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An optimized method for the determination of perfluorooctanoic acid, perfluorooctane sulfonate and other perfluorochemicals in different matrices using liquid chromatography/ion-trap mass spectrometry

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ABSTRACT

Perfluorochemicals (PFC's) are widely spread in the environment and have been detected in blood of wildlife and humans world-wide. Recently, various toxic effects of PFC's in laboratory rats have been demonstrated, resulting in increased government concerns regarding the presence of PFC's in the environment and the implications they have on human health. In the last decade, various analytical methods have been developed for the analysis of PFC's in different matrices whereby the majority of methods have utilised liquid chromatography coupled with mass spectrometry (LC–MS). Here we describe an optimized method for the quantitation of PFC's, including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), in food packaging, polytetrafluoroethylene (PTFE) sealant tape and drinking water. The method involved PFC's extraction via off-line SPE followed by separation using reversed-phase liquid chromatography on a Phenyl–Hexyl column coupled with ion-trap (IT) mass spectrometric detection. The optimized approach minimized ion-suppression effects commonly seen with conventional elution buffers, improving detection limits down to 25 pg/mL and allowed effective quantitation down to 50 pg/mL for PFOA and PFOS. The optimized LC–MS method detected PFOA and other PFC's in microwave popcorn packaging and PFOA in PTFE sealant tape in the low μg/kg. In all samples, PFOS was not detected.

1. Introduction

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) belong to the family group of perfluorochemicals (PFC's). These anionic compounds have a molecular structure in which all hydrogen atoms from the hydrophobic alkylic framework are replaced by fluorine atoms. The 'van der Waals' radius of a fluorine atom is larger than that of a hydrogen atom, increasing the chemical stability and providing unique water and oil repellent properties [1]. PFC's are routinely used in industry as a coating in food packaging (*e.g.* baking paper and microwave popcorn bags to prevent oil leaching into the paper [2]), as stain- and water-resistant coatings for fabrics and carpets and in firefighting foam [3]. In Particular, PFOA is used as an emulsifier for the production of polytetrafluoroethylene (PTFE), well known by the DuPont brand name Teflon [4]

The chemical stability of PFC's pose an issue for human safety as they do not degrade naturally and hence may accumulate in the body. Recent toxicity studies in rats showed that PFOA induces various toxic effects, such as reproductive and liver toxicity, as well as the development of cancer [5]. Since the introduction of PFC's over 50 years ago, studies dating to the early 1960s indicated the presence of fluorinated compounds in human serum samples [6]. However, due to the lack of knowledge regarding PFC's toxicity, this research did not result in the implementation of government regulations. As such, PFC's have since been detected in blood samples collected from humans as well as wildlife [7–10], which has resulted in increased awareness of PFC's as potential environmental pollutants [11–14]. The United States Environmental Protection Agency's (EPA) scientific advisory board issued a draft report in 2005, implicating PFOA as a "likely" carcinogen. However, EPA has not yet introduced any new regulations [15].

The development of methods for PFOA and PFOS detection in different matrices is of critical importance, considering the potential implications of these PFC's for both human and wildlife health. Several methods have been developed employing liquid chromatography mass spectrometry (LC–MS) to achieve this. These LC–MS methods typically employ the use of C8 or C18 reversed-phase high-performance liquid chromatography (RP-HPLC) columns under aqueous-ammonium conditions. Following LC separation, MS detection is typically performed using a triple-quadrupole (QQQ) mass spectrometer in the multiple-reaction-monitoring (MRM) mode, where quantitation limits in

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the order of low pg/mL have been reported [3,16]. One drawback of using this MRM approach is that only a selection of known compounds or compounds where standards are available can be monitored. Hence, in order to obtain information on all compounds present in a sample, a broad m/z range must be selected in full-scan MS mode. Although quadrupole mass spectrometers are capable of collecting ions in the full-scan MS mode, the scan speed and sensitivity is lower compared to scans performed in the MRM mode. As an alternative, ion-trap mass spectrometry (IT-MS) can be employed, as in the full-scan MS mode, IT mass spectrometers have the advantage of accumulating ions prior to scanning the entire mass range. The increase in trapping efficiency of modern 3D IT mass spectrometers in full-scan MS mode result in detection limits comparable with those achieved in single ion monitoring (SIM) or MRM in quadrupole devices. Further technical improvements of IT-MS has lead to higher resolution (up to $R = 20{,}000$) at reasonable scan speeds (>2 Hz), resulting in mass accuracies better than 0.1 Da to provide full information on the sample, including unknown compounds. However, the disadvantage of ion-trap mass spectrometers is the restricted mass range in the MRM mode. Due to physical limitations of ion-trap mass spectrometers, the fragment ion m/z must be >25% of the parent ion m/z for sensitive and quantitative detection. The major fragment ion from PFOS, 99 m/z ($[FSO_3]^-$), is below 25% m/z of the parent ion 499 m/z and hence could not be used for quantitation. In contrast to PFOS, PFOA shows a major fragment at 369 m/z ($[M\text{-}COOH]^-$), which is clearly above the 25% cut-off [17–19].

QQQ and IT mass spectrometers have been used frequently in conjunction with electrospray ionization (ESI) for detection of positively or negatively charged ions. ESI is susceptible to ion-suppression of the analyte by several interfering substances such as fluorinated carboxylic acids like trifluoroacetic acid (TFA). In particular, the use of ammonium in the mobile phase has been reported to result in ion-suppression, resulting in higher detection limits [20].

In this study we describe the development of an ammonium free LC–MS method for the separation and identification of PFC's extracted from microwave popcorn bags, popped popcorn after microwaving, non-stick baking paper, a French fry box, sandwich wrapper and a hamburger box from a major fast food company, PTFE sealant tape and bottled drinking water. The approach was subsequently utilised to quantify levels of PFOA and PFOS by IT-MS.

2. Experimental

2.1. Chemicals and materials

The PFOA (98.6%) and PFOS (98%) standards were purchased from Sigma–Aldrich (Steinheim, Germany). LC/MS grade acetonitrile and acetic acid were obtained from Fluka (Buchs, Switzerland). Methanol was purchased from Burdick and Jackson (SK Chemicals, Ulsan, Korea), acetone from Spectrosol (NSW, Australia) and ammonia from Ajax Finechem (VIC, Australia). The C18, 300 mg SPE cartridges were purchased from Alltech (NSW, Australia) and water was purified using a MilliQ Gradient A10 system (Millipore, Molsheim, France). Two different microwave popcorn bags (brands A and B), PTFE sealant tape, two different brands non-stick baking paper (brands A and B) and bottled drinking water were all purchased at local retail shops. A French fry box, sandwich wrapper and a hamburger box were obtained from a major fast food company on site.

2.2. Sample preparations

From the microwave popcorn bag brands A and B, non-stick baking paper brands A and B, a French fry box, sandwich wrapper, a hamburger box and PTFE sealant tape, one piece of 50 mg, which in case of the microwave popcorn bags represents 2.5 cm^2, and one piece of popped popcorn after microwaving brand A (230 mg), were transferred to a reagent vial filled with 10 mL water and sonicated at 70 W for 1 h. Water, acetonitrile and acetone were all tested as extraction solvents. However, acetonitrile and acetone showed lower amounts of PFC's, compared with water. After sonication, the extracts were centrifuged at 14,500 rpm (17,500 × g) using an Eppendorf Minispin Plus centrifuge (Eppendorf South Pacific, NSW, Australia). The supernatant was subsequently concentrated using solid phase extraction (SPE). To increase trapping efficiency of PFC's, the supernatant was acidified with acetic acid to a final concentration of 5 mM prior to SPE. 100 mL bottled drinking water was also acidified with acetic acid to a final concentration of 5 mM. SPE cartridges containing 300 mg octadecylsilyl particles (50 μm particle size, 60 Å pore size, 496 m^2/g silica surface area) were conditioned with 5 mL methanol and 5 mL water. After sample loading, the SPE cartridge was washed with 1 mL water. Bound PFCs were eluted with 1 mL acetone and collected into 1.5 mL low-bind Eppendorf tubes. The acetone, chosen for its high elution strength and high vapor pressure, was evaporated under a stream of nitrogen. After evaporation, the concentrated eluate was diluted with MilliQ water to 1 mL.

2.3. Instrumentation and configuration

All chromatographic experiments were performed using an UltiMate 3000 system (Dionex, Germering, Germany) equipped with a quaternary low pressure mixing gradient pump (LPG-3400) with a built-in membrane degasser-unit, a temperature controlled pulled-loop autosampler (ACC-3000 T) equipped with a 20 μL sample-loop and thermostatted column compartment. The LC system was coupled on-line to an ion-trap mass spectrometer (amaZon, Bruker Daltonik, Bremen, Germany). The LC–MS system was controlled by Hystar V3.2 where the data have been processed by Data Analysis V4.0 SP2 and QuantAnalysis V2.0 SP2 (Bruker Daltonik, Bremen, Germany). 20 μL injections were performed on a Luna Phenyl–Hexyl, 50 mm × 2 mm i.d., 3 μm particle size column (Phenomenex, Lane Cove, Australia) where PFC's were separated applying an acetonitrile gradient in water from 20% to 50% in 6 min. As a cleaning step, the column was washed with 95% acetonitrile for 2 min and equilibrated for 7 min at 20% acetonitrile before the next injection. The flow-rate was set to 200 μL/min and the column was operated at 40 °C.

For the detection of all PFC's in full-scan MS and MRM mode, the mass spectrometer was operated in the negative-ionization mode in the scan range from 200 to 800 m/z under the following optimized conditions; nebulizer pressure 25 psi, dry gas 8 L/min with a dry temperature of 220 °C, capillary voltage 4500 V with an end plate off-set of −500 V. In the MRM mode applied for the detection of PFOA, the isolation mass was set to 413 m/z with an isolation width of 1 m/z, fragmentation cut-off of 112 m/z and fragmentation amplitude of 0.25 V. PFC's were detected in full-scan MS mode by creating extracted ion chromatograms from the parent ion plus the fragment ion shown in Table 1.

3. Results and discussion

3.1. Background contamination

One of the main challenges in the determination of PFC's using LC–MS involves the reduction of background contamination. PFC contaminations in the laboratory environment have not yet been well-characterized and are therefore a common source of background ions seen in analytical blanks. The increase in

Table 1
Selected ions monitored for the determination of PFC's.

PFC's	Precursor ion (m/z)	Product ion (m/z)
Perfluorohexanoic acid (PFHxA, $C_6HF_{11}O_2$)	313	269
Perfluoroheptanoic acid (PFHpA, $C_7HF_{13}O_2$)	363	319
Perfluorooctanoic acid (PFOA, $C_8HF_{15}O_2$)	413	369
Perfluorononanoic acid (PFNA, $C_9HF_{17}O_2$)	463	419
Perfluorodecanoic acid (PFDA, $C_{10}HF_{19}O_2$)	513	469
Perfluoroundecanoic acid (PFUA, $C_{11}HF_{21}O_2$)	563	519
Perfluorooctane sulfonate (PFOS, $C_8HF_{17}O_3S$)	499	–

background contamination ultimately prevents the lowering of detection limits down to parts-per-quadrillion (ppq) concentrations [11]. Previously described sources of contaminations in the laboratory environment are polypropylene sample bottles, C18 SPE materials and purified water [3]. In addition, the analytical instrumentation can also contribute to sources of contamination, particularly when components are made from fluoropolymers like Teflon. In this study, a major source of PFC's contamination was found to be the HPLC degasser, as the degasser chambers are made from Teflon and resulted in high signals for PFHxA, PFOA, PFNA, PFDA and PFUA in the MS analysis (abbreviations are explained in Table 1). In order to overcome this source of contamination, the solvent line from mobile phase A (water) was disconnected from the degasser and connected directly to the pump. This could only be done for mobile phase A, as disconnecting the solvent line from mobile phase B (acetonitrile) from the degasser resulted in air-bubble formation and subsequent oscillating pressures. However, the contamination observed from the degasser in mobile phase B was below the quantitation limit of PFOA due to the low extraction efficiency of acetonitrile as determined in Section 2.2.

3.2. Method optimization for the determination of PFOA, PFOS and other PFC's

Previously reported LC–MS strategies analyzing PFC's utilised a C8 or C18 column separation applying a water/methanol or a water/acetonitrile gradient with ammonium acetate in the elution buffer. Holcapek et al. [20] showed that ammonium acetate caused ion-suppression in negative-ESI for the detection of sulfonic acid dyes. In addition, Inoue et al. [16] investigated ion-suppression of PFOA and PFOS in negative-ESI using MS in response to varying ammonium concentrations and concluded that the addition of 1 mM ammonium to the mobile phase showed the highest PFOA and PFOS MS response. To investigate ion-suppression effects of ammonium on PFOA and PFOS, standards were separated on a C8 and C18 column with different concentrations of ammonium added to the mobile phase. Ion-suppression in the electrospray interface was observed at concentrations as low as 1 mM. However, without the addition of ammonium to the mobile phase, over 90% acetonitrile was required to elute the compounds of the column, which resulted in poor separation efficiency. In order to overcome the need for ammonium without sacrificing separation efficiency, a Phenyl–Hexyl column was successfully employed, yielding baseline separation of PFOA and PFOS using a water/acetonitrile gradient. The Phenyl–Hexyl stationary phase consists of a phenyl group on a C6 backbone. This functional group shows a high affinity for free electrons found in an aromatic ring (π–π interaction). Although the PFC's discussed here do not have an aromatic ring, fluorine is extremely electronegative and attracts the electron pair from the C–F bond. The high electron density around fluorine showed affinity for the phenyl functional group of the Phenyl–Hexyl column. With the addition of acetic acid to the mobile phase, a secondary retention mechanism can possibly be introduced. The ammonia added to the mobile phase may act as a counter ion, eliminating secondary interactions. Fig. 1 shows the extracted ion chromatogram (EIC) from the separation of a 2.5 ng/mL PFOA and PFOS standard mixture separated on a 50 mm × 2 mm i.d. Phenyl–Hexyl column. A linear gradient was applied from 20 to 50% B in 10 min with 95% B for 5 min. The following mobile phase compositions were examined; (a) 5 mM acetic acid in water as mobile phase A and 5 mM acetic acid in acetonitrile as mobile phase B, (b) 5 mM acetic acid, 1 mM ammonium in water as mobile phase A and 5 mM acetic acid in acetonitrile as mobile phase B and (c) water as mobile phase A and acetonitrile as mobile phase B.

The formations of branched isomers are common by-products during manufacturing of PFC's standards. Possible branched isomers present in the PFOA standard eluted with the un-branched PFOA as a single peak (1), where the branched isomers present in the PFOS standard (2) were separated from the non-branched PFOS (3).

In the presence of acetic acid (Fig. 1(a)), PFOA and PFOS eluted in the 95% acetonitrile wash step, which resulted in high signal intensities caused by enhanced desolvation under high organic conditions. The presence of acetic acid also resulted in co-elution of branched PFOS isomers with un-branched PFOS in the 95% acetonitrile wash step. However, these conditions were not favorable since other PFCs were not separated and co-eluted as a single chromatographic peak.

The addition of ammonium was necessary for the efficient elution of PFOA and PFOS from the Phenyl–Hexyl column in the presence of acetic acid, yet resulted in ion-suppression as demonstrated in Fig. 1(b). The replacement of both the acetic acid and ammonium containing mobile phase A and acetic acid containing mobile phase B with water and acetonitrile respectively, resulted in a comparable retention profile, with a two times increase in signal intensity obtained for PFOA and PFOS as demonstrated in Fig. 1(c). In order to improve the separation efficiency of PFC's on the Phenyl–Hexyl column and to decrease the total analysis time, the solvent line from mobile phase A was reconnected back to the degasser, which provided the ability to utilise the detectable amounts of PFC's; PFHxA, PFNA, PFDA and PFUA as standards. The gradient from 20 to 50% B in 10 min was optimized such that all PFCs were baseline separated in a retention time window of 8 min. This resulted in a gradient from 20 to 50% B in 6 min, and this was subsequently utilised in all further experiments. After optimization of the method gradient, the solvent line from mobile phase A was disconnected from the degasser and re-connected directly to the pump.

3.3. PFOA and PFOS limit of detection (LOD) and limit of quantitation (LOQ)

To determine the detection and quantitation limits applying the optimized method, 20 μL of PFOA and PFOS standard mixtures were injected in triplicate in a concentration range from 10 pg/mL to 1000 pg/mL. The obtained peak areas were processed by GraphPad Prism version 5.03, December 10, 2009 (GraphPad Software, Inc., CA, USA). Fig. 2 shows the obtained calibration curve for PFOA (a) and PFOS (b), with the standard deviation from the triplicate injections shown as vertical bars, whilst Table 2 shows the linear regression results obtained.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined based on the extracted ion chromatograms obtained from PFOA and PFOS. The LOD was set to 3 times the average noise and LOQ was set to 5 times the average noise obtained 0.1 min before elution till 0.1 min after elution of the main compound and was determined by Data Analysis. 25 pg/mL was found to be the LOD and 50 pg/mL was found to be the LOQ for both PFOA and PFOS.

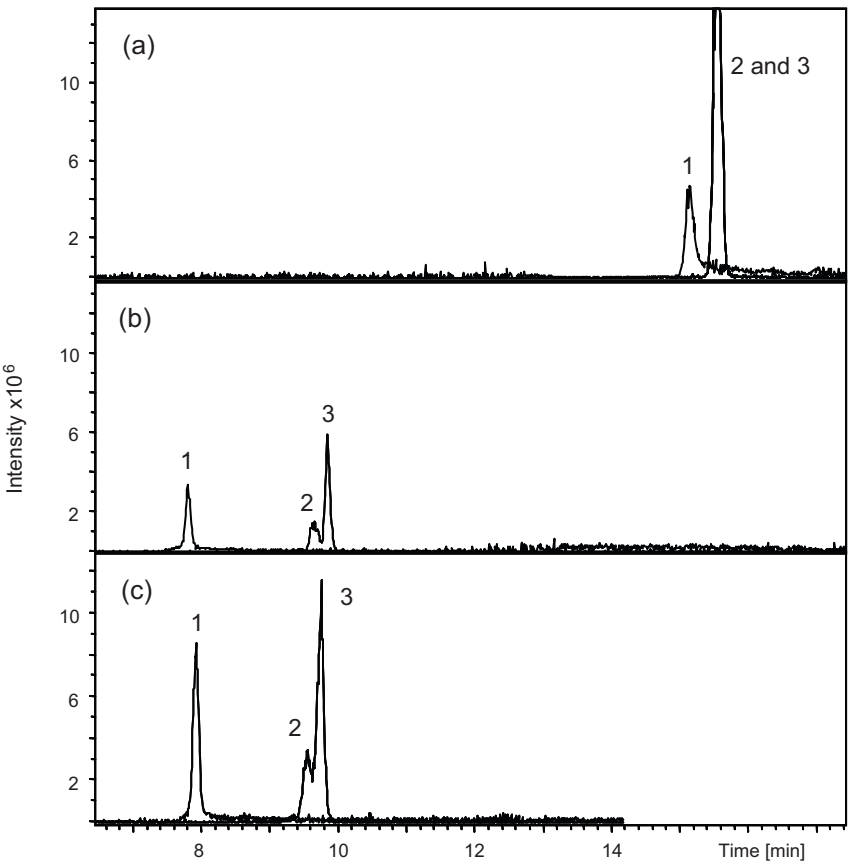

Fig. 1. Extracted ion chromatogram of a PFOA and PFOS standard mixture separated on a 50 mm × 2 mm i.d. Phenyl–Hexyl column applying different mobile phase compositions.

3.4. PFOA and PFOS recovery

It has been previously reported that PFOA may bind to glass surfaces and as such, only polypropylene low-bind Eppendorf tubes and sample vials were used for all PFC's analyses to improve overall recovery [21]. Despite this, the loss of PFOA and PFOS due to hydrophobic absorption to the polypropylene low-bind Eppendorf tubes and sample vials was examined. This involved utilising known standards at concentrations of 10, 25 and 50 pg/mL in acetone, which were prepared in 1.5 mL low-bind polypropylene Eppendorf tubes. After evaporation of the acetone, the residue was reconstituted in water to a final volume of 1 mL. 200 μL was then transferred to a 250 μL polypropylene sample vial where 20 μL was injected. Both PFOA and PFOS showed no hydrophobic absorption to polypropylene, which can be explained by the repelling nature of PFC's against the plastic surfaces of the vials. The loss of PFOA and PFOS during SPE experiments was examined. PFOA recoveries between 75 and 78% and PFOS recoveries between 81 and 88% have been reported in spiked de-ionized water after SPE [17,19]. The recovery of PFOA and PFOS after SPE was determined, in which 400 μL of a 2.5 ng/mL PFOA and PFOS standard mixture was spiked to 10 mL MilliQ water. Matrix effect were determined, in which 200 μL of a 2.5 ng/mL PFOA and PFOS standard mixture was spiked to 50 mg microwave popcorn bag brands A and B in 10 mL water

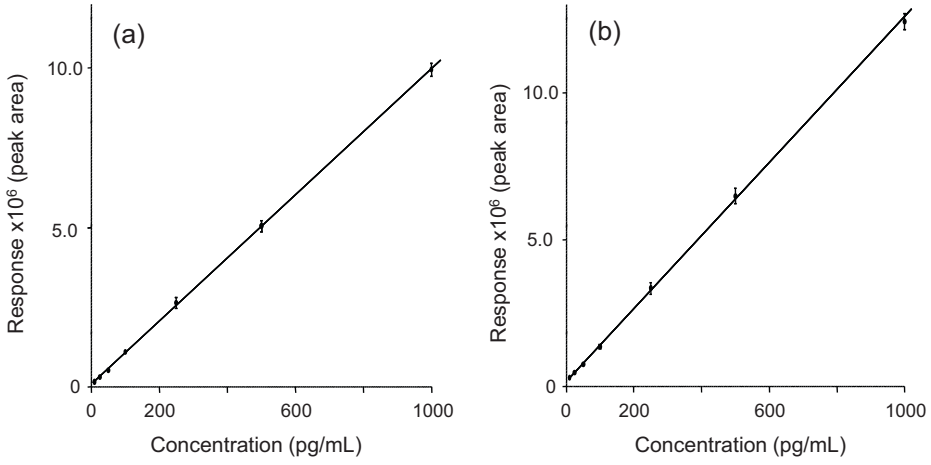

Fig. 2. Calibration curve for the triplicate injections of PFOA (a) and PFOS (b) in the concentration range from 10 pg/mL to 1000 pg/mL.

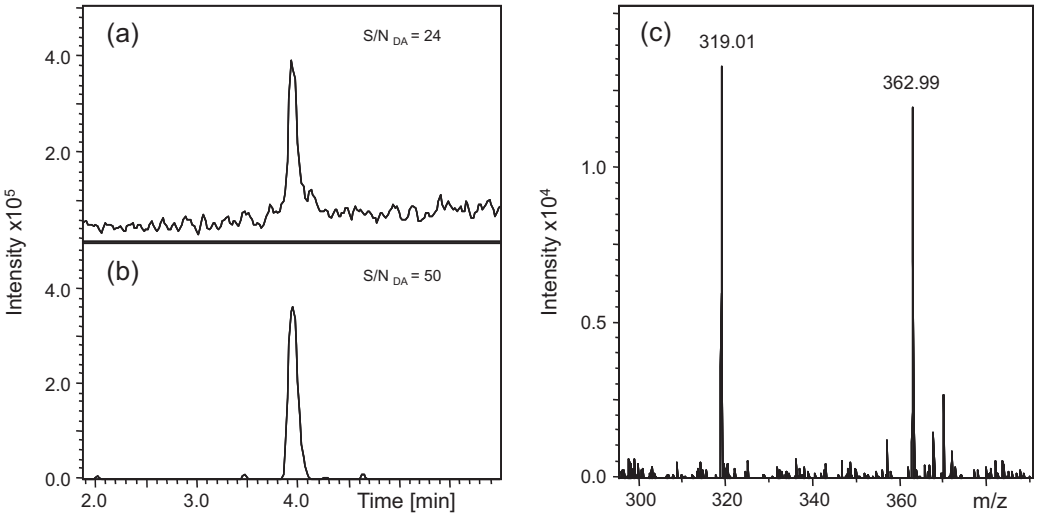

Fig. 3. Extracted ion chromatogram of perfluoroheptanoic acid obtained in microwave popcorn bag brand A.

Table 2
Linear regression results for the calibration curves of PFOA and PFOS.

Best-fit values	PFOA	PFOS
Slope	9892 ± 74	12,304 ± 105
Y-intercept when *X* = 0.0	77,372 ± 32,104	192,279 ± 45,794
X-intercept when *Y* = 0.0	−7.8	−15.6
95% confidence intervals		
Slope	9738–10,047	12,084–12,525
Goodness of fit		
R^2	0.9989	0.9986

prior to sonication. All samples were then processed as described in Section 2.2. The recovery of the spiked MilliQ water was determined by the obtained peak area after SPE divided by the peak area of a 1 ng/mL standard x 100%. The PFOA and PFOS recovery from the spiked popcorn bags was determined by QuantAnalysis. The obtained recovery from spiked MilliQ water after SPE was 96.1 and 94.8% with a relative standard deviation (RSD) of 1.9 and 2.8% for PFOA and PFOS respectively (n = 9). The obtained recovery of PFOA and PFOS when spiked to microwave popcorn bag brand A and B ranged from 79.2 to 89.6% with an RSD < 10.8% as shown in Table 3. PFCs were identified in microwave popcorn bag brand A prior to spiking. The average peak area of PFOA, as determined in Section 3.5, was subtracted from the peak area of PFOA after spiking. A recovery of 88.7% with a RSD of 10.8% was obtained, which includes the deviation in SPE recovery, the deviation in PFOA measured in the microwave popcorn bag and the deviation in the spiking experiment. The recovery of PFOA and PFOS from microwave popcorn bag brand B, where no PFC's could be quantified in Section 3.5, is lower compared to spiking to microwave popcorn bag brand A. Most likely the PFOA and the PFOS were partially absorbed by the microwave popcorn bag and could not be extracted.

3.5. Analysis of commercial samples for PFC's

To prevent the migration of fatty acids from food into the packing paper, a common strategy involves the treatment of the paper with PFC's. Two different brands of microwave popcorn bags and non-stick baking paper, a French fry box, sandwich wrapper and a hamburger box from a major fast food company were selected for analysis as these packaging papers were likely treated with PFC's. Popcorn from brand A was examined for absorbed PFC's after microwaving. PTFE sealant tape was examined for PFOA residues from production as was bottled drinking water for PFC's originating from environmental pollution. The high mass accuracy of the ion-trap mass spectrometer allowed the identification of PFC's using the extracted ion chromatograms of both the parent ion and fragment ion from the calculated theoretical mass. From microwave popcorn bag brand A, three bags out of a pack of five were analyzed in triplicate. Fig. 3(a) shows the extracted ion chromatogram of perfluoroheptanoic acid with a theoretical parent ion 362.97 m/z and fragment ion 318.98 m/z, applying a mass accuracy of ±0.5 Da (a) and ±0.05 Da (b). The MS signal was averaged between 3.9 and 4.0 min with background subtraction between 4.1 and 4.2 min (c) measured in full-scan MS mode.

The high mass accuracy of the ion-trap mass spectrometer allowed the identification of perfluoroheptanoic acid with a signal-to-noise ratio of 50, as determined by Data Analysis software (S/N_{DA}). From PFHxA, PFHpA, PFOA, PFNA, PFDA, and PFUA the theoretical masses were calculated and the overlays of the extracted ion chromatograms identified in microwave popcorn bag brand A are shown in Fig. 4.

Fig. 4 highlights the peak area observed from the PFOA trace corresponding with a concentration of 461.5 pg/mL obtained from the calibration standards corresponding to 9.2 μg/kg PFOA. Beside PFOA, PFHxA, PFHpA, PFNA, PFDA, PFUA were detected by accurate mass measurement, which shows that the paper from microwave

Table 3
Recovery levels of PFOA and PFOS spiked to water and microwave popcorn bag brand A and B.

Sample	Spike amount PFOA and PFOS (ng/ml)	PFOA		PFOS	
		Recovery average (%)	(%RSD) (n)[a]	Recovery average (%)	(%RSD) (n)[a]
Water	1	96.1	1.9 (9)	94.8	2.8 (9)
Popcorn bag brand A	0.5	88.7	10.8 (6)	89.6	4.9 (6)
Popcorn bag brand B	0.5	81.5	7.9 (6)	79.2	7.6 (6)

[a] Number of replicates in brackets.

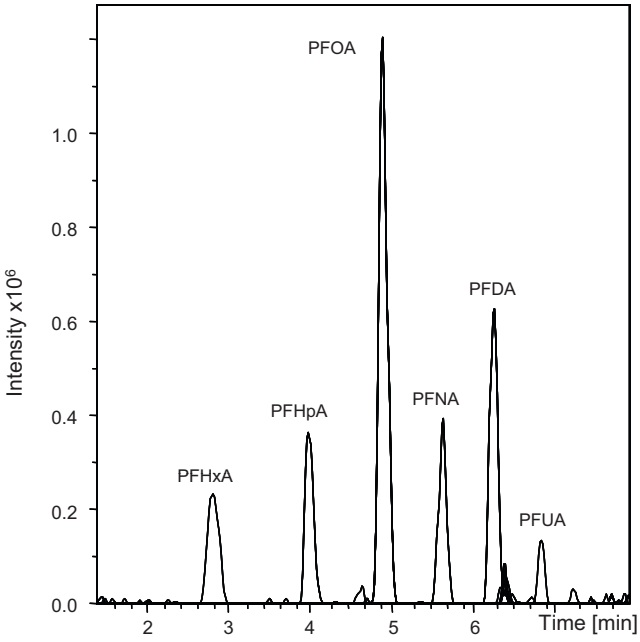

Fig. 4. Extracted ion chromatograms of PFHxA, PFHpA, PFOA, PFNA, PFDA and PFUA identified in microwave popcorn bag brand A.

popcorn bag brand A was treated with PFC's. No PFC's could be detected in the popped popcorn from brand A after microwaving, which suggests that PFCs were absorbed by the popcorn and could not be extracted. Alternatively, the inability to detect PFC's could indicate that PFC's remains in the coating of the popcorn bag and does not migrate into the popcorn. This has also been suggested previously by Begley et al., who investigated the migration of PFC's present in the microwave popcorn bag into the popcorn oil during microwaving [2].

In microwave popcorn bag brand B, PFOA was detected but could not be quantified since the amount was below the quantitation limit. In addition, other PFC's were not detected.

PFOA presence in PTFE sealant tape was also determined. Fig. 5 shows the total ion chromatogram (TIC) obtained from the PTFE extract (a), with the corresponding mass spectrum from 350 *m*/*z* until 440 *m*/*z* (b) at the elution time of PFOA. The signals 429 *m*/*z* and 385 *m*/*z* are part of a polymer distribution of Polyethylene Glycol (PEG) with a mass difference of 44 *m*/*z* which is co-eluting with

Table 4
Analyzed samples with the detected amount of PFOA.

Sample	Concentrations of PFOA (μg/kg)	(%RSD) (*n*)[a]
Non-stick baking paper brand A	n.d.	–
Non-stick baking paper brand B	n.d.	–
Bottled drinking water	n.d.	–
PTFE sealant tape	2.8	5.9 (6)
Microwave popcorn bag brand A	9.1	6.1 (9)
Microwave popcorn bag brand B	<0.05[b]	– (6)
French fry box	n.d.	–
Sandwich wrapper	n.d.	–

n.d. Indicates PFOA and PFOS concentrations lower than 0.025 μg/kg in samples.
[a] Number of replicates in brackets.
[b] PFOA was detected below the quantitation limit.

PFOA. The signals 404 *m*/*z* and 355 *m*/*z* originate from the PTFE polymer chain.

The PFOA ions were isolated from co-eluting polymer ions by creating an extracted ion chromatogram based on the parent ion of 413 *m*/*z* and the product ion of 369 *m*/*z* which is shown in Fig. 6a. Fig. 6b shows the average spectrum view of PFOA between 4.8 and 4.9 min with background subtraction between 4.7 and 4.8 min. Even with background subtraction, polymers like PEG (385 *m*/*z*) were observed, reducing signal-to-noise ratio and increase detection limits.

Instead of isolating the relevant ions in the software, the ions can also be isolated in the ion-trap where all other ions are discarded. The mass spectrometer is then operated in the MRM mode where only the parent ion 413 *m*/*z* was detected, isolated and fragmented resulting in the 369 *m*/*z* product ion. The parent ion and product ion are used for subsequent identification and quantitation of the compound of interest.

To examine the influence on the signal-to-noise ratio, the same PTFE extract was analyzed using the ion-trap mass spectrometer in the MRM mode. Fig. 7a shows the extracted ion chromatogram of 413 *m*/*z* and 369 *m*/*z* and Fig. 7b shows the average spectrum view without baseline subtraction.

As can be seen from Figs. 6 and 7, PFOA was detected in PTFE tape in both full-scan MS and MRM scan mode, however an increase in signal intensity was observed in the extracted ion chromatogram of PFOA in full-scan MS mode, which is caused by co-eluting polymers with an *m*/*z* close to 413 and 369. In this instance, the signal intensity from the average spectrum view was comparable. Using the MRM scan mode resulted in a dramatically improved signal-

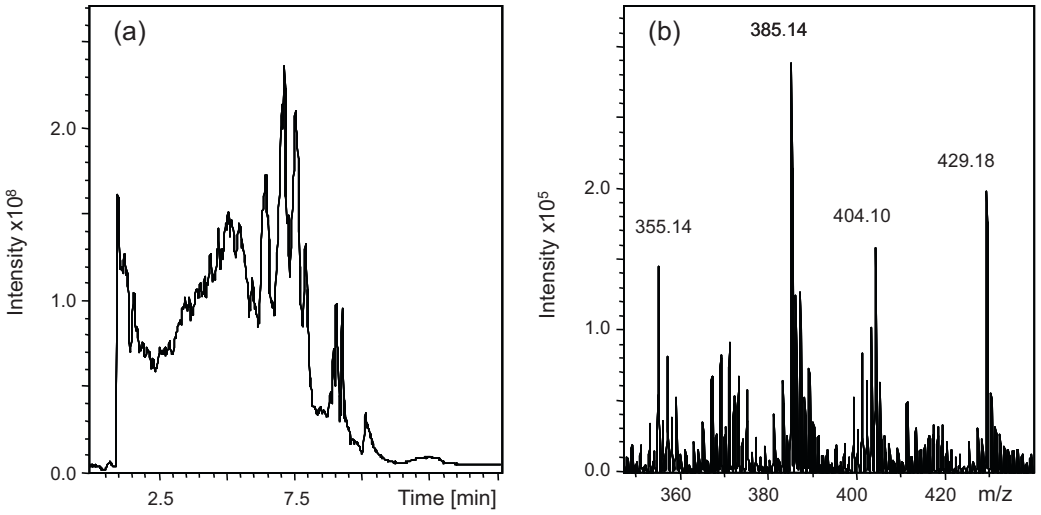

Fig. 5. Total ion chromatogram from PTFE sealant tape extract (a) with MS signal average between 4.5 and 5.5 min (b).

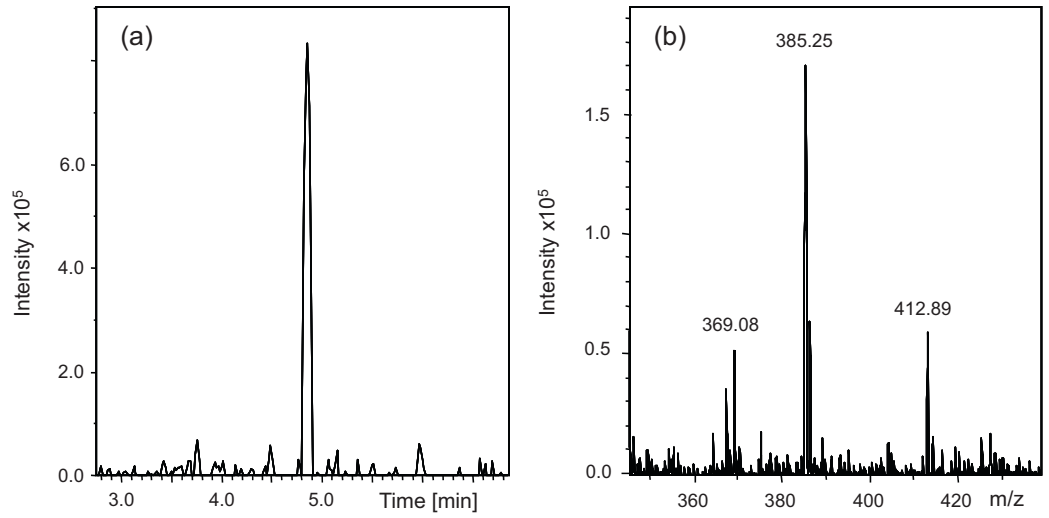

Fig. 6. Extracted ion chromatogram of PFOA (a) with the MS signal average between 4.8 and 4.9 min (b) measured in the PTFE sealant tape in full-scan MS mode.

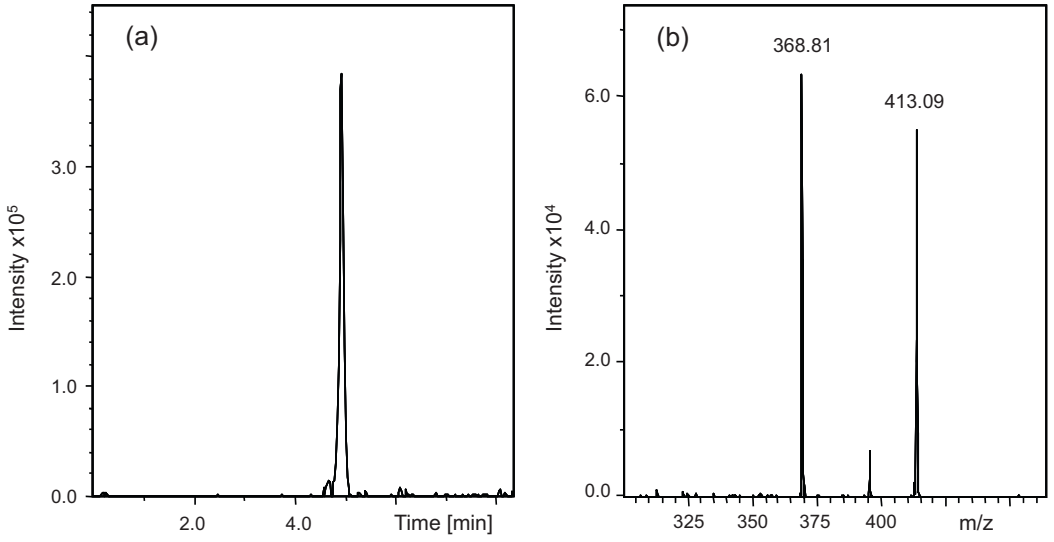

Fig. 7. Extracted ion chromatogram of PFOA (a) with the MS signal average between 4.8 and 4.9 min (b) measured in the PTFE sealant tape in MRM scan mode.

to-noise ratio without a loss in sensitivity. The extractable amount of PFOA from the PTFE sealant tape was 140 pg, which correspond to 2.8 μg/kg. PFOA was the only PFC detected in the PTFE sealant tape. The amount of PFOA detected in all samples is summarized in Table 4. In all samples, PFOS was not detected above the detection limit of 25 pg/mL.

4. Conclusions

The present study sought to develop and optimize a robust and sensitive LC–MS method for the quantitation of PFOA, PFOS and detection of other PFC's in different matrices. This was achieved by using solid phase extraction followed by the separation of PFC's on a Phenyl–Hexyl column applying a water/acetonitrile gradient coupled on-line to an ion-trap mass spectrometer. The advantage of this configuration was the ability to separate, identify and quantify PFC's without the need for ammonium which minimized ion-suppression effects. Detection limits of 25 pg/mL and quantitation limits of 50 pg/mL were achieved for both PFOA and PFOS in calibration standards.

Microwave popcorn bag brand A was treated with PFC's, as demonstrated by the presence of 9.1 μg/kg PFOA as well as other PFC's. In the microwave popcorn bag brand B, PFOA was detected, however could not be quantified. PTFE sealant tape showed a high amount of interfering polymers in full-scan MS. An MRM approach was utilised as a targeted approach for the detection of PFOA. This resulted in an improved signal-to-noise ratio without loss in sensitivity. In the PTFE sealant tape, PFOA was found to be present at 2.8 μg/kg, yet no other PFCs were detected. The analysis of popped popcorn from band A, French fry box, sandwich wrapper, hamburger box, non-stick baking paper and bottled drinking water showed that no PFOA or other PFC's were present in detectable amounts. None of the samples analyzed were found to contain any detectable amounts of PFOS.

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