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# Validation of a screening method based on liquid chromatography coupled to high-resolution mass spectrometry for analysis of perfluoroalkylated substances in biota

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**Abstract**

A screening method for analysis of perfluoroalkylated substances (PFAS) in biota samples has been developed and validated using liver samples from polar cod (*Boreogadus saida*) and glaucous gull (*Larus hyperboreus*). The method was based on extraction of target compounds from homogenised samples into the solvent mixture used as mobile phase in high-performance liquid chromatography (HPLC), i.e. methanol/water (50:50; 2 mM ammonium acetate). The extract was filtered and directly injected into a HPLC/time-of-flight mass spectrometry (TOF-MS) system. Quantification was performed using 7*H*-perfluoroheptanoic acid as internal standard and a calibration standard solution dissolved in sample extract for each matrix type (matrix-matched calibration standard). The method is very time and cost efficient. Except for long-chain compounds and perfluorooctane sulfonamide (which cannot be covered by this method), recoveries were between 60% and 115% and method detection limits were in the range 0.04–1.3 ng/g wet weight. Blank values could be neglected with the exception of perfluorooctane sulfonate (PFOS), perfluorohexanoic acid (PFHxA) and perfluorooctanoic acid (PFOA). One of the major challenges in PFAS analysis is ionisation disturbance by co-eluting matrix in the ion source of the mass spectrometer. Both matrix and analyte specific signal enhancement and suppression was observed and quantified. Repeated extractions ($n = 3$) gave relative standard deviations (RSD) <35% for all PFAS. Accuracy was examined by comparing the screening method to the generally applied ion pair extraction (IPE) method. PFAS concentration values of a glaucous gull liver sample deviated by less than 30% for the two methods, provided that matrix-matched calibration standards were employed in both methods.


*Keywords:* Perfluoroalkylated substances; Perfluorooctane sulfonate; PFOS; High-resolution MS; Method validation; Screening method; Liver samples

## 1. Introduction

Perfluoroalkylated substances (PFAS) have been industrially produced for several decades [1–3] and are applied as stain and water repellents for surface treatment of textiles, carpets, leather and paper products. Furthermore, they are employed in the large-volume production of fire-fighting foams and in the chromium-plating industry. Perfluorooctane sulfonate (PFOS) has recently gained considerable attention because of its ubiquitous distribution in biota [4–7] and its presence in human blood plasma [8]. Though most of the production of PFOS-based chemicals has been phased out voluntarily by the main manufacturer, similar compounds with polyfluorinated chains continue to be employed for comparable applications [3]. Such compounds include shorter chain perfluorinated sulfonates, perfluorinated carboxylic acids (usually produced by electrochemical fluorination, as PFOS-based chemicals) and telomerisation products such as 1*H*,1*H*,2*H*,2*H*-tetrahydro-PFOS.

In recent years, many articles about environmental monitoring of PFAS in biota have been published [4–13]. All these studies employed the same analytical method, based on ion pair extraction of the analytes [14,15] and subsequent

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HPLC/MS quantification by tandem mass spectrometry [15]. This method is flexible and can be used for a wide range of matrices, such as egg, blood plasma, liver and other biological tissues. However, the method is quite time consuming and matrix-matched calibration standards are not routinely employed, i.e. matrix induced ionisation disturbances in the ion source of the mass spectrometer are usually not accounted for. Furthermore, tandem MS suffers from considerable sensitivity loss due to low fragmentation yields for some PFAS [16]. Recently, HPLC coupled to high-resolution time-of-flight (TOF) MS was suggested as alternative and more sensitive technique compared to tandem MS [16].

The aim of this study was to develop and validate a quick and cost efficient screening method for analysis of PFAS in biological tissues. The method should involve as few steps as possible to avoid loss of the surface-active analytes, which may occur during extract transfer between containers, solvent change or solvent evaporation to dryness. Applying TOF-MS instead of tandem MS was expected to compensate for the sensitivity loss implied by omitting a concentration step. The screening method was to be validated for liver samples from polar cod and glaucous gull, which are important species in the Arctic marine food web. Though PFAS are known to be ubiquitously present in liver [4–7], low contamination was expected in the test samples due to the remote sampling location. This was expected to facilitate spiking experiments.

## 2. Experimental

### 2.1. Chemicals

The analysed PFAS are listed in Table 1, including the here used abbreviations as well as the purity and supplier of the standard compounds. Additionally, $^{13}C_2$-PFOA (≥90%, Perkin-Elmer, Shelton, CT, USA) and 2*H*,2*H*-dihydro-PFDcA (>98%, Wellington Laboratories, Guelph, Canada) were tested as internal and recovery standard, respectively. The abbreviations PFBS, PFHxS, PFOS and PFDcS are used for the sulfonate ions. All concentrations given in this study are based on these ions, not the respective salts. *n*-Hexadecane (≥98%, for synthesis) and tetra-*n*-butylammonium hydrogensulfate (≥99%, for synthesis) were purchased from Merck-Schuchardt (Hohenbrunn, Germany). All other solvents and reagents used in this work were of analytical or HPLC grade. Gas (quality 5.0) was purchased from Hydrogas (Porsgrunn, Norway).

### 2.2. Samples, extraction and clean-up

Polar cod (*Boreogadus saida*) and glaucous gull (*Larus hyperboreus*) were collected from the marginal ice zone in the Barents Sea northwest of Hopen (Norwegian Arctic; 77°1′N, 29°5′E) during one week in late May 2004. The liver was removed and homogenised using a blender (Ultraturrax

Table 1
The target analytes and analytical standards, their abbreviation, purity and supplier as well as cone voltage and ion mass to charge ratio (*m*/*z*) used in extracted mass chromatograms for quantification (typical mass tolerance 0.06 u)

| Compound | Abbreviation | Purity (%) | Supplier | Cone voltage (V) | Quantification mass (*m*/*z*) |
|---|---|---|---|---|---|
| Tetrabutylammonium perfluorobutane sulfonate | PFBS | ≥98 | Fluka[a] | 40 | 298.94 |
| Potassium perfluorohexane sulfonate | PFHxS | 98 | Interchim[b] | 40 | 398.94 |
| Potassium perfluorooctane sulfonate | PFOS | ≥98 | Fluka[a] | 40 | 498.93 |
| Ammonium perfluorodecane sulfonate 25% (weight) in 2-butoxyethanol in water (37%) | PFDcS | n.i.[c] | Sigma–Aldrich[d] | 40 | 598.92 |
| Perfluorohexanoic acid | PFHxA | 98 | ABCR[e] | 20 | 268.98 |
| Perfluoroheptanoic acid | PFHpA | 99 | Sigma–Aldrich[d] | 20 | 318.98 |
| Perfluorooctanoic acid | PFOA | ∼95 | Fluka[a] | 20 | 368.98 |
| Perfluorononanoic acid | PFNA | 97 | Sigma–Aldrich[d] | 20 | 418.97 |
| Perfluorodecanoic acid | PFDcA | ≥97 | Fluka[a] | 20 | 468.97 |
| Perfluoroundecanoic acid | PFUnA | 95 | Sigma–Aldrich[d] | 20 | 518.97 |
| Perfluorododecanoic acid | PFDoA | 95 | Sigma–Aldrich[d] | 20 | 568.96 |
| Perfluorotetradecanoic acid | PFTeA | 97 | Sigma–Aldrich[d] | 20 | 668.96 |
| 1*H*,1*H*,2*H*,2*H*-Tetrahydro perfluorooctane sulfonic acid | TH-PFOS | n.i.[c] | Interchim[b] | 40 | 426.97 |
| Perfluorooctane sulfonamide | PFOSA | 97 | ABCR[e] | 40 | 497.95 |
| 7*H*-Perfluoroheptanoic acid[f] | 7H-PFHpA | 98 | ABCR[e] | 20 | 280.98 |
| 3,5-Bis(trifluoromethyl)phenyl acetic acid[g] | BTPA | 98 | ABCR[e] | 40 | 227.03 |

[a] Fluka, Buchs, Switzerland.
[b] Interchim, Montluçon Cedex, France.
[c] No information available.
[d] Sigma–Aldrich, Steinheim, Germany.
[e] ABCR, Karlsruhe, Germany.
[f] Used as internal (surrogate) standard.
[g] Used as recovery (volume) standard.

T 25, Janke & Kunkel, IKA Labortechnik, Staufen, Germany). For polar cod, pooled samples consisting of livers from three fish were employed, due to the small size of the liver. To 1.0 g of the homogenized sample in a polypropylene (PP) centrifuge tube, 20 ng internal standard (7H-PFHpA, see Table 1, 20 μL of a 1 ng/μL solution in methanol) and 2.7 g (corresponding to 3.0 mL) of methanol/water (50:50; 2 mM ammonium acetate ($NH_4OAc$)) were added. The PP tube was capped and the sample was thoroughly mixed using a Vortex chemical mixer, and then allowed to extract for 30 min in an ultrasonic bath at room temperature. After extraction, the mixture was first coarsely filtered through Kleenex covering the tip of a Pasteur pipette, and the resulting solution was filtered through a Microcon YM-3 centrifugal filter (Millipore, Billerica, MA, USA) at 14 000 rpm. Approximately 100 μL of the final extract were transferred to an autoinjector vial, weighed and 2 ng recovery standard (BTPA, see Table 1, 20 μL of a 0.1 ng/μL solution in methanol) were added before HPLC/MS analysis. For each species, calibration standards were dissolved in an unspiked liver extract, which proved to contain only minor amounts of the relevant PFAS.

### 2.3. Ion pair extraction for method comparison

For method comparison, a modification of the ion pair extraction (IPE) method originally developed by Ylinen et al. [14] and later adapted by Hansen et al. [15] was applied. In short, to 0.5 g of homogenous liver sample 10 ng 7H-PFHpA, 2 mL water, 2 mL buffer (0.25 M $NaHCO_3$ plus 0.25 M $Na_2CO_3$ in water) and 1 mL TBA solution (0.5 M tetra-*n*-butylammonium hydrogensulfate in water, adjusted to pH 10 with 2 M NaOH in water) were added. Extraction was performed twice with 5 mL *tert*-butyl methyl ether by shaking for 15 min on a wrist-action-shaker, and the combined extracts (10 mL) were evaporated to dryness. The residues were suspended in 2 mL methanol/water (50:50; 2 mM $NH_4OAc$) and 5 ng BTPA were added. Finally, the extract was filtered through a Microcon YM-3 centrifugal filter. Calibration standards were dissolved in an unspiked glaucous gull liver extract.

### 2.4. HPLC/MS analysis

A 1100 series low-pressure quaternary gradient pump and auto-injector (Agilent Technologies, Palo Alto, CA, USA) were coupled to a time-of-flight (TOF) mass spectrometer (LCT, Micromass, Manchester, England). The mobile phase solvents for chromatography were degassed with a gentle flow of helium. A sample volume of 25 μL was injected into a $C_{18}$ reversed phase column (Ace 3 C18, 3 μm particles, 100 Å pore size, 150 mm length, 2.1 mm i.d., Advanced Chromatography Technologies, Aberdeen, Scotland). The target compounds were separated at a flow rate of 200 μL/min using 2 mM $NH_4OAc$ in both methanol (A) and water (B). The following binary gradient was applied: 0 min, 50% A; 0–5 min, linear gradient to 85% A; 5–10 min, 85% A; 10–10.5 min, linear gradient to 99% A, followed by 4.5 min rinsing with 99% A. The void volume of the HPLC pump led to approximately 6 min delay of the gradient on the column. The equilibration time with 50% A was 8 min and the total run time 23 min. The TOF-MS was employed in the negative ion electrospray ionisation (ESI(−)) mode. Mass spectra were registered in full scan mode (mass range *m*/*z* 165–720). The following optimised parameters were applied: Capillary voltage, 3 kV; sample cone voltage, alternating 20/40 V; desolvation temperature, 325 °C; source temperature, 120 °C; nitrogen cone, desolvation and nebuliser gas flows, 20, 400 L/h and maximum, respectively. Quantitative analysis was performed employing extracted mass chromatograms from full scan recording using the cone voltages and mass to charge values (*m*/*z*, typical mass tolerance of 0.06 u) given in Table 1 for the different analytes.

### 2.5. Detection limits, linear range and recoveries

The instrumental limit of detection (LOD, based on a signal-to-noise ratio of three) and the linear range of the mass spectrometer were determined by triplicate injections of a dilution series (20 μL injections of each concentration) of all analytes in methanol/water (50:50; 2 mM $NH_4OAc$) with concentrations ranging from below LOD to 10 ng/μL (eight measuring points). The method detection limit (MDL) was defined as the higher value of the following two alternatives: (1) Average blank signal plus three standard deviations of a series of five blind extractions from *n*-hexadecane (surrogate matrix) on five different days of the validation experiments; (2) signal-to-noise ratio of three in the chromatogram of a sample extract from spiked liver (the selected liver did not contain detectable amounts of PFAS and was fortified with all analytes close to the MDL). For recovery determination, all analytes including the internal standard 7H-PFHpA were spiked into *n*-hexadecane or low contaminated liver samples (spiking levels 50 ng/g). After extraction, PFAS recoveries were determined relatively to the recovery standard BTPA. External calibration standards were dissolved in methanol/water (50:50; 2 mM $NH_4OAc$) for the *n*-hexadecane experiments, or in the respective unspiked liver extracts for the experiments with spiked liver samples. This was done in order to determine recoveries independent of matrix effects.

### 2.6. Matrix effects

For determination of matrix effects on ionisation, equal concentrations of standard solutions were prepared in unspiked liver extract and in pure methanol/water (50:50; 2 mM $NH_4OAc$). The solutions were analysed consecutively and the absolute signal areas in the two chromatograms were compared. The matrix effect of a given liver matrix on a given analyte was quantified as the ratio of these areas, i.e. a matrix effect >1 represents signal enhancement, and a value <1 represents signal suppression by co-eluting matrix compounds.

### 2.7. Determination of precision and accuracy

The precision of the screening method was evaluated by determining its repeatability and reproducibility. For repeatability testing, three aliquots of a glaucous gull liver sample (GG1 a–c) were extracted and analysed. Reproducibility was tested with another glaucous gull liver sample (GG2). Three aliquots were analysed on different days over a period of nine months, which included freezing and thawing of the sample homogenate between the different extractions. Since no certified reference material exists yet for PFAS analysis, accuracy was only estimated by comparing the screening method to the IPE method.

## 3. Results and discussion

### 3.1. Internal and recovery standard

The lack of pure calibration standards (free from isomers and homologues) and well-suited, isotopically labelled internal standards for PFAS analysis has been discussed in literature [17]. The selection of internal and recovery standard is crucial for reliable quantification using the internal standard method. The only isotopically labelled PFAS, which were commercially available at the time of this study, were [M + 2] compounds, i.e. their masses are only 2 u larger than the native compounds. The screening method was tested using $^{13}C_2$-PFOA or 7H-PFHpA as internal standard and 2*H*,2*H*-dihydro-PFDcA or BTPA as recovery standard. These four compounds were not expected to be present in samples. $^{13}C_2$-PFOA might be a good surrogate for perfluorinated acids, especially for PFOA. However, it was not suited as internal standard for the following reasons. Perfluorinated acids are most sensitively analysed by TOF-MS using the ion $[M - CO_2 - H]^-$. Due to loss of $^{13}CO_2$, $[M - CO_2 - H]^-$ of $^{13}C_2$-PFOA (*m*/*z* 370) has only 1 u mass difference to the corresponding ion of native PFOA (*m*/*z* 369). Signal overlap in the mass chromatograms of both these masses was observed. Furthermore, the $^{13}C_2$-PFOA standard contained about 10% $^{13}$C-PFHpA, which interfered with the quantification of native PFHpA. Both, $^{13}C_2$-PFOA (retention time 10.9 min) and 2*H*,2*H*-dihydro-PFDcA (12.0 min) eluted from the HPLC column in the time window where most matrix effects were observed. Therefore, their signal abundance varied strongly between liver extracts from different species. The earlier eluting 7H-PFHpA (retention time 7.0 min) and BTPA (9.0 min) were much less influenced by matrix effects and chosen as internal and recovery standard, respectively.

### 3.2. Instrumental detection limits and linearity

The instrumental limits of detection of LC/TOF-MS are given in Table 2. They were in the fg to low pg range for all analytes. These LODs are approximately factor 10 lower than in LC/MS–MS (triple quadrupole instrument) [16]. The linear range of the TOF-MS instrument for the PFAS typically covered three orders of magnitude from the LOD upwards (results for selected compounds previously shown [16]).

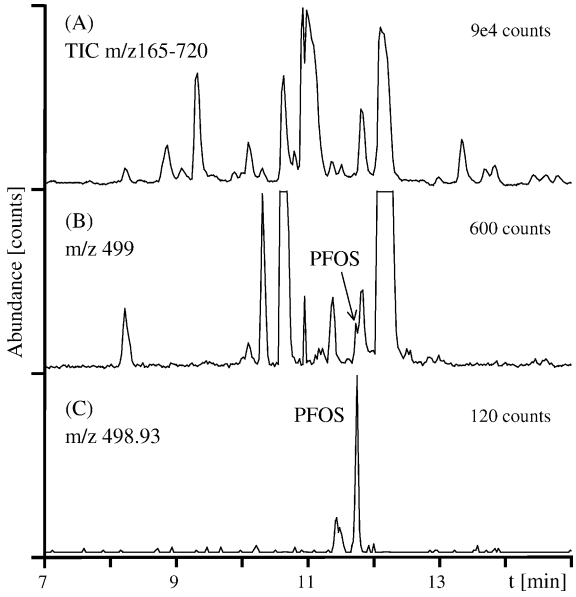


Fig. 1. Extracted chromatograms from full scan (*m*/*z* 165–720) TOF-MS analysis of a black guillemot liver extract demonstrating the specificity of high mass resolution. (A) Total ion current (TIC) chromatogram; (B) extracted mass chromatogram at *m*/*z* 499 (PFOS, mass tolerance ±0.5 u; the shown range of 600 counts corresponds to 3% of the full abundance range for the base signal at 12.1 min); and (C) extracted mass chromatogram at *m*/*z* 498.93 (mass tolerance ±0.03 u). The PFOS isomers were quantified to 28 ng/g in the liver.

### 3.3. Specificity of LC/TOF-MS

The high mass resolution of the TOF-MS instrument (5000 FWHM) offers excellent specificity for unequivocal compound identification after a crude sample clean-up. This is illustrated in Fig. 1 with extracted chromatograms from LC/TOF full scan MS analysis of a black guillemot liver extract. A considerable amount of matrix compounds remained in the extract, which coeluted with the PFAS in LC separation (see TIC chromatogram in Fig. 1A). Fig. 1B shows the extracted mass chromatogram for PFOS (*m*/*z* 498.5–499.5) the way it would be expected for single MS analysis with a low-resolution (unit resolution) instrument. Only the extracted high-resolution mass chromatogram (Fig. 1C; *m*/*z* 498.90–498.96) resolves the PFOS isomer signals from virtually all matrix compounds. Tandem mass spectrometry shows excellent specificity as well, but has the drawback that the matrix background is eliminated by the instrument, thus it cannot be visualised. Applying TOF-MS gives an estimation of the amount of matrix left in the extract, which could impair the ionisation performance.

### 3.4. Recoveries

Recoveries for all compounds spiked to *n*-hexadecane at a spiking level of 50 ng/g are listed in Table 2. Most

Table 2
Validation parameters for the selected PFAS: instrumental limit of detection (LOD); recovery from spiked *n*-hexadecane; method detection limit (MDL), recovery from spiked liver samples and matrix effect in spiked extracts for polar cod liver and glaucous gull liver

| Compound | LOD (pg injected) | Recovery from hexadecane (%) | Polar cod liver | | | Glaucous gull liver | | | Northern fulmar liver |
|---|---|---|---|---|---|---|---|---|---|
| | | | MDL (ng/g ww) | Recovery (%) | Matrix effect in ionisation[a] | MDL (ng/g ww) | Recovery (%) | Matrix effect in ionisation[a] | Matrix effect in ionisation[a] |
| PFBS | 0.5 | 114 | 0.16 | 109 | 2.13 | 0.18 | 93 | 2.44 | 1.06 |
| PFHxS | 0.2 | 99 | 0.04 | 89 | 1.96 | 0.06 | 76 | 1.43 | 0.82 |
| PFOS | 0.3 | 70 | 0.23 | 79 | 1.68 | 0.30 | 90[b] | 0.11 | 1.26 |
| PFDcS | 0.5 | 28 | >15 | n.q.[c] | 0.05 | >15 | n.q.[c] | 0.00 | 0.67 |
| PFHxA | 2 | 99 | 0.22 | 83 | 1.56 | 0.22 | 87 | 2.07 | 1.03 |
| PFHpA | 1 | 102 | 0.44 | 84 | 2.58 | 0.56 | 82 | 2.31 | 0.98 |
| PFOA | 1 | 73 | 1.25 | 84 | 0.31 | 1.28 | 83[b] | 0.17 | 0.85 |
| PFNA | 1 | 61 | 0.24 | 67 | 2.15 | 1.00 | 51 | 0.50 | 1.08 |
| PFDcA | 2 | 51 | 0.32 | 86 | 1.41 | 3.40 | 46 | 0.19 | 0.95 |
| PFUnA | 3 | 28 | 0.75 | 175 | 0.30 | >15 | n.q.[c] | 0.01 | 0.92 |
| PFDoA | 5 | 13 | >15 | n.q.[c] | 0.00 | >15 | n.q.[c] | 0.00 | 0.62 |
| PFTeA | 8 | 2 | >15 | n.q.[c] | 0.00 | >15 | n.q.[c] | 0.00 | 0.05 |
| TH-PFOS | 5 | 107 | 0.40 | 104 | 1.33 | 0.70 | 110 | 0.74 | 1.22 |
| PFOSA | 0.5 | 49 | 5.59 | 27 | 0.05 | 9.80 | 9 | 0.08 | 0.71 |
| 7H-PFHpA | 5 | 112 | n.d.[d] | 98 | 2.17 | n.d.[d] | 79 | 2.51 | 1.23 |

For comparison, also the matrix effect in northern fulmar liver extracts is given. For definitions and experimental details see text. Two parallel extractions were performed for all recovery experiments and the average value is given.

[a] Signal area of PFAS standard diluted in liver extract relative to signal area of the standard diluted in LC mobile phase. Values >1 and <1 represent matrix induced signal enhancement and suppression, respectively.

[b] Single determination, the second parallel could not be quantified due to disturbance in the chromatogram (low signal abundance due to strong matrix suppression).

[c] No quantification possible due to low signal abundance.

[d] Not determined.

values were between 60% and 115%. The internal standard 7H-PFHpA, TH-PFOS and short chain PFAS were quantitatively recovered, while the recovery rates decreased with increasing chain length for both the sulfonates and the acids. PFDcS, PFDcA to PFTeA and the non-ionic PFOSA had recoveries below 60%, probably due to poor solubility in the extraction solvent mixture of methanol and water. Recovery experiments from spiked (50 ng/g) polar cod liver and glaucous gull liver were performed in duplicates and the average recovery rates are given in Table 2. In most cases, the parallel determinations did not deviate more than 10%. With the exception of lower recoveries for PFOSA, the values found for spiked liver were comparable to the values from spiked *n*-hexadecane, i.e. the matrix did not have a strong influence on extraction and *n*-hexadecane was a good matrix surrogate for blank experiments. The only peculiarity was that the recoveries of PFHxA to PFDcA from polar cod liver did not seem to decrease with increasing chain length.

A weakness of recovery determination in the screening method is that it is based on the ratio of the weight of the final extract to the total weight of the extraction solvent with the assumption that no solvent evaporation occurs during extraction and filtration. This might lead to overestimation of recoveries due to evaporation loss. On the other hand, samples with considerable water content would add to the total solvent volume and result in underestimation of recovery rates.

### 3.5. Matrix effects

Matrix effects in LC/TOF-MS were observed as effects on signal intensity (ionisation efficiency) of the PFAS in ESI(−). Neither retention times nor signal shapes in LC were influenced by the presence of liver matrix in the extract. The matrix effect on ionisation was quantified as described in Section 2.6. Results for liver matrix of polar cod, glaucous gull and northern fulmar (*Fulmarus glacialis*, for comparison) are summarised in Table 2. Large species-specific differences were observed. The matrix residues of northern fulmar liver had only a minor effect on ionisation efficiencies, with exception of strong suppression of PFTeA. In polar cod and glaucous gull liver extracts signal enhancement of up to a factor 2.6 was observed for the smaller compounds, compared to pure solvent solutions of the PFAS. Ionisation of PFOSA and the long chain sulfonates and acids, however, was strongly suppressed by the presence of matrix residues from the same species. These data emphasis the necessity of using external calibration standards dissolved in authentic sample extracts for proper quantification, especially in connection with a crude sample clean-up and a vulnerable ionisation technique such as ESI.

### 3.6. Blank values and method detection limits

For most analytes the MDL was governed by recovery, matrix effects, instrumental LOD and chromatographic noise,

rather than laboratory blank values. However, laboratory contamination by PFOS, PFHxA, PFOA, TH-PFOS and PFOSA was present. Blank values were the limiting factor for the MDL of PFOS, PFHxA and PFOA in polar cod liver as well as PFHxA in glaucous gull liver. Typical MDL for all analytes are given in Table 2. They varied slightly between individual samples, however, the variation between livers from different species was much more pronounced. For most compounds (except PFOSA and long chain PFAS) the MDL was in the range 0.04–1.3 ng/g wet weight. These values compare favourably with literature values, being about one order of magnitude lower than the MDL (or quantification limits) reported in the years 2001–2003 [4,5,8,9,15] for the method described in [15]. Newer publications (2004–2005) using this method [6,7,11–13] achieved values, which correspond to the values for the here described screening method.

Due to the uncertainties in recovery calculation (see Section 3.4), it is not advisable to correct quantified PFAS levels for recovery difference between the analyte and the internal standard. Thus, levels of compounds with lower recoveries than the internal standard (i.e. PFOSA, PFDcS and PFDcA to PFTeA) are underestimated and can therefore be lower than the MDL. Furthermore, these were basically the same compounds that suffered from strong matrix suppression (see Table 2), which additionally increased their MDL. If at all detected, these compounds could only be analysed qualitatively with the screening method.

### 3.7. Precision

The results of the repeatability and reproducibility experiments are given in Table 3. Single determinations in the repeatability study (GG1 a–c) varied with up to factor 2 for PFOS and PFHxA, resulting in relative standard deviations (RSD) of 35% and 34%, respectively ($n = 3$). The repeated analysis of PFHxS, PFNA and PFDcA showed RSD of 21% or less. Similar variations were found in the reproducibility experiment (GG2 day *x–z*; RSD 10–39%). This indicates that the deviations are rather due to method uncertainty than due to day-to-day variations.

### 3.8. Accuracy/comparison with IPE method

Due to the lack of certified reference material for PFAS analysis, accuracy testing was limited to comparison of values obtained with the screening method and the IPE method. The results are summarised in Table 3. The mean concentrations for GG1 found in repeatability testing with the screening method are compared to mean levels ($n = 3$) found applying the IPE method. As described in Section 2, the two methods are different in extraction and clean up. Even though matrix-matched calibration standards are not usually used in combination with the IPE method, this was done here to provide better comparability with the screening method. The mean concentrations of PFAS in GG1 found applying the screening method compared very well with the mean values for the IPE method (Table 3). The biggest discrepancy was found for PFHxS, which was the lowest concentrated of all detected PFAS. However, it has to be kept in mind that this excellent agreement between the two methods is based on comparison of mean values of three repeated analyses and application of the same instrumental method and quantification approach.

### 3.9. Advantages and limitations of the screening method

The screening method was successfully applied to liver samples of 12 different species (fish, birds and marine mammals). As an illustration, extracted mass chromatograms of the glaucous gull liver sample GG2 are shown in Fig. 2.

Table 3
Repeatability, reproducibility and accuracy data for the screening method using two different glaucous gull liver samples (GG1 and GG2)

| | PFHxS | PFOS | PFDcS | PFHxA | PFOA | PFNA | PFDcA | PFUnA | PFDoA | PFOSA |
|---|---|---|---|---|---|---|---|---|---|---|
| Repeatability | | | | | | | | | | |
| GG1 a | 0.13 | 225 | <15 | 0.17 | <1.28 | 4.74 | 9.43 | <15 | <15 | <15 |
| GG1 b | 0.17 | 214 | <15 | 0.09 | <1.28 | 5.78 | 6.94 | <15 | <15 | <15 |
| GG1 c | 0.13 | 109 | <15 | 0.18 | <1.28 | 4.02 | 6.47 | <15 | <15 | <15 |
| Reproducibility | | | | | | | | | | |
| GG2 day x | 0.35 | 117 | <15 | 0.24 | <1.28 | 2.47 | 2.27 | <15 | <15 | <15 |
| GG2 day y | 0.23 | 119 | <15 | 0.14 | <1.28 | 1.32 | <3.40 | <15 | <15 | <15 |
| GG2 day z | 0.34 | 140 | <15 | 0.32 | <1.28 | 2.14 | 2.65 | <15 | <15 | <15 |
| Accuracy | | | | | | | | | | |
| GG1 mean (RSD)[a], screening method | 0.14 (16) | 183 (35) | <15 | 0.15 (34) | <1.28 | 4.85 (18) | 7.61 (21) | <15 | <15 | <15 |
| GG1 mean (RSD)[a] IPE method | 0.19 (5) | 182 (11) | 0.65 (9) | <0.67[b] | <1.34[b] | 5.10 (10) | 8.04 (6) | 14.0 (4) | 2.74 (8) | <0.67[b] |

All values are given in ng/g ww. The triplicate repetitions for GG1 (a–c) were performed as parallels, while the repeated extractions of GG2 were done on different days covering a period of nine months. For accuracy estimation, mean values for GG1 obtained by the screening method and the ion pair extraction (IPE) method were compared.

[a] Mean value and relative standard deviation (%) of three replicates.

[b] Method detection limit defined as three times blind value (single determination) for the IPE method.

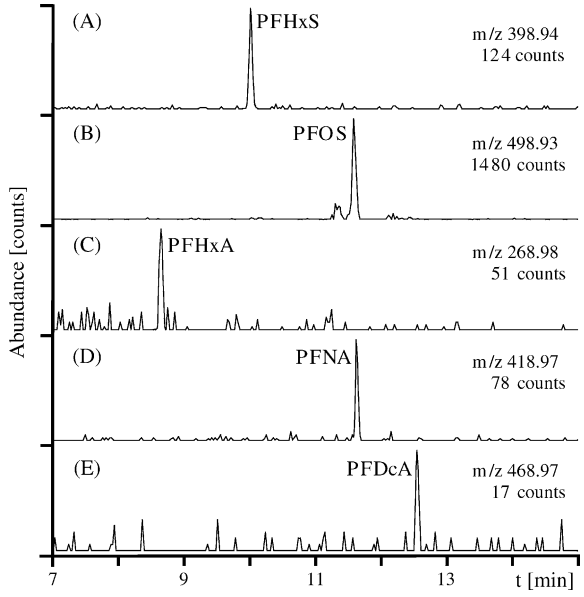


Fig. 2. Extracted mass chromatograms (mass tolerance 0.06 u) from full scan (*m/z* 165–720) TOF-MS analysis of a glaucous gull liver extract (GG2 day *x*, see Table 3). Quantified concentrations are (A) PFHxS 0.35 ng/g; (B) PFOS 117 ng/g; (C) PFHxA 0.24 ng/g; (D) PFNA 2.47 ng/g; and (E) PFDcA 2.27 ng/g.

Furthermore, also brain samples of fish and birds were successfully analysed with the screening method (results not shown). They showed much less matrix effects compared to liver samples, but were sometimes difficult to filter.

The main advantages of the screening method over the IPE method are the time and cost efficiency and the very short and straightforward sample handling, reducing the risk for lab contamination (blank values) and loss of analytes. The IPE method has furthermore the weakness of an evaporation step to dryness, which might lead to loss of PFOSA possessing a relatively high vapour pressure. The screening method works technically very well also for lipid rich samples such as polar cod or burbot liver, whereas lipids are extracted into *tert*-butyl methyl ether when applying the IPE method. This poses a big challenge for evaporation to dryness and re-dissolution in methanol or methanol/water.

The major drawback of the screening method is the inability to comprise the less polar PFAS (PFDcS, PFUnA to PFTeA and PFOSA) at low method detection limits. Depending on the type of matrix and the chosen internal standard, very strong matrix effects due to the crude clean up procedure can lead to reduced precision compared with the IPE method (see RSD values in Table 3). However, since the screening method corrects for matrix effects, accuracy is not expected to be influenced other than by precision.

## 4. Conclusions

The validation experiments showed that the screening method produced as accurate results as the IPE method, despite a very quick and crude sample extraction and clean up. Typical applications for the screening method could be large sample series of the same matrix type, where the time and cost efficiency are beneficial. At least one low-contaminated sample of the same matrix type has to be available for obtaining the sample extract needed for dilution of the calibration standards. The method could also qualitatively be used to select the most contaminated samples of a large sample set for more comprehensive analysis. Furthermore, the screening method is a valuable alternative to verify quantitative results and systematic errors (e.g. due to matrix effects if no matrix-matched standards are employed) of the IPE method. It is not beneficial for analysis of samples from many different matrix types or when long-chain PFAS are of particular interest. Independent of the applied method, matrix effects are a big challenge in PFAS analysis. Although the limited availability of suitable standards and the problems connected to matrix residues in ESI-MS have been discussed before [17], measures to avoid systematic errors due to these issues are not yet routinely applied.

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