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Development and characterization of a solvent extraction–gas chromatographic/mass spectrometric method for the analysis of perfluorooctanesulfonamide compounds in solid matrices

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Abstract

A method utilizing solvent extraction and analysis by gas chromatography–positive chemical ionization mass spectrometry (SE–GC–PCI-MS) was developed for the analysis of three neutral hydrophobic perfluorooctanesulfonamide compounds [perfluorooctanesulfonamide (PFOSA), *N*-ethyl perfluorooctanesulfonamide (*N*-EtPFOSA), and *N*,*N*-diethyl perfluorooctanesulfonamide (*N*,*N*-Et_2PFOSA)]. These compounds are suspected metabolic precursors of perfluorooctane sulfonate. The SE–GC–PCI-MS method was used to analyze all three perfluorooctanesulfonamides in fast food, fish, and Arctic marine mammal liver samples. The SE–GC–PCI-MS method produced relatively higher recoveries of the analytes (averaging 83 ± 6%, 84 ± 9%, and 89 ± 19% for *N*,*N*-Et_2PFOSA, *N*-EtPFOSA, and PFOSA, respectively) with lower coefficients of variation, and less susceptibility to matrix effects, than ion pair extraction–liquid chromatography–tandem mass spectrometric methods. Method detection limits (MDLs) were 100, 120, and 250 pg/g for *N*,*N*-Et_2PFOSA, *N*-EtPFOSA, and PFOSA, respectively. The three compounds were found at concentrations ranging from below the MDL to 22 ng/g wet weight in fast food, fish, and Arctic marine mammal liver samples.

Keywords: Perfluorinated chemicals (PFCs); Surfactants; Food; Method detection limit; PFOS-precursors

1. Introduction

Perfluorinated chemicals (PFCs) are an emerging class of persistent organohalogen contaminants. Anionic species, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are widespread environmental contaminants, found in biota sampled from numerous locations worldwide [1] including remote locations such as the Canadian Arctic [2]. In addition to a widespread geographical distribution in biota, PFOS and PFOA have been observed in human serum at the low ng/mL range in many non-occupationally exposed human populations including Canada [3], the United States [4–8], and Japan [9]. The widespread distribution of these, and structurally related, compounds is due to a number of causes, including their use in a wide variety of consumer/industrial products and applications, ranging from personal care products and cleaning solutions, to grease resistant coatings for fabric and paper and emulsifiers in the production of polymers [10].

In addition to their global distribution, reports of toxicological activity are driving research in the area of PFCs. PFOS has a relatively long serum half life (estimated at 9 ± 6 years) [11], and both in vitro and in vivo animal assays have indicated that PFOS and other PFCs are bioactive. PFOA has been shown to act as a peroxisome proliferator [12] and induce atrophy of the thymus and spleen in mice [13]. An in vitro study demonstrated that PFOA, PFOS, and perfluorooctanesulfonamide (PFOSA) all inhibit gap junctional intercellular communication [14]. However, in one recent study, serum PFOS and PFOA concentrations in occupationally exposed subjects were not correlated with any changes in lipid,

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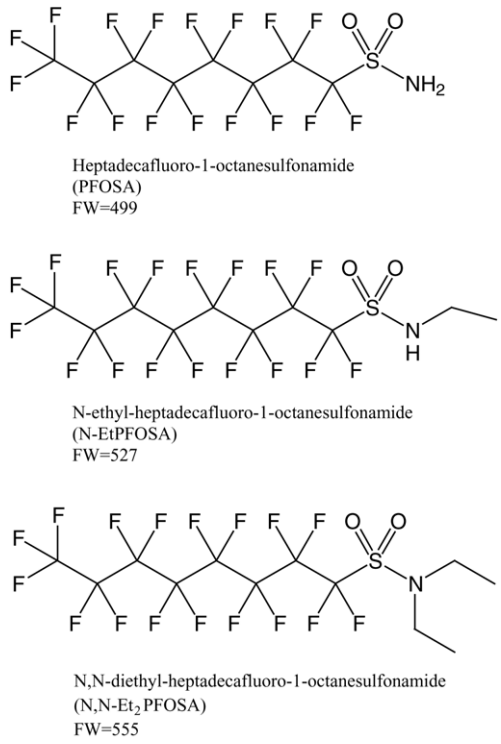

Fig. 1. Molecular structures and formula weights of the three perfluorooctanesulfonamide analytes.

hepatic, haematological, or thyroid parameters typically associated with exposure to perfluorooctyl compounds in laboratory animals [15].

Most of the work already reported in the literature has focused on the analysis of anionic perfluorinated compounds such as PFOS and PFOA using ion pair extraction (IPE) or solid-phase extraction (SPE) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) methodology [4,8]. These methods are not well suited to the analysis of neutral hydrophobic perfluorooctanesulfonamide compounds such as PFOSA, *N*-ethyl perfluorooctanesulfonamide (*N*-EtPFOSA), or *N*,*N*-diethyl perfluorooctanesulfonamide (*N*,*N*-Et_2PFOSA) (Fig. 1), since they were developed for the analysis of anionic perfluorinated sulfonates and carboxylates. Such neutral hydrophobic perfluorooctanesulfonamides have been used in grease and stain resistant coatings for textiles and paper products [10] and are implicated as potential precursors in the formation of PFOS [16]. Neutral perfluorooctanesulfonamides generally have low and variable recoveries, plus high method detection limits, when determined by IPE–LC–MS/MS [6,17–19].

A method specifically geared towards the analysis of neutral perfluorooctanesulfonamides was developed for three related compounds (PFOSA, *N*-EtPFOSA, and *N*,*N*-Et_2PFOSA) in solid matrices, including food and biota samples. The method involves solvent extraction (SE) and analysis by gas chromatography–positive chemical ionization mass spectrometry. Average analyte recoveries, and method detection and quantitation limits of the developed SE–GC–PCI-MS method were characterized. Performance of the SE–GC–PCI-MS method in the analysis of PFOSA and *N*-EtPFOSA was compared to that of an IPE–LC–MS/MS method. The SE–GC–PCI-MS method was also used to obtain data on the presence of PFOSA, *N*-EtPFOSA, and *N*,*N*-Et_2PFOSA in selected food and biota samples.

2. Materials and methods

2.1. Chemicals

N-EtPFOSA (96%) was purchased from Interchim (Montluçon, France). PFOSA (>95%) was provided by Griffin LLC (Valdosta, GA, USA). *N*,*N*-Et_2PFOSA was synthesized as described further. Methyl perfluorodecanoate (98%) and methyl perfluorotetradecanoate (95%) were purchased from SynQuest Laboratories (Alachua, FL, USA). Labelled *N*-ethyl-d_5-perfluorooctanesulfonamide (*N*-Et-d_5-PFOSA, >98% chemical and ≥98% isotopic purity) and *N*-methyl-d_3-perfluorooctanesulfonamide (*N*-Me-d_3-PFOSA, >98% chemical and ≥98% isotopic purity) were provided by Wellington Laboratories (Guelph, Ont., Canada).

2.2. Synthesis, characterization, and quantitiation of *N*,*N*-Et_2PFOSA

A 0.001 mg/L solution of *N*-EtPFOSA in ethyl acetate was mixed with an equal volume of 10 M aqueous KOH. The organic layer was removed, and 1 mL bromoethane (Aldrich, Madison, WI, USA) was added to the remaining aqueous layer. The aqueous mixture was heated in a water bath at 40 °C for 3 h. Synthesized *N*,*N*-Et_2PFOSA was extracted from the aqueous layer with ethyl acetate (3 × 5 mL). The combined organic phases were washed with 3 × 5 mL Milli-Q purified water (Millipore, Billerica, MA, USA). Residual water was removed from the organic phase using Na_2SO_4 that had been heated for 24 h at 650 °C.

Synthesized *N*,*N*-Et_2PFOSA was separated from any residual starting material using Florisil column chromatography. Approximately 8 g of 4.5% deactivated Florisil (60–100 mesh) was wet packed into a 1 cm I.D. × 30 cm column using hexane. *N*,*N*-Et_2PFOSA was eluted off of the column using hexane, leaving the more polar *N*-EtPFOSA on the Florisil column. Full scan and selected ion monitoring gas chromatography–electron capture negative ionization and positive chemical ionization mass spectrometry analysis of the fraction containing *N*,*N*-Et_2PFOSA showed that it was free of *N*-EtPFOSA and PFOSA. Florisil eluates containing *N*,*N*-Et_2PFOSA from a number of syntheses were combined. The reaction yield of *N*,*N*-Et_2PFOSA was estimated to be 8.2%.

The combined Florisil eluate containing *N*,*N*-Et_2PFOSA contained other reaction by-products (purity was approximately 3.2%), thus the synthesized *N*,*N*-Et_2PFOSA was quantified using gas chromatography with flame ionization

detection (GC–FID). Yieru et al. [20] demonstrated that carbon weight response factors for a variety of organic compounds are constant even though FID responses are affected by the presence of heteroatoms [21]. Since the C–F bond is particularly strong and may not completely be oxidized in the FID jet, only compounds with similar structures to *N*,*N*-Et_2PFOSA (i.e. containing the $C_8F_{17}SO_2$ moiety) were used as standards during the quantification. Solutions of *N*-EtPFOSA, PFOSA, and *N*-ethyl, *N*-ethanol perfluorooctanesulfonamide in the 5–200 μg/mL range were used as standards.

2.3. Samples

A variety of samples were analyzed in this initial survey for *N*-EtPFOSA, *N*,*N*-Et_2PFOSA, and PFOSA. Samples included fillets from a large predatory fish [shark ($n = 5$)], fast food composite samples [pizza ($n = 3$), hamburger ($n = 3$), and French fries ($n = 3$)]. Shark fillets were purchased from Canadian stores in 2001; fast food samples were composites generated from the 1992 to 1994 Canadian Total Diet Studies [22]. All samples were stored in I-Chem series 200 (Chase Scientific Glass, Rockwood, TN, USA) glass jars at −20 °C until analysis.

Marine mammal liver samples used in the comparison of the IPE–LC–MS/MS and SE–GC–PCI-MS methods were obtained from the Canadian Arctic. Beluga samples ($n = 4$) were collected from Iqaluit, Nunavut in 1992. Narwhal samples ($n = 4$) were obtained from Grise Ford, Nunavut in 1998 and 1999. Liver samples were stored in polyethylene Whirlpak bags at −20 °C during sampling and storage.

2.4. Analysis of N-EtPFOSA, N,N-Et_2PFOSA, and PFOSA by SE–GC–PCI-MS

Approximately 10 g of each sample was placed in a polypropylene centrifuge tube. Samples were spiked with methyl perfluorotetradecanoate ($C_{15}H_3F_{27}O_2$, MePFTeD; 50.0 μL of a 2000 pg/μL solution) as a recovery internal standard. Solvent [2:1 (v/v) hexane/acetone] was added to the tube and the samples were homogenized using a Polytron mixer. Homogenates were centrifuged (10 min, 1400 × *g*) to separate the organic layer from solids. The organic layer was then removed and transferred into a round bottom flask through a bed of dry Na_2SO_4 to remove any residual water. The solvent extraction was repeated, and organic layers were combined, and reduced in volume on a rotary evaporator. Extracted lipids were removed by washing with concentrated sulphuric acid. The organic layer was again reduced in volume, and passed through a silica gel column containing 8 g of 40% acidified (using concentrated sulphuric acid) and 4 g neutral silica gel using 100 mL dichloromethane as the eluant. Isooctane was added (1.0 mL), and the eluate was reduced in volume to 500 μL and spiked with methyl perfluorodecanoate ($C_{11}H_3F_{19}O_2$, MePFD; 10.0 μL of a 2000 pg/μL solution) as an instrument performance internal standard. A sample containing Milli-Q purified water was concomitantly run through the method as a blank with each set of fast food composites analyzed to monitor laboratory sources of the perfluorooctylsulfonamide analytes.

Samples were analyzed by gas chromatography–positive chemical ionization mass spectrometry (GC–PCI-MS) using an Agilent 5973N mass spectrometer coupled to a 6890 GC (Palo Alto, CA, USA). The GC was fitted with a retention gap (1 m × 0.530 mm I.D., deactivated fused silica) and a DB-1701 (30 m × 0.25 mm I.D., 0.250 μm film thickness; Agilent) column. Samples were injected (2.0 μL) by an Agilent 7683 Automatic Liquid Sampler using cool on-column injection under the following conditions: initial oven temperature 60 °C, initial ramp 3 °C/min to 75 °C, 20 °C/min to 280 °C, GC transfer line temperature 280 °C, and mass spectrometer source temperature 200 °C. The injector port temperature program tracked the GC oven temperature program plus 3 °C. Helium (99.999%; constant flow 0.9 mL/min) and methane (99.97%) was used as the carrier and reagent gases, respectively. The selected ion monitoring mode was used to monitor the quasimolecular ion $[M + H]^+$ of all fluorinated compounds.

Compounds were positively identified by the presence of the $[M + H]^+$ ion eluting within 0.07 min of the retention time of the corresponding standard. No abundant fragment ions were formed during PCI-MS, so confirmation of analytes in samples for which the retention time deviated more than 0.07 min of the corresponding standard was performed in electron capture negative ionization mass spectrometry in the selected ion monitoring mode. In these cases, presence of the $[M - H]^-$ and $[C_8F_{17}SO_2]^-$ ions were used to confirm the identity of perfluorooctanesulfonamides.

Quantitation was performed using the quasimolecular ion $[M + H]^+$ as the target ion. Perfluorooctanesulfonamide analyte areas were normalized to areas of the instrument performance internal standard (MePFD) prior to quantitation using a calibration curve constructed from three external standards prepared in isooctane (spanning 10–100 pg/μL). Two smaller peaks were present in the GC–PCI-MS chromatograms of *N*-EtPFOSA, *N*,*N*-Et_2PFOSA, and PFOSA standards and some samples (approximately 13%, 20%, and 3% as large as the main peak for *N*-EtPFOSA, *N*,*N*-Et_2PFOSA, and PFOSA, respectively), eluting roughly 0.1 min after the main peak. Mass spectra indicated that these smaller peaks were isomers of *N*-EtPFOSA and *N*,*N*-Et_2PFOSA, and were attributed to branched chain isomers [23]. Quantitation was performed using only the large peak of the straight chain isomers.

2.5. Method recoveries

To examine the extent to which the analytes (*N*-EtPFOSA, *N*,*N*-Et_2PFOSA, PFOSA) and the recovery internal standard (MePFTeD) were recovered by the method, recent (2002) chicken burger, hamburger, and freshwater fish composite samples (5 g) were fortified with each compound and run through the method. These recent samples were previously

Table 1
Average (±standard deviation, $n = 5$) percent recoveries of recovery internal standards and perfluorooctanesulfonamide analytes in samples fortified at three different levels, method detection limits (MDLs), and method quantitation limits (MQLs) using the SE–GC–PCI-MS method

Compound	Percent recoveries			MDL (pg/g)	MQL (pg/g)
	Low (250 pg/g)	Medium (500 pg/g)	High (5000 pg/g)		
MePFTeD[a]	77 ± 8	–	–	–	–
N-Et-d_5-PFOSA[a]	83 ± 2	–	–	–	–
N,*N*-Et_2PFOSA	74 ± 8	79 ± 7	97 ± 4	100	330
N-EtPFOSA	82 ± 9	83 ± 9	88 ± 9	120	400
PFOSA	–[b]	81 ± 19	101 ± 12	250	830

[a] MePFTeD and *N*-Et-d_5-PFOSA (recovery internal standards) were added to give one concentration (10000 and 2500 pg/g, respectively) only. Average and standard deviation calculated from $n = 10$ fortified samples.
[b] Not determined.

analyzed, and found to be free of the perfluorinated compounds. Perfluorooctylsulfonyl compounds were spiked at three levels: low (hamburger; final sample concentration 250 pg/g), medium (chicken burger; final sample concentration 500 pg/g), and high (freshwater fish; final sample concentration 5000 pg/g). Since the fluorinated ester was used as a recovery internal standard as opposed to an analyte, it was spiked only at the level it was added during sample analysis (final sample concentration 10 000 pg/g). Five replicate samples were analyzed for each of the three fortification levels.

At the end of the study, deuterium labelled *N*-EtPFOSA and *N*-MePFOSA became available for use as internal standards. The usefulness of *N*-Et-d_5-PFOSA as a recovery internal standard was evaluated in a similar fashion as for MePFTeD. Ten samples consisting of a variety of food matrices (chicken burger, hamburger, French fries, pizza, hot dog) were spiked with *N*-Et-d_5-PFOSA (50 μL, 500 pg/μL) and MePFTeD (10 000 pg/g), and taken through the analytical method. *N*-Me-d_3-PFOSA replaced MePFD as the instrument performance internal standard for these analyses.

2.6. Method detection limits

Method detection limits (MDLs) were calculated as the lowest concentration required to produce a signal greater than three times the standard deviation of a matrix blank signal [24]. Seven replicate analyses of a spiked hamburger extract previously determined to contain no perfluorooctanesulfonamides were used to estimate the matrix blank signal standard deviation. The hamburger extract was fortified with perfluorooctanesulfonamides at a concentration of 5 pg/μL for each analyte. Method quantitation limits (MQLs) were calculated in a similar fashion as MDLs, but used a value of 10 times the standard deviation of the matrix blank signal to ensure measurements greater than the MQL are not due to fluctuations in noise.

3. Results

3.1. Method recoveries, MDLs, and MQLs

Percent recoveries of the three perfluorooctanesulfonamide analytes and MePFTeD and *N*-Et-d_5-PFOSA recovery internal standards using the SE–GC–PCI-MS method from fortified samples are listed in Table 1. Mean recoveries of all compounds were greater than 74%. Recoveries of analytes and recovery internal standards were not significantly different [$p = 0.160$, analysis of variance (ANOVA)], suggesting that MePFTeD and *N*-Et-d_5-PFOSA are adequate recovery internal standards for *N*,*N*-Et_2PFOSA, *N*-EtPFOSA, and PFOSA. However, the mean recovery of *N*-Et-d_5-PFOSA appeared greater, and the coefficient of variation lower, than that of MePFTeD. This, along with the obvious greater structural similarity of *N*-Et-d_5-PFOSA to the analytes, indicates that *N*-Et-d_5-PFOSA is the better recovery internal standard.

Analyte recoveries were significantly lower ($p = 8 \times 10^{-5}$, ANOVA) in the hamburger (low fortification level) than the freshwater fish matrix (high fortification level). It is unclear whether this is an effect of the matrix or of the fortification level, since *N*-Et-d_5-PFOSA recoveries did not differ amongst the five various matrices.

Calculated MDLs and MQLs for *N*,*N*-Et_2PFOSA, *N*-EtPFOSA, and PFOSA are also listed in Table 1.

Table 2
Recovery corrected mean (range) perfluorooctanesulfonamide concentrations in food and biota samples (ng/g weight wet)

	n	*N*,*N*-Et_2PFOSA	*N*-EtPFOSA	PFOSA
Shark fillet	5	<0.10	22 (<0.12–58)	0.50 (<0.25–0.78)
Pizza	3	1.3 (0.63–2.6)	1.25 (<0.12–3.2)	<0.25
Hamburger	3	0.70 (0.56–0.80)	0.23 (<0.12–0.58)	<0.25
French fries	3	3.6 (3.5–3.7)	9.2 (6.7–12)	<0.25
Beluga liver	4	1.2 (0.52–2.1)	3.3 (0.12–12)	14 (3.9–28)
Narwhal liver	4	3.6 (<0.10–7.2)	11 (2.5–32)	6.2 (<0.25–11)

3.2. Perfluorooctanesulfonamide concentrations

Fish, fast food, and marine mammal liver concentrations of *N*,*N*-Et_2PFOSA, *N*-EtPFOSA, and PFOSA are given in Table 2. Concentrations are all recovery corrected using the individual sample recoveries of MePFTeD. Recoveries of MePFTeD in the samples averaged 80 ± 9%.

4. Discussion

4.1. SE–GC–PCI-MS method

Since the perfluorooctanesulfonamide compounds are estimated to have relatively high log K_{ow} values (7.99, 6.85, and 4.5 for *N*,*N*-Et_2PFOSA, *N*-EtPFOSA, and PFOSA, respectively) [25,26], a solvent extraction GC–MS analytical method (SE–GC–PCI-MS) suitable for hydrophobic compounds was developed. This approach was taken in an attempt to overcome some of the disadvantages of the IPE–LC–MS/MS method, used in the past to analyze PFOSA alongside anionic fluorinated compounds such as PFOS and PFOA. A SE–GC–PCI-MS method was also pursued to widen the scope to include *N*,*N*-Et_2PFOSA.

The new SE–GC–PCI-MS method can be applied to the analysis of three perfluorooctanesulfonamide compounds—*N*,*N*-Et_2PFOSA, *N*-EtPFOSA, and PFOSA. Recoveries of all three analytes are approximately 83 ± 6%, 84 ± 9%, and 89 ± 19% for *N*,*N*-Et_2PFOSA, *N*-EtPFOSA, and PFOSA, respectively. The average recovery of *N*-EtPFOSA and PFOSA is in the range of recoveries (83–127%) reported for an enzyme digestion, solvent extraction, and GC–ECD analysis of rat tissue [25]. Average PFOSA recoveries in fortified samples obtained using the SE–GC–PCI-MS method are greater than reported recoveries (<40% in salmon liver to roughly 86% in human sera) from the IPE–LC–MS/MS method [6,17–19]. It also appears that the average recovery coefficients of variation are smaller for the SE–GC–PCI-MS method (7–24%) than for the IPE–LC–MS/MS method (24-71%) [6,18], indicating the SE-GC–PCI-MS method generates more reproducible results than the IPE–LC–MS/MS method. The improvement in method performance is most probably driven by the differences in extraction efficiency and reproducibility of the IPE and SE methods, as opposed to the mass spectrometric analysis.

In addition to improved recoveries, the new SE–GC–PCI-MS method has lower MDLs and MQLs than other methods developed for the analysis of animal and human tissue. Two IPE–LC–MS/MS methods used to analyze avian and human liver for PFOSA had MQLs of 6.3 and 75 ng/g [6,17], whereas a method utilizing enzyme digestion, solvent extraction, and GC–ECD achieved an MDL of 310 ng/g [25]. There are fewer references to the analysis of *N*-EtPFOSA; the GC–ECD method achieved an MDL of 220 ng/g [25] and an IPE–LC–MS/MS method was able to quantitate samples containing as low as 1.9 ng/g of *N*-EtPFOSA (G. Tomy, Freshwater Institute, unpublished data). These reported MDLs and MQLs are from 10 to 1000 times higher than those obtained by the SE–GC–PCI-MS method.

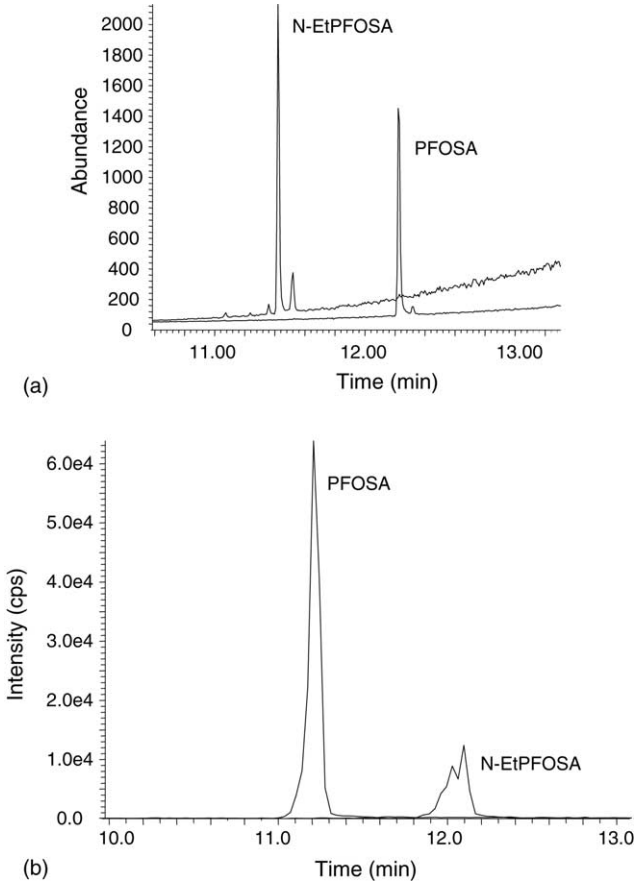

Fig. 2. (a) PCI-MS extracted ion chromatograms of *N*-EtPFOSA (*m*/*z* 527) and PFOSA (*m*/*z* 500) standards clearly showing branched chain isomers eluting approximately 0.1 min after the large peak of the straight chain isomer. (b) The branched chain isomers are unresolved by the LC–MS/MS method, and are not clearly visible in the selected reaction monitoring chromatograms for *N*-EtPFOSA (*m*/*z* 526 → 169) or PFOSA (*m*/*z* 499 → 78).

The SE–GC–PCI-MS method also has the required resolving power to easily separate branched and straight chain *N*-EtPFOSA, *N*,*N*-Et_2PFOSA, and PFOSA isomers. The IPE–LC–MS/MS method was unable to achieve the separation of these isomers (Fig. 2). The resolving power of the SE–GC–PCI-MS method will facilitate isomer-specific analyses. The branched *N*-EtPFOSA and *N*,*N*-Et_2PFOSA that co-eluted after the main peak were present in all samples containing their respective straight chain isomers. Branched PFOSA was observed in about 40% of the samples, when the straight chain isomer was present at concentrations greater than 0.5 ng/g.

4.2. Comparison of results generated by SE–GC–PCI-MS and IPE–LC–MS/MS methods

Four narwhal and four beluga liver samples previously analyzed for PFOSA and *N*-EtPFOSA by the IPE–LC–MS/MS

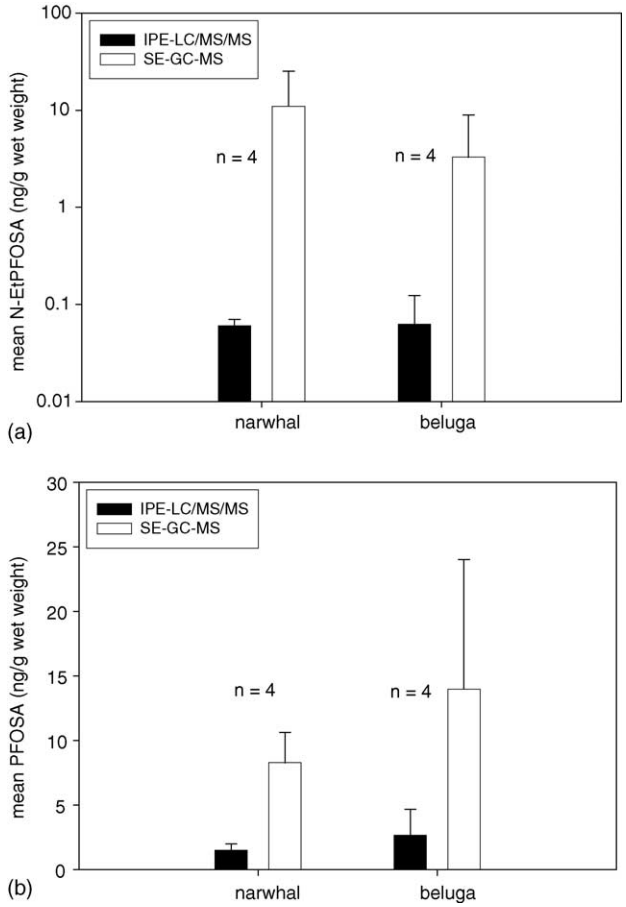

Fig. 3. Comparison of average (±standard deviation) concentrations of (a) *N*-EtPFOSA and (b) PFOSA in beluga and narwhal liver obtained using the solvent extraction GC–MS and ion pair extraction LC–MS/MS methods.

method [27] were analyzed by the SE–GC–PCI-MS method. Since no adequate recovery internal standard was used in the IPE–LC–MS/MS method, the compared concentrations obtained by both methods were not corrected for losses due to sample processing. The concentrations measured by SE–GC–PCI-MS are two orders of magnitude higher (for *N*-EtPFOSA) and approximately three-fold higher for PFOSA (Fig. 3).

The discrepancy in results obtained by the two methods is not solely accounted for by higher perfluorooctanesulfonamide recoveries in the SE–GC–PCI-MS method, since recovery corrected values (analyte recoveries in the IPE–LC–MS/MS were estimated using average recoveries of spiked water) still differed by at least one order of magnitude. It is possible that estimated recoveries of fortified water overestimate actual recoveries, especially since actual matrix conditions such as protein binding (which has been demonstrated for *N*-EtPFOSA [28]) are not approximated by this estimate.

It is also possible that there was suppression of the perfluorooctanesulfonamide analyte signals during analysis by LC–MS/MS. Standards used in the IPE–LC–MS/MS method were not prepared in matrix extracts that resembled matrix conditions of the samples, thus ion pair reagents used during sample preparation, or co-extracted matrix material, may have interfered with analyte ionization and affected quantitation.

The effect of ion pair reagents and co-extracted material on the LC–MS/MS signals of PFOSA and *N*-EtPFOSA were examined to determine if signal suppression occurred. Pork liver extracts ($n = 3$) prepared and analyzed using the IPE method described in [27] were spiked with PFOSA and *N*-EtPFOSA just prior to analysis by LC–MS/MS and compared to solutions of PFOSA and *N*-EtPFOSA prepared in methanol at the same concentration ($n = 3$). The pork liver was previously analyzed by the SE–GC–PCI-MS method and was not found to contain any PFOSA and *N*-EtPFOSA. Absolute peak areas of both compounds were approximately three times lower in the solution made up in pork liver extract ($p < 0.01$, *t*-test), indicating that matrix effects do cause perfluorooctanesulfonamide signal suppression. These matrix effects were not observed when fortified pork liver extracts ($n = 3$) were similarly compared to solutions prepared solely in isooctane ($n = 3$) using the SE–GC–PCI-MS method.

The main drawback to the SE–GC–PCI-MS method as compared to the IPE–LC–MS/MS method is that anionic perfluorinated organic compounds such as PFOA or PFOS cannot be determined. The SE–GC–PCI-MS method is specific towards neutral hydrophobic compounds.

4.3. Perfluorooctanesulfonamides in food and biota samples

The newly developed SE–GC–PCI-MS method was applied to the analysis of six different sample matrices for PFOSA, *N*-EtPFOSA, and *N*,*N*-Et_2PFOSA. All three compounds were observed in the environmental and food samples. Concentrations of PFOSA found in the marine mammal livers and shark fillets were lower than those reported for other biota, such as dolphin liver (878 ng/g) [18] and cormorant liver (100–215 ng/g) [17], but more often PFOSA is not detected above the MQL in biota. There are no comparable reports of food or biota concentrations of *N*-EtPFOSA and *N*,*N*-Et_2PFOSA.

The results of this small survey indicate that humans and wildlife are exposed to perfluorooctanesulfonamides and suggest that exposure occurs via multiple routes. Human exposure will include a dietary component, as indicated by the presence of PFOSA, *N*-EtPFOSA, and *N*,*N*-Et_2PFOSA in the fast food and shark fillet samples. It is likely that the presence in food is related to the use of perfluorinated grease and oil repellent coatings on food packaging materials [10]. This dietary exposure route may also contribute to human body burdens of PFOS, since PFOSA and *N*-EtPFOSA are precursors for the formation of PFOS in vitro [16]. The finding of these compounds in beluga and narwhal liver obtained from the Arctic also supports the hypothesis that perfluorooctanesulfonamides are not confined to the immediate vicinities of their use, but undergo long range transport to remote areas [29].

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