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Development of Extraction Methods for the Analysis of Perfluorinated Compounds in Leather with High Performance Liquid Chromatography Tandem Mass Spectrometry

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Abstract. Perfluorinated compounds (PFCs), used to provide water, oil, grease, heat and stain repellency to a range of textile and other products, have been found to be persistent in the environment and are associated with adverse effects on humans and wildlife. This study presents the development and validation of an analytical method to determine the simultaneous presence of eleven PFCs in leather using solid-phase extraction followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The perfluorinated compounds were primarily extracted from the samples by a liquid extraction procedure by ultrasonic, in which the parameters were optimized. Then the solid-phase extraction (SPE) is the most important advantages of the developed methodology. The sample volume and elution conditions were optimized by means of an experimental design. The proposed method was applied to determine the PFCs in leather, where the detection limits of the eleven compounds were 0.09-0.96 ng/L, and the recoveries of all compounds spiked at 5 ng/L concentration level were in the range of 65-96%, with a better RSD lower than 19% (n = 7).

1. Introduction

Perfluorinated compounds (PFCs), a group of widely used surfactants, are designated as a broad range of compounds containing a perfluorinated alkyl chain, with a typical structure of: $F\text{-}(CF_2)_n\text{-}OH/COO^-/SO_3^-/PO_3^-/NH_3^+$ (n = 1-14) [1]. The physicochemical properties of the strong carbon-fluorine (C-F) bond, results in a high stability to the perfluorinated substance. The structure makes PFCs water, oil, grease, heat and stain repellency, for which they have been employed as surfactants and surface protectors for paper, food containers, leather, carpets, upholstery and textile for over 40 years in the market [2]. However, at around 2000, it became common knowledge that perfluorooctane carboxylate, known as PFOA, and its homologues with longer carbon chain were almost everywhere in the environment including human blood, though at very low levels. Then the PFCs have been concerned about their long elimination half-lives and bioaccumulative properties. The increased awareness of the potential risk of exposure to such PFCs to consumer health has led to European Union Directives that the occurrence of PFOA below 0.005 % in product, 0.1 % in semi-finished products and 1 $\mu g/m^2$ in textile or coating. Today it is clear that some PFCs can disrupt thyroid hormones, proteins, high-density cholesterol, and triglycerides [3]. Sweden-based retailer H&M Hermes & Mauritz AB (H&M Group) has banned the use of perfluorinated compounds (PFGs) in all products it sells. All products ordered on or after Jan.1, 2013, must be PFG-free. In addition, some other countries have issued similar regulations. To meet the requirements of these international

regulations, OEKO-TEX standard 100 banned the use of perfluorooctanesulfonic acid (PFOS) and PFOA, and different chain length (C_6-C_{15}) PFCs similar to PFOS and PFOA in textiles (max. content < 1.0 μg/m^2) in 2016 [4].

PFCs have been used in the textile and leather industry for decades. One of their main usage areas is to confer water and oil repellent properties to textile materials and can be found on most functional apparels and also and on all kinds of technical textiles like car seats, sofas, medical gowns and uniforms. In market, most of water repellent shoes made of leather are favored by consumers, which use PFCs as a founctional agents. Due to the concern on exposure to PFCs, a special interest has grown to develop robust analytical methods in the last years. In most cases, the analytical method used is liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS), which is regarded as a desirable method for trace-level analysis, and can achieve very low levels of quantitation. Hansen et al. applied solid-phase extraction (SPE) method coupled with HPLC-negative-ion electrospray tandem mass spectrometry to determine PFOS and PFOA in drinking water and surface water, certain PFCs can be quantitatively measured down to 25 ppt [5]. The SPE / LC-MS-MS technique has been used successfully for the determination of PFOS and PFOA in different matrices such as whole blood [6], mollusks [7], sludge [8], human hair and nail [9], soil and other experimental samples. To the best of our knowledge, the determination of PFOA and PFOS in textiles and leather matrix has seldom been reported. However, different from environmental samples, textile and leather come directly into contact with human skin, so it is of great significance to accurately determine the PFOA and PFOS at trace levels in fabric materials. In the present work, an effective and simple pretreatment method which integrated of extraction and clean-up into a single step, and a test method of LC/MS/MS were developed. The experimental results showed that the developed method could be satisfactorily used to monitor PFCs concentrations in leather and textile.

2. Experimental

2.1. Chemicals

Eleven PFCs were analyzed in this study. The standard solutions containing perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic Acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA) and perfluorooctanesulfonate (PFOS), perfluorooctane sulfonamide (PFOSA) were obtained from Sigma-Aldrich (Steinheim, Germany) with chemical purities of ≥ 98%. High performance liquid chromatography (HPLC) grade methanol and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany), and Milli-Q water was used throughout the study. Tetrabutylammonium hydrogen sulfate (TBA) and methyl-tert-butyl ether (MTBE) were purchased from J&K Chemical company. HPLC grade ammonium acetate and formic acid were purchased from Fisher Scientific (USA), and analytical grade sodium carbonate and guarantee grade sodium hydrogen carbonate (99 %) were purchased from the Jinke Institute of Fine Chemicals (Tianjin, China). Individual stock solutions at ca. 2,000 μg/mL and a mixture of all the analytes at 20 μg/mL were prepared in methanol. The solutions were stored in the dark at -20 °C. Waters Oasis-WAX (poly (divinylbenzene-co-N-vinylpyrrolidone) + secondary amine polymer, 150 mg) SPE cartridges were purchased from Waters Corporation (Milford, USA). For extraction, a KQ600VDE ultrasonica extractor (60 kHz; KunShan, China) was used. Fractions were evaporated in a KL-512 Organomation (BeiJing, China) using a gentle stream of nitrogen. After the extraction step, the supernatant was filtered through PTFE filters (0.45μm, 25 mm, Macherey-Nagel, Germany) and nylon microfilters (0.2μm, 13 mm, Pall, USA) were used to filter extracts before LC-MS/MS analysis. Leather samples and textiles were obtained from a local market.

2.2. Samples Treatment and Ultrasound Assisted Solid-Liquid Extraction

Leather samples were cut as close to the scalp as possible and were stored in 50 mL polypropylene (PP) centrifuge tubes at room temperature until analysis. The efficiencies of different extraction methods were compared using parallel samples. The optimal extraction conditions were as follows: 1.0 g of

ground sample was placed into a 50 mL polypropylene (PP) centrifuge tubes, and then 3 mL of TBA (169.8 g/L) and 10 ml MTBE were added. Then, the tube was immersed in the in a 59 kHz ultrasonic bath at 30 °C for 1 h. This extraction procedure was repeated again with 3 mL of MTBE and the yielded extracts were combined. The final extract was concentrated under a stream of high-purity nitrogen, and then made up to 1 mL with methanol prior to SPE cleanup.

2.3. Cleanup Method for Extracts

This clean-up approach was referred to the method published by Olatz Zuloaga [10]. The 150 mg/6 mL Waters Oasis-WAX cartridges were preconditioned with 12 mL of 0.1 % ammonium hydroxide in MeOH followed by 12 mL of Milli-Q water. The sample (extract) was loaded onto the cartridge and then washed with 12 mL of 2 ‰ NaAc in water (pH = 4). The target compounds were eluted twice with 8 mL of 0.1 % ammonium hydroxide in methanol and collected to a polypropylene graduate tube. The extract was concentrated to dryness and reconstituted in 1 mL of LC-MS grade MeOH. Finally, the reconstituted extract was filtered through a 0.2 μm nylon filter before the LC-MS/MS analysis.

2.4. LC-MS/MS Analysis

The separation and detection of analytes were performed on an Agilent 1200 LC system coupled with a MS/MS triple quadrupole mass spectrometer (USA). The MS/MS data for all perfluorinated compounds were collected in negative electrospray ionization mode (ESI) by selected reaction monitoring (SRM). Two different transitions (precursor / product ion) were monitored per compound. Nitrogen was the gas used in both the nebulizer and the collision cell. The nebulizer pressure was 50 psi, drying gas temperature was 350 °C, the capillary voltage was 4.0 kV and a drying flow rate was 8 L/min. The optimized settings for MS data acquisition in SRM mode were obtained by flow injection analysis of each analyte and are shown in Table 1. Instrumental operations, data acquisition and peak integration were performed with the Workstation Software (Qualitative Analysis, Version B.06.00, Agilent Technologies).

Separation of analytes was tested with Acquity UPLC^{TM} BEH C18 (1.7μm, 2.1×100mm, Waters, USA). The column temperature was set to 35 °C. A gradient program was employed using 5 mM aqueous formic acid solution and methanol mobile phases. The flow rate of 0.08 mL/min and the volume injected was 5μL. The gradient started at 35% methanol followed by an 8 min ramp to 87 % methanol. At 20 min, the ramp was decreased to 85 % methanol and at 25 min the ramp was decreased to 80 % methanol. The method then reverted back to initial conditions at 35 min and a 15 min stabilization time was maintained before the next injection.

Table 1. The mass condition for compounds.

Compound	Structure	Precursor ion	Product ion(SRM1/SRM2)	Collision energy(eV) (SRM1/SRM2)
PFTeDA	$CF_3(CF_2)_{12}COOH$	713	669/169	15/20
PFTrDA	$CF_3(CF_2)_{11}COOH$	663	619/169	5/30
PFDoA	$CF_3(CF_2)_{10}COOH$	613	569/169	5/20
PFUnA	$CF_3(CF_2)_9COOH$	563	519/269	5/17
PFDA	$CF_3(CF_2)_8COOH$	513	469/219	5/15
PFNA	$CF_3(CF_2)_7COOH$	463	419/219	5/15
PFOA	$CF_3(CF_2)_6COOH$	413	369/169	3/15
PFHpA	$CF_3(CF_2)_5COOH$	363	319/169	5/15
PFHxA	$CF_3(CF_2)_4COOH$	313	269/119	5/15
PFOS	$CF_3(CF_2)_7SO_3K$	499	99/80	70/35
PFOSA	$CF_3(CF_2)_7SO_2NH_2$	498	498/78	5/24

3. Results and Discussion

3.1. HPLC-MS/MS Conditions

Before starting the analysis of PFCs in real samples, the chromatographic and mass spectrometric parameters were optimized by injecting standard solutions of different concentrations. The elution order of the 11 investigated compounds was determined and gradients were optimized to obtain an acceptable separation of the compounds in as less time as possible. Fig. 1 shows a chromatogram for standard solutions of 11 PFCs obtained under optimized conditions.

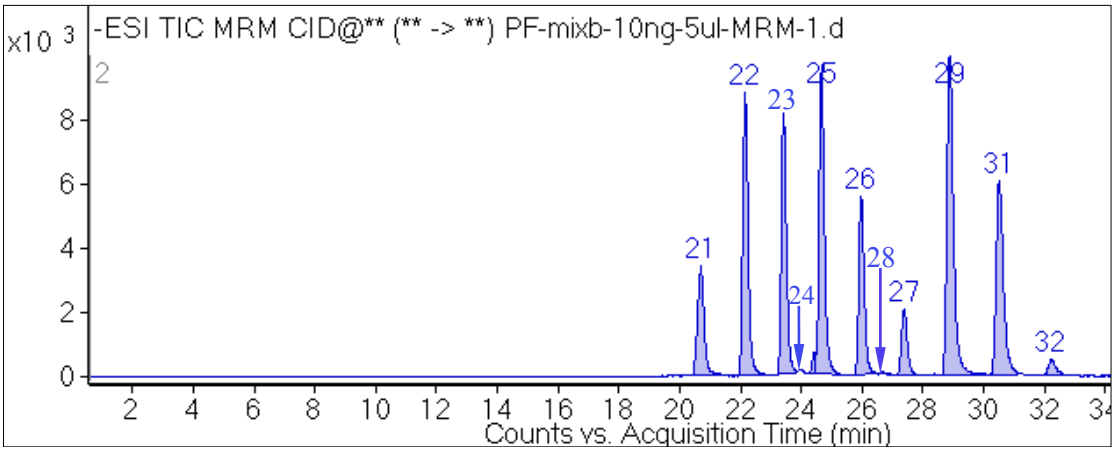

Figure 1. Chromatogram of standard solutions (2 ng/ml). (21) PFHxA, (22) PFHpA, (23) PFOA, (24) PFOS, (25) PFNA, (26) PFDA, (27) PFUnA, (28) PFOSA, (29) PFDoA, (31) PFTrDA, (32) PFTeDA

PFAs contain a large group of highly stable compounds, which are amphiphilic and consist of a perfluorinated hydrophobic. A good separation of PFCs was achieved in Acquity UPLC^{TM} BEH C18 reversed chromatographic column. Several mobile phases were considered to reach the best chromatographic separation of 11 compounds and to achieve the best signal/noise ratio (S/N), and consequently, the lowest limits of detection (Table. 2). The mobile phase selected was 5 mM aqueous formic acid solution (A) and methanol (B) because they allowed achieving higher S/N ratio and an adequate chromatographic separation of the compounds. The chromatographic run was kept for 35 min and a post run time of 10 min was required to properly stabilize the initial conditions of the mobile phase, which helps to ensure an acceptable repeatability between consecutive runs. As can be seen the run time for the UHPLC-column is not drastically shortened with respect to the use of the HPLC-column because the small column diameter leads to high column pressure, thereby decreasing the low flow rate of 0.08 mL/min. It is also important that peaks 24 and 28 (PFOS and PFOSA) attain a baseline separation. However, the use of selective MS detection provided clearly different signals for each compound that allow its quantification.

Since the simultaneous determination of up to 11 PFCs was aimed in the present work, only ESI was optimized. In the study, good responses for most of the PFCs were obtained in negative mode. The results showed that the peak of $[M\text{-}H]^-$ was stronger. After determining the parent ion, its daughter ion was scanned. The fragment ions with the maximum abundance were used for quantitative analysis, and the other ions were used for qualitative analysis. The optimum parameters of negative ionization mode, capillary voltage and collision energy were displayed in Table 1. The optimized parameters were fixed as follows: capillary voltage at 4000 V, drying gas flow at 8 L/min, drying gas temperature was 350 °C and nebulizer pressure at 50 psi.

3.2. Development of Ultrasound Assisted Solvent Extraction Method

For solid samples extraction was performed with ultrasonic with a polar organic solvent. According to the published methods, PFAs could be extracted by methanol, acetonitrile, 2 % formic acid and MTBE in the presence of TBA in extraction procedures. Before extraction, 1.0 g of leather sample was cut

into small pieces (3-5mm). To evaluate the efficiency of four different solvents for extracting PFCs from leather samples, 8 mL of the organic solvent was added to 1.0 g sample spiked with 5 ng/mL of standard solution in a 15 mL prewashed PP tube, respectively. The mixture was extracted in 59 kHz ultrasonic bath at 30 °C for 1h. The concentrations of detected PFCs obtained by 10 mL MTBE in presence of 3 ml of TBA (169.8 g/L) extraction were higher than those of other solvents.

3.3. Selection of Clean-Up Step

Preconcentration was necessary to determine low concentrations of PFCs in the water samples. To this end, solid phase extraction is certainly the most suitable and commonly used technique. Recently, the use of newly developed polymeric sorbents with ion exchange (i.e., Oasis WAX) characteristics for SPE is gradually increasing. These types of sorbents exhibit good water-wetting properties, multiple retention characteristics and higher binding capacities compared to silica based sorbents, and are adequate for enrichment of hydrophilic or ionic compounds and removal of the interfering salts from the matrix. In this study, relatively high rates of recovery were observed using an Oasis WAX-SPE cartridge, which provides weak ionic binding between the carboxylic acid, sulfonate group and the ionized amino-group. Optimization of the SPE-procedure was also performed by varying the sample volume and elution conditions.

3.4. Analytical Performance

The method performance data, such as linear response range, reproducibility and quantitation limits, are summarized in Table 2. The linearities of eleven PFCs were from the calibration curve in the range from 0.2 to 5 ng/mL. The precision of the curves as indicated by RSD of response factor (RF) were 2.3-8.4 %, and the correlation coefficients (r^2) exceeded 0.99. The instrumental detection limits were 0.09-0.96 ng/L. For the "SPE" method, the recoveries of all the PFCs compounds spiked at 5 ng/L concentration level were in the range of 65-96 %, with a better RSD lower than 19 % (n = 7). Therefore, the proposed method was applicable for the determination of PFCs residues in leather.

Table 2. Calibration curve, Recoveries (%), RSD (%), and LODs obtained from the SPE followed by LC-MS/MS method

PFCs	Calibration curve	r^2	RSD (%,n=7)	LOD (ng/L)	Recovery (%)
PFTeDA	y=691.38x+171.19	0.9967	4.6	0.96	85.83
PFTrDA	y=8660.30x+766.07	0.9967	2.3	0.51	84.38
PFDoA	y=11952.32x+2199.96	0.9973	5.1	0.65	89.48
PFUnA	y=1803.38x+872.12	0.9915	5.0	0.62	79.51
PFDA	y=4596.50x+620.10	0.9997	4.7	0.09	71.01
PFNA	y=8242.05x-572.57	0.9964	4.9	0.16	65.97
PFOA	y=6032.64+236.80	0.9968	8.4	0.18	86.89
PFHpA	y=7804.99x-52.89	0.9973	6.0	0.13	71.68
PFHxA	y=3189.34x+2998.35	0.9950	3.3	0.10	89.40
PFOS	y=478.05x-53.01	0.9996	8.0	0.30	92.77
PFOSA	y=106.67-12.80	0.9902	5.5	0.33	96.26

4. Conclusions

The simultaneous determination of trace amounts of 11 PFAs in leather was developed in this study. Ultrasonic assisted solid-liquid extraction step was developed by comparing the extraction efficiency of different extractants. The combination of SPE cleanup and HPLC-MS/MS analysis showed accurately when analysing PFCs in leather samples. This method allows the extraction of analytes in less solvent consumption than required by many other techniques, integrating sample cleanup and without dedicated instrumentation. The whole method has been validated by showing good repeatability with RSDs below 20 % and LODs below 0.96 ng/L. Satisfactory recovery values between 65 % and 97 %. Therefore, the proposed method was applicable for the determination of PFCs residues in leather.

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