Bull Environ Contam Toxicol (2009) 83:630–635
DOI 10.1007/s00128-009-9756-y

Long Chain Perfluorinated Alkyl Acids Derivatisation and Identification in Biota and Abiota Matrices Using Gas Chromatography

Francis Orata · Natalia Quinete · Rolf-Dieter Wilken

Received: 11 December 2008 / Accepted: 24 April 2009 / Published online: 12 May 2009

Abstract An analytical method involving derivatisation of perfluorocarboxylic acids for their analysis in abiotic and biotic matrices is presented. Derivatisation of the acid group to form a suitable alkyl ester provided a suitable compound for mass spectrometric detection in gas chromatography/mass spectroscopy (GC/MS) instrumental analysis. The acid is esterified by an alkyl halide i.e. benzyl bromide as the alkylating agent for perfluorocarboxylic acids quantification in fish and water by GC/MS. The gas chromatography method can be applied in the analysis perfluoro alkyl acids in water and biological matrices, especially where high levels of these compounds are expected. Typical values for precision obtained were 0.1%–10.0% with concentrations ranging from 0.2 to 1 μg/mL). Results demonstrate that GC/MS can supplement liquid chromatographic/mass spectroscopy method for quantification of fluorocarboxylic acid surfactants. The result indicates that there is need for more research on method analysis of perfluorinated acids in environmental matrices.

F. Orata (✉) · R.-D. Wilken
IWW Rheinisch-Westfälisches Institut für Wasserforschung gemeinnützige GmbH, Justus-von-Liebig Str. 10, 64584 Biebesheim, Germany
e-mail: fraora@yahoo.com; francis.orata@hessenwasser.de

F. Orata · R.-D. Wilken
Institute of Geowissenschaft/Geologie, Johannes Gutenberg-Universität, Joachim-Becher-Weg 21, 55099 Mainz, Germany

N. Quinete
Department of Chemistry, Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio), Rua Marquês de São Vicente, 225, Gávea, Rio de Janeiro, RJ 22453-900, Brazil

Keywords Derivatisation · Perfluorinated alkyl acids · Benzyl esters · GC/MS · Fish muscles · Water

Besides being an industrially important group of compounds, perfluorinated alkyl surfactants are regarded as highly toxic and extraordinarily persistent chemicals that pervasively contaminate human blood (Olsen et al. 2003; Hansen et al. 2001) and wildlife throughout the world (Gonza'lez-Barreiro et al. 2006; Lau et al. 2006; Abbott et al. 2007). They are therefore regarded as PBT [persistent, bioaccumulative, and toxic chemicals] (Gonza'lez-Barreiro et al. 2006). Issues and concerns regarding analytical methods for determination of PFCAs and other perfluorinated substances in the environment have been reviewed. In the review, sample contamination, calibration, recovery, and separation were noted as recurrent difficulties to be overcome (Belisle and Hagen 1980). Determination of PFCs in environmental and biological matrixes is quite challenging. This is due to their lack of volatility for gas chromatographic (GC) analysis and the lack of a suitable chromophore for liquid chromatographic (LC) analysis using ultraviolet detection (Belisle and Hagen 1980). Gas chromatography with electron capture detection (Martin et al. 2003) or mass spectrometric (MS) detection (Ylinen et al. 1985) can be used to sensitively and selectively measure derivatized fluorinated carboxylates (Moody and Field 1999). Fluoroalkyl carboxylates have been derivatized by means of diazomethane (Martin et al. 2003), a liquid/liquid extractive alkylation method that utilizes benzyl bromide (Ylinen et al. 1985), and by a strong anion exchange extraction method coupled with methyl iodide derivatization (Moody and Field 1999). By contrast, perfluorinated sulfonates like PFOS, which do not

form stable, volatile derivatives, can not be analyzed by GC/MS. A quantitative MS analysis of perfluoroalkyl sulfonates was reported in which samples with no preparation were injected directly into the mass spectrometer (Hebert et al. 2002). Although this technique is less time consuming, its developers do not recommend it for the analysis of blood serums, whole tissues, or wastewaters because of possible, interfering matrix effects. The widely used method in perfluorinated compounds (PFC) analysis is HPLC–MS/MS, and has a detection limit in the range of pg/L in water and pg/g levels in biological matrices (Taniyasu et al. 2005). Sinclair et al. (2004) found that ion-pairing, liquid liquid extraction method was suitable for measurements of concentrations in ng/L and μg/L levels for PFOA and PFOS analysis and we have used this method to extract Perfluorocarboxylic acids in water, fish liver and muscles. We have used GC/MS as an alternative method which can also supplement LC/MS in the analysis of long chain perfluorinated acids namely; perfluoro-*n*-octanoic acid (PFOA), 2H-perfluoro-2-octenoic acid (FHUEA), 2H-perfluoro-2-decenoic acid (FOUEA) and 2H-perfluoro-2-dodecenoic acid (FNUEA) in water and fish. The method involves derivatisation of the acid to form a suitable ester for GC analysis. Derivatisation procedure for GC instrumental analysis involved using benzyl bromide solution and acetone to form benzyl-perfluorooctanoate (benzyl ester) as in the example equation.

$$CF_3(CF_2)_6COOH + C_7H_7Br \rightarrow C_{15}H_7F_{15}O_2 + HBr$$

This work is an improvement as well as a continuation of the research on methodology done by (Ylinen et al. 1985). Due to the unique properties of the carbon–fluorine bond and the polarity of perfluoroalkyl groups, potential substitutes in most cases continue to be perfluoroalkyl based and with acidic group in functionality. Thus, issues of persistence in the environment remain. Continuous research on method development to analyse these types of compounds is necessary.

Materials and Methods

Perfluorinated compounds analysis methods differ depending on the matrices of samples to be analysed. All measuring instruments and equipments were subjected to quality control processes with quality control charts used in checking their performance. To evaluate the accuracy and reliability of the method as a whole, spike/recovery experiments were performed for water, and fish samples. A known concentration of target compounds was spiked into an aliquot of the sample matrix (i.e., matrix spikes) and then run through the analytical procedure as a check for matrix effects, through calculation of the recoveries. External calibration standards were prepared in methanol at concentrations range up to 1,000 ng (absolute amount). A midpoint calibration standard was injected after every ten samples, throughout the instrumental analysis, to check for instrument response and drift. Procedural blanks were analyzed by passage of water and reagents through the entire analytical procedure, to check for contamination in reagents and glassware. Repeatability of results was also done in intervals of time where a sample of known concentration was passed through the whole analytical procedure to verify the method. Each experiment was performed to evaluate different components of the method's accuracy. First, to evaluate whether the method's extraction procedure was capable of completely removing the analyte from the biota and abiotic matrixes, sequential extraction experiments were performed. To determine the accuracy of the GC/MS analysis step, standard addition experiments were performed. Because these experiments were performed with cleaned-up sample extract, they only reflect the accuracy of the GC/MS analysis step and account for any matrix-induced ion suppression or enhancement. Reproducibility tests and in this case interlaboratory comparison tests were performed. The limit of quantification (LOQ) of target chemicals was evaluated for each sample based on the average blank concentrations plus five times its standard deviation of ten blanks. This value was then verified by the concentration in a standard that yielded a standard to noise (S/N) ratio of >10:1.

Perfluorocarboxylic acids were dissolved in methanol at a concentration of 50 μg/mL. The stock solutions were prepared weekly. The counter-ion solution, 0.5 M tetrabutylammonium (TBA) hydroxide, was prepared by dissolving TBAhydrogen sulphate (Merck, Darmstadt, GER) in water; the pH value of the solution was adjusted to 10 with 2 M NaOH-solution. Benzyl bromide (Merck, Darmstadt, GER), 100 mM, was dissolved in acetone. Analytical grade acetone, methanol and methylene chloride were obtained from Merck (Darmstadt, GER). Gas chromatography–Mass spectrum analyses were carried out with a Saturn Varian 4D, Version 5.2 Column: apillary SGE BPX35 (30 m length, 0.25 mm i.d, 0.25 mm film thickness). Separation: the temperature program was 30°C isothermal for 10 min, then 10°C/min–120°C, 30°C/min–280°C.

Muscles of two fish species namely *Lates niloticus* (Nile perch) and *Oreochromis niloticus* (Nile Tilapia) obtained from Lake Victoria and water samples obtained from Rhine River (Biebesheim, Germany) were used as representative samples. The fish and water were analysed to determine the levels of perfluorinated acids before they were spiked for derivatisation, followed by GC analysis.

For fish, more than 5 g muscles were homogenised with five times its weight of water. One gram of the

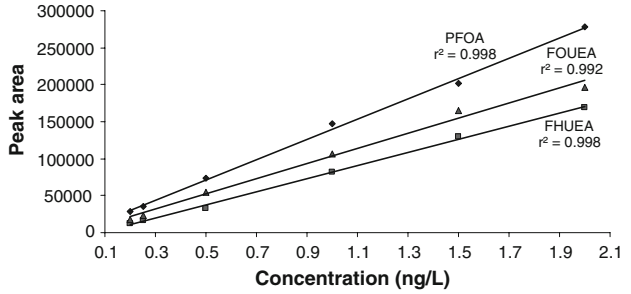

Fig. 1 Calibration curve for PFOA, FHUEA and FOUEA for water samples using GC/MS

homogenate, to which known amounts of perfluorinated acids analytes and an internal standard was added, was thoroughly mixed with 1 mL of 0.5 M tetrabutylammonium hydrogen sulphate solution (adjusted to pH 10) and 2 mL of 0.25 M sodium carbonate buffer. Target analytes were then extracted by adding 5 mL of methyl-tert butyl ether (MTBE) to the aqueous sample mixture and shaking for 20 min. The MTBE supernatant was then recovered and the extraction repeated two more times. The MTBE fractions were all combined, concentrated to 1 mL and methylene chloride (1 mL) added before proceeding to the extract and clean-up. Sample extracts were cleaned up by low pressure chromatography using silica. For this, 2 g of activated silica were mixed with methylene chloride and poured in a glass column. The sample extract was then added to the column and eluted using 15 mL of dichloromethane to remove fat, followed by 30 mL of acetone which contained all target analytes. The acetone fraction was evaporated to dryness at room temperature under a stream of nitrogen. The residue was dissolved in 0.5 mL of acetone and 0.1 mL of benzyl bromide solution added to it. The tube was heated at 80°C for 15 min, cooled, and evaporated to dryness with nitrogen. The residue was dissolved in 1 mL of methylene chloride for GC analysis. For water samples, 1 L previously spiked with known amounts of perfluorinated acid analytes was loaded for solid phase extraction. An internal standard was added in the sample bottle prior to solid phase extraction. The sample was let to run through water Oasis HLB SPE cartridges (60 mg) previously conditioned, at a flow rate of one drop per second (3–6 mL/min). Target analytes were then eluted with 6 mL of methanol. The eluate was evaporated with a gentle stream of nitrogen gas to a final volume of 1,000 µL. For GC analysis of water samples, derivatisation followed the procedure above. Blanks were treated with exactly the same manner as the samples, except for replacing the sample by the appropriate amount of water (MilliQwater). Procedural blanks were analyzed with each batch of samples (a maximum of ten samples).

Results and Discussion

Analytical curves of perfluoro-*n*-octanoic acid (PFOA), 2H-perfluoro-2-octenoic acid (FHUEA), 2H-perfluoro-2-decenoic acid (FOUEA) and 2H-perfluoro-2-dodecenoic acid (FNUEA) enrichment standards in water and fish muscles from concentration of 0.2–1.5 µg/L were measured by GC/MS. Calibration curve for both perfluoro alkyl acids analysed gave a $r^2 > 0.98$ as shown in Figs. 1, 2 and 3. The mass spectrometry mornitored ions m/e were 504,

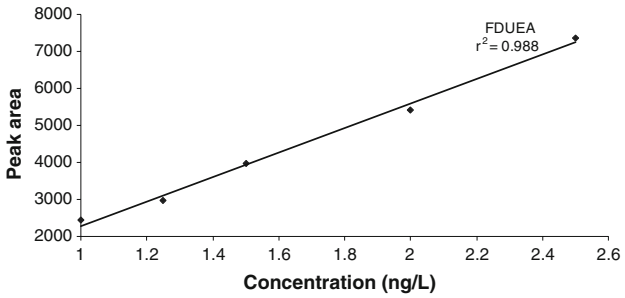

Fig. 2 Calibration curve for FDUEA for water samples using GC/MS

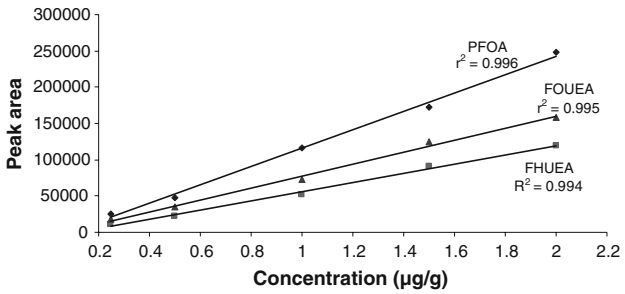

Fig. 3 Calibration curve for PFOA, FHUEA and FOUEA for fish muscles samples using GC/MS

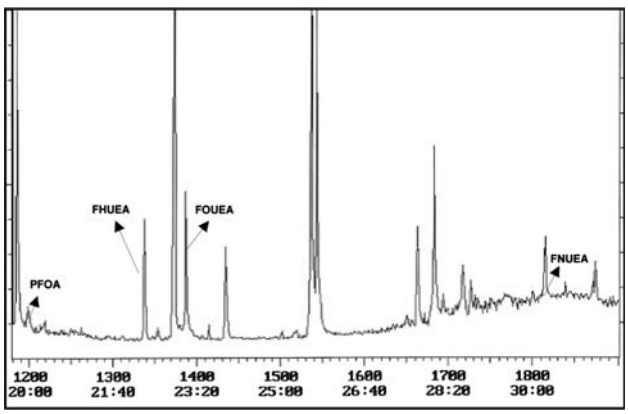

Fig. 4 GC/MS Chromatogram for PFOA, FHUEA, FOUEA, FNUEA

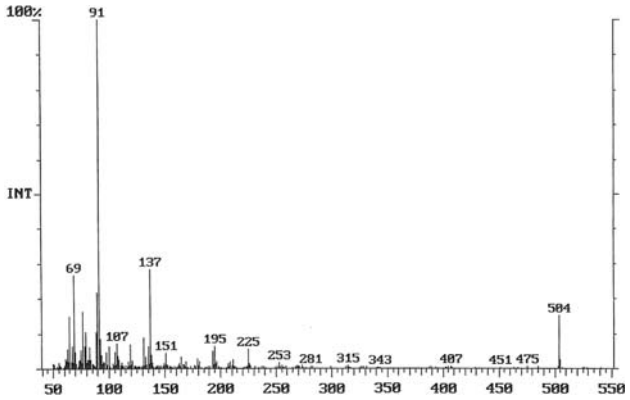

Fig. 5 MS Spectrum for PFOA showing 504 m/e ($C_{15}H_7F_{15}O_2$)

448, 548 and 648 for benzyl- (perfluorooctanoate, 2H-perfluoro-2-octenoate, 2H-perfluoro-2-decenoate and 2H-perfluoro-2-dodecenoate) esters formed respectively. Their corresponding chromatograms are as indicated in Fig. 4. Other monitored ions m/e originating from the elimination of the esters fragments were 91, 107, 108, 135, and 137 corresponding to the fragments C_7H_7+, C_7H_7O+, C_7H_7OH+, $C_7H_7CO_2+$ and $C_8H_9O_2-$ as shown in Fig. 5. Figure 6 shows fragment ions obtained in mass spectrum of benzyl-per-fluorooctanoate.

Fish and water were spiked and analyzed to test the precision of the method. Results of accuracy and precision of analyses for the present investigation were found to be satisfactory as shown in Table 1. Recoveries ranging from 77.3% to 101.0% for water samples and 65.0%–92.6% of spiked samples of fish were obtained. The quantification analysis of spiked 2H-perfluoro-2-dodecenoate (FNUEA) was not done. Although FNUEA was detected, quantification limit (LOQ) was difficult to determine in GC method using derivatisation probably due to matrix interference. The concentrations of the water and muscles were previously determined. All fish muscles samples were below detection limit, while Rhine water sample previously determined concentration before spiking was 2.45 ng/L. Typical values for precision obtained were 2.1%–8.6% for fish muscles samples in GC/MS with concentrations ranging from 0.3 to 1.0 μg/g). For water samples the corresponding precision range was 0.1%–10.0% in GC analysis with concentrations ranging from 0.2 to 1.0 μg/L). Instrumental limit of quantification (LOQ) of ≈0.2 for PFOA, 0.15 for FHUEA and FOUEA and 0.5 μg/L for FNUEA in GC/MS/MS respectively for water samples. The limit of quantification was ≈0.3 μg/g for PFOA, 0.25 μg/g for FHUEA and FOUEA respectively for fish muscles samples. Precision range of 6.38%–10.97% from interlaboratory analysis/reproducibility and liquid chromatography hyphenated with mass spectroscopy which is the instrumental method of choice for analysis of these compounds was also used to verify the results. Results of repeatability experiments done in intervals of 4 days for analysis of PFOA in water sample spiked concentration of 1 μg/L gave a precision value of 6.9% for n = 9 analysis. The ion pair extraction with TBA is selective for acidic organic compounds allowing efficient transfer of the anion of perfluorinated compounds into the organic phase. The ratio of the derivatising agent (Benzyl bromide) to that of perfluorinated acid was calculated to be 2.2×10^4:1 for GC method.

The LOQ in the GC/MS method does not have the sensitivity to study potential human exposure to perfluorinated compounds, but it is applicable to contaminated environmental samples. Long chain perfluorinated acids have been determined in biota (fish) and abiota (water) by derivatisation and subsequent analysis by GC/MS. The ion pair extraction with TBA is selective for acidic organic

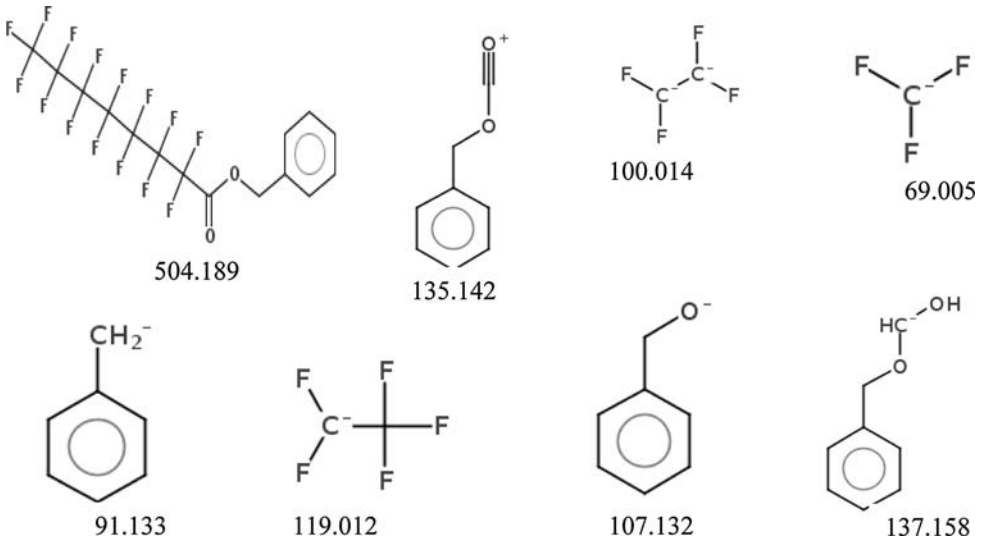

Fig. 6 Fragment ions obtained in mass spectrum of $C_{15}H_7F_{15}O_2$

Table 1 Recovery concentrations (ng/L or ng/g) of perfluorocarboxylic acids from spiked samples of fish muscles and water samples in GC/MS

Matrice	Analyte	Initial concentration in ng	Added amounts (ng)	Recovered amounts (ng)	% recoveries
Rhein river water	PFOA	2.45	300	285.1 ± 11.7	95.0
		2.45	500	490.6 ± 10.6	98.1
		2.45	1,000	915.4 ± 9.9	91.5
	FHUEA	ND	150	151.5 ± 15.1	101.0
		ND	300	279.6 ± 28.4	93.2
		ND	500	485.8 ± 4.9	97.6
		ND	1,000	977.9 ± 11.0	97.8
	FOUEA	ND	200	183.7 ± 7.4	93.9
		ND	300	283.1 ± 6.8	94.4
		ND	1,000	883.5 ± 7.7	88.4
	FNUEA	ND	500	477.0 ± 13.6	95.4
		ND	800	713.7 ± 21.3	89.2
		ND	1,000	890.2 ± 33	89.0
Fish muscles	PFOA	ND	300	195.2 ± 16.8	65.0
		ND	500	407.3 ± 30.1	81.5
		ND	1,000	904.5 ± 18.6	90.5
	FHUEA	ND	350	295.3 ± 9.0	84.4
		ND	500	402.5 ± 13.1	80.5
		ND	1,000	898.0 ± 23.8	89.8
	FOUEA	ND	350	288.2 ± 14.7	82.3
		ND	1,000	926.5 ± 36.5	92.6

ND not detectable

compounds allowing efficient transfer of the anion of perfluorinated acids into the organic phase. Results demonstrate that GC/MS can be used to supplement the LC/MS method for quantification of perfluorinated acids in contaminated areas, and where higher concentration of the analyte is expected. Overall, the methods developed in this study for the measurement of poly- and perfluorinated acids is a continuation of the method by (Ylinen et al. 1985), and may be applied to measure fluorotelomer acids. More research on the analysis of fluorotelomer acids using the method used in this study is required. The gas chromatography method can be applied in the analysis of water, and biological matrices, so that we can better understand the fate of per- and polyfluorinated compounds in the environment.

Acknowledgments We thank the research team at IWW (Rheinisch-Westfaelisches Institut für Wasser, Moritzstr. 26 D-45476 Mülheim an der Ruhr especially Werres Friedrich and Prof T. Hofmann and his research group at Mainz university. Many thanks go to Jörg Warke who helped us a lot in our inter-laboratory analysis as part of analytical quality management (AQM). We acknowledge DAAD for their financial support.

References

Abbott BD, Wolf CJ, Schmid JE, Das KP, Zehr RD, Helfant L, Nakayama S, Lindstrom AB, Strynar MJ, Lau C (2007) Perfluorooctanoic acid induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferators activated receptor-alpha. Toxicol Sci 98(2):571–581. doi:10.1093/toxsci/kfm110

Belisle J, Hagen DF (1980) A method for the determination of perfluorooctanoic acid in blood and other biological samples. Anal Biochem 101:369–376. doi:10.1016/0003-2697(80)90202-X

Gonza'lez-Barreiro C, Martı'nez-Carballo E, Sitka A, Scharf S, Gans O (2006) Method optimization for determination of selected perfluorinated alkylated substances in water samples. Anal Bioanal Chem 386:7–8. doi:10.1007/s00216-006-0557-4

Hansen KJ, Clemen LA, Ellefson ME, Johnson HO (2001) Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. Environ Sci Technol 35:766–770. doi:10.1021/es001489z

Hebert GN, Odom MA, Craig PS, Dick DL, Strauss SH (2002) Method for the determination of sub-ppm concentrations of perfluoroalkylsulfonate anions in water. J Environ Monitor 4:90–95. doi:10.1039/b108463c

Lau C, Thibodeaux JR, Hanson RG, Narotsky MG, Rogers JM, Lindstrom AB, Strynar MJ (2006) Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol Sci 99(2):366–394. doi:10.1093/toxsci/kfm128

Martin JW, Mabury SA, Solomon KR, Muir DCG (2003) Identification of long-chain perfluorinated acids in biota from the Canadian arctic. Environ Toxicol Chem 22:196–204. doi:10.1897/1551-5028(2003)022<0196:BATDOP>2.0.CO;2

Moody CA, Field JA (1999) Determination of perfluorocarboxylates in groundwater impacted by fire-fighting activity. Environ Sci Technol 33:2800–2806. doi:10.1021/es981355+

Olsen GW, Hansen KJ, Stevenson LA, Burris JN, Mandel JH (2003) Human donor liver and serum concentrations of perfluorooctanesulfonate and other perfluorochemicals. Environ Sci Technol 37:888–891. doi:10.1021/es020955c

Sinclair E, Taniyasu S, Yamashita N, Kannan K (2004) Perfluorooctanoic acid and perfluoroocatane sulfonate in Michigan and New York waters. Organohalog Compd 66:4069–4073

Taniyasu S, Kannan K, So MK, Gulkowska A, Sinclair E, Okazawa T, Yamashita N (2005) Analysis of fluorotelomer alcohols, fluortelomer acids and short- and long-chain perfluorinated acids in water and biota. J Chromatogr A 1093:89–97. doi:10.1016/j.chroma.2005.07.053

Ylinen M, Hanhijarvi H, Peura P, Ramo O (1985) Quantitative gas chromatographic determination of perfluorooctanoic acid as the benzyl ester in plasma and urine. Arch Environ Contam Toxicol 14:713–717. doi:10.1007/BF01055778