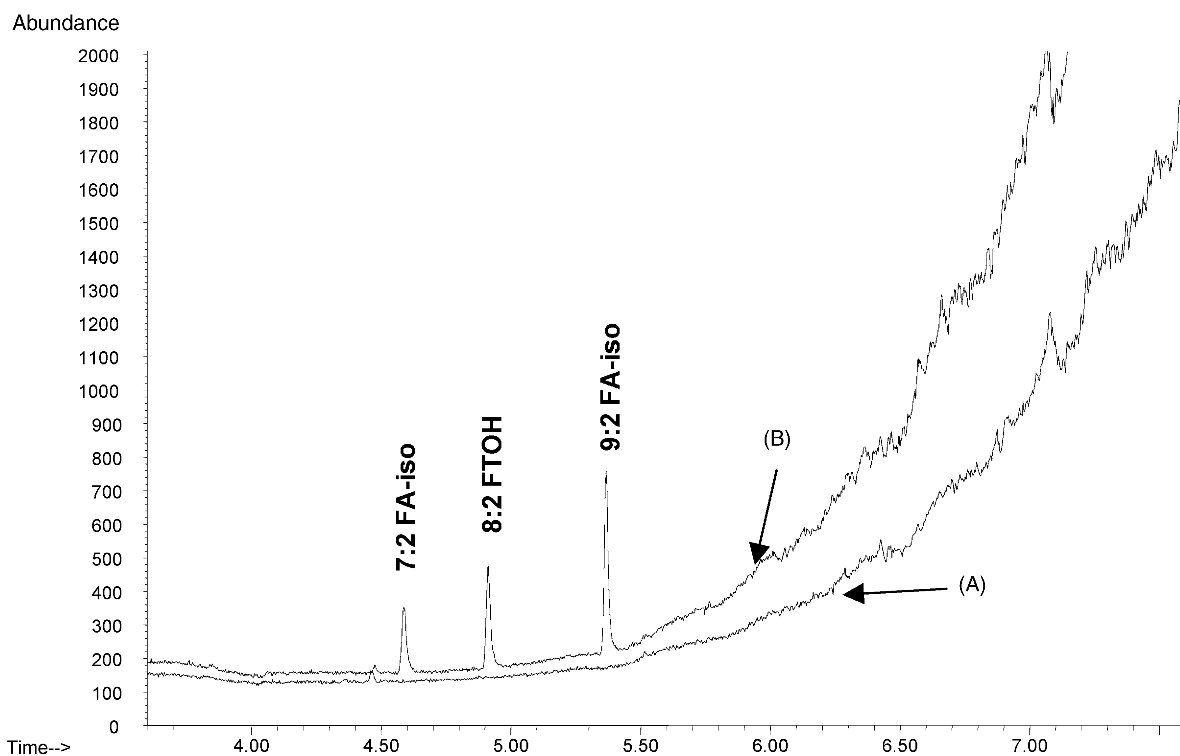

Fig. 5. Chromatogram of ion *m/z* 131 for rat adipose tissue: blank tissue (A), tissue spiked with 190 ng/g 8:2 FTOH (B).

The recoveries for 8:2 FTOH spiked samples closely follow the recoveries of the 7:2 FA-iso surrogate, confirming that 7:2 FA-iso is an appropriate surrogate for this method. Fig. 4A presents a chromatogram of MTBE rat plasma extract for 100 ng/mL 8:2 FTOH spike in plasma. Using the chromatograms of rat plasma spikes, the limit of detection (LOD) was estimated by calculating signal-to-noise (S/N) ratios for selected four 100-ng/mL spike samples. The signal-to-noise ratio was calculated as the ratio of peak height divided by the standard deviation of baseline in proximity to the analyte peak. The concentration equivalent to the S/N ratio of three was calculated as the LOD. The LOD for 8:2 FTOH in rat plasma was estimated to be 5 ng/mL in rat plasma.

The recoveries for spiked liver and kidney samples were generally in the range of 70–100% with recoveries of the surrogate closely following the recoveries of 8:2 FTOH (Table 2). Fig. 4B presents a chromatogram of a liver sample resulting from 200 ng/g 8:2 FTOH spiked into blank liver tissue. Clearly, the background signal is much higher than the one observed for plasma extracts; however, the baseline signal in the retention window of analytes is relatively flat and clear of interferences. The LOD for liver and kidney tissues were estimated using the chromatograms of spiked samples and following the same procedure as described for plasma extracts. The LOD for 8:2 FTOH in liver and kidney tissues were estimated to be 6 ng/mL and 4 ng/g, respectively (Table 2).

The recoveries for spiked rat adipose tissue samples were generally around 100% for high-level spiking, and approximately 60% for low-level spiking (Table 2). However, the recoveries of the surrogate closely followed the recoveries of 8:2 FTOH, allowing correction for recoveries for each sample analyzed. Fig. 5 presents a chromatogram of a blank adipose sample (A) and a blank adipose sample spiked at 190 ng/g 8:2 FTOH (B). The LOD for adipose tissues were estimated using the chromatograms of spiked samples and following the same procedure as described for plasma extracts. The LOD for 8:2 FTOH in adipose tissues were estimated to be 12 ng/g (Table 2).

4. Conclusions

The physico-chemical properties of 8:2 FTOH, especially its volatility and low water solubility, mandate a different analytical approach than that used for other PFAS such as perfluorinated carboxylic acids or perfluorinated sulfonates. The fluorotelomer alcohols can be readily analyzed by GC–MS with either electron impact or chemical ionization [10] for relatively clean matrices. Analysis of 8:2 FTOH from complex biological matrices such as plasma or tissues presents a challenge as efficient extraction and clean-up methods have to be devised without the possibility of incorporating solvent evaporation or solvent exchange. The degradation of chromatographic column performance manifested as peak broadening and loss of sensitivity for the FTOHs is very sensitive to the cleanliness of the injected extracts. Therefore, development of appropriate sample extraction and clean up is necessary to maintain the method performance for analysis of authentic samples. These procedures were developed for rat plasma and rat tissues (liver, kidney, and adipose). The method validation data (Table 2) indicated that, depending on the spike level and the matrix, the 8:2 FTOH recoveries might be lower than 80%. The surrogate compound was developed to identify the recovery for each sample analyzed and introduce additional level of data quality assurance.

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