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25 December 2024

Determination of perfluoroalkyl substances (PFAS) in human hair by liquid chromatography- high accurate mass spectrometry (LC-QTOF)

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Abstract

Biomonitoring of perfluoroalkyl substances (PFAS) in hair is conventionally achieved by SPE extraction and liquid chromatography-triple quadrupole analysis, with sensitivity in the range of ng/g. The aim of this study was to develop and validate a rapid method to detect 20 perfluoroalkyl substances (PFAS) in human hair from general populations by SPE purification and liquid-chromatography coupled to accurate mass measurement (LC-QTOF). The obtained sensitivity (LOQ), linearity and RSD accuracy were respectively in the range of 0.07-0.5 ng/g, 0.1 (or 0.2 or 0.5)-10 ng/g, 1-16%. To verify the applicability of the method, 11 hair samples from volunteers were tested. The detected PFAS were PFBA (range 0.240-14.580 ng/g), PFBS (0.496 ng/g), PFOA (range 0.080-0.178 ng/g) and PFOS (<LOQ-0.239 ng/g). The results were compared in terms of detection frequency and abundance with previously published studies. The method proved useful for the determination of the tested PFAS in the hair matrix.

Keywords

1 Introduction

Perfluoroalkyl substances (PFAS) are a group of fluorinated compounds, judged as persistent organic pollutants (POPs) by the Stockholm Convention [1] in which they are listed. They are produced by anthropogenic industrial activities and can be found in surfactants, carpets, fire-retarding and food packaging. The most studied PFAS are the short chain PFOA (perfluorooctanoic acid) and PFOS (perfluorooctane sulfonic acid) [2]. PFOA is highly stable and persistent in the environment with the capacity to undergo long

range transport. Monitoring data in air, water, soil/sediment and biota in both local and remote locations like the Arctic have shown a wide diffusion of PFOA through the environment. Furthermore, PFOA can bioaccumulate and biomagnify in air-breathing mammals and other terrestrial species including humans. PFOS is both intentionally produced and an unintended degradation product of related anthropogenic chemicals. The current intentional use of PFOS is widespread and specifically includes electric and electronic parts, fire-fighting foam, photo imaging, hydraulic fluids and textiles. It is extremely persistent and has substantial bioaccumulating and biomagnifying properties, although it does not follow the classic pattern of other POPs by partitioning into fatty tissues but instead binds to proteins in the blood and the liver. Like PFOA, it also has the capacity to undergo long-range transport. Recent studies have suggested that exposure to high levels of PFAS may impact the immune system [3,4]. However, the human health effects from enduring exposure to low environmental levels of PFOA are unknown, mainly because PFOA can remain stored in the body for long periods of time. Major health issues [5] such as kidney cancer, testicular cancer, high cholesterol, thyroid disease [6], hypertension [7], endocrine disruption [8] have been linked to PFOA. The main route of PFAS exposure is, beside air pollution, the diet. Water can be considered the major vector of PFAS ingestion for the taken daily amount. For this reason, the two main agencies recognised for environment and human safety, USA Environmental Protection Agency (EPA) and European Food Safety Authority (EFSA) have recognised the problem of PFAS diffusion by establishing methods and limits in PFAS exposure. In particular, EPA [9] has established guidelines to detect, quantify and advisory levels of PFAS [10] presence in drinking water, while the European Food Safety Authority (EFSA) has promulgated provisional tolerable weekly intake (TWI) values for PFOS and PFOA of 13 ng/kg bw/week and 6 ng/kg bw/week respectively [11]. No alert levels are proposed so far for any of the human matrix investigated (i.e., human blood/serum [12-14] and breast milk [15-17]), since

the finding of measurable amounts of PFAS in biological matrices does not directly imply an adverse health effect. In fact, biomonitoring studies on levels of PFAS provide physicians and public health officials with reference values so that they can determine whether people have been exposed to higher levels than are found in the general population. Furthermore, biomonitoring data can also help scientists plan and conduct research on exposure and health effects. As an alternative matrix, hair has recently gained popularity in human biomonitoring exposure to organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), dioxins, polychlorinated biphenyls (PCBs) and pesticides [18], only to cite some. The characteristics of extended window of detection, compared with biological fluids, easy of collection and stability of both the compounds and the matrix have contributed to consider hair analysis a most relevant biomarker in the assessment of chronic consumption/exposure [19,20]. It is thus quite straightforward that also the emerging issue of perfluoroalkyl substances (PFAS) environmental contamination has seen some applications on hair to detect human direct and indirect exposure. A review of the current literature has highlighted only a small number of studies [21-24] on PFAS determination in hair (see tab. 1) when compared to the analysis of other biological fluids. This may rely on the extreme low sensitivity required to detect contaminants exposure in this peculiar matrix, in the order of ng/g and on the relatively novelty of PFAS issue. All the proposed methods were based on sample purification by Weak-Anion-eXchange or Carbon Surface sorbents and LC triple quadrupole analysis. The number of PFAS varied from 8 to 22, the LOQs ranged from as low as 0.006 up to 0.796 ng/g among the proposed procedures. The aim of the present study was to develop and validate a new LC-MS method based on accurate mass measurements (QTOF) to detect 20 PFAS in hair. As a proof of concept of the proposed method, it was applied to the determination of PFAS in 11 real samples from volunteers living in three different areas. As further perspectives of this application, 1) the

present method would be applied to a wider cohort of samples for biomonitoring of the Italian population, 2) the QTOF acquisition could be exploited to detect possible direct and indirect biomarkers of PFAS exposure (metabolomic approach).

2 Materials and methods

2.1 Materials

PFAS compounds (> 98% purity): potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (11Cl-PF3OUdS); potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS); perfluoro-n-butanoic acid (PFBA); potassium perfluorobutanesulfonate (PFBS); perfluorodecanoic acid (PFDA); perfluorododecanoic acid (PFDoA); perfluoro(2-ethoxyethane)sulfonic acid (PFEESA); sodium perfluoroheptanesulfonate (PFHpS); perfluoroheptanoic acid (PFHpA); potassium perfluorohexanesulfonate (PFHxS); perfluorohexanoic acid (PFHxA); perfluoro-3-methoxypropanoic acid (PFMPA); perfluoro-4-methoxybutanoic acid (PFMBA); Perfluoro-n-nonanoic acid (PFNA); potassium perfluoro-1-octanesulfonate (PFOS); Perfluoro-n-octanoic acid (PFOA); Perfluoro-n-pentanoic acid (PFPeA); sodium perfluoro-1-pentanesulfonate (PFPeS); perfluoroundecanoic acid (PFUnA); 4,8-Dioxa-3H-perfluorononanoic acid (ADONA) were obtained from Wellington Laboratories (Guelph, Ontario, Canada). Mass-labelled (^{13}C) perfluoro-n-[1,2-^{13}C$_2$] octanoic acid (M2PFOA) and mass-labelled (^{13}C) sodium perfluoro-1-[1,2,3,4-^{13}C$_4$] octanesulfonate (MPFOS) at chemical purities > 98% and isotopic purities of > 99% were used as internal standards (I.S.) and were also from Wellington Laboratories. API-TOF reference mass solution was from Agilent Technologies (Santa Clara, USA). Perfluoroalkyl compounds and mass-labelled analogues were diluted in methanol to working standard solution at concentration of 10 ng/ml. Solutions were stored at -20 °C and left at room temperature at least 2 h for equilibration prior use. Solvents like methanol and acetonitrile for mobile phases and purification steps (all LC–MS grade) were

purchased from Merck (Darmstadt, Germany). Formic acid 98-100% for LC-MS was also from Merck. Water for mobile phase was obtained by Sartorius Arium mini apparatus (Sartorius, Goettingen, Germany). Ammonium acetate was acquired from Sigma-Aldrich (S.Louis, MO, USA). Bond Elut-ENV (200mg, 6 ml) cartridges for solid-phase extraction were from Agilent Technologies (Santa Clara, US).

2.2 Hair samples preparation

All hair samples for analysis were collected from the vertex posterior region of volunteers. The volunteers or equivalent legal representatives were informed about the study and gave their written informed consent. From these samples a proximal 3 cm segment was used. Hair sample was rinsed by shaking with 10 ml of water 10 minutes and then twice with 10 ml of acetone for 10 minutes with careful attention to soak all the material. Hair samples were subsequently left to dry at room temperature and then cut into small pieces. For validation and analysis, 100 mg of hair were weighted in a polypropylene vial and 10 µl of mass labelled I.S. were added. To extract PFAS from hair matrix, 2 ml of acetonitrile were added. The samples were then placed in an ultrasound bath at 45°C for 45 minutes. After that time, the extracts were collected in a separated polypropylene vial and the procedure was repeated twice (4 ml total volume of extract for each sample). Sample clean-up was by solid phase extraction (SPE) by using Bond elut-ENV cartridges. Briefly, a three steps procedure was employed. Cartridges conditioning was by 3 ml of methanol followed by sample loading (4 ml). Eluate from sample was collected in a propylene vial. Two ml of methanol were used for elution. Extracts were taken to dryness under a flow of nitrogen at 40°C using a metal heating block. Finally, samples were reconstituted in 500 µl water/methanol, 90/10 (v/v).

2.3 Apparatus

The LC MS system consisted of an Agilent 1290 Infinity II high pressure liquid-chromatography (HPLC) system coupled to an Agilent 6546 quadrupole- time-of-flight mass spectrometer (Q-TOF, Agilent Technologies, Santa Clara, CA). Separations were carried out in an EC-C18 column (2.1 x 100 mm, 1.9 μm), (Agilent Technologies, Santa Clara, CA), while a second LC C18 column (EclipsePlus -C18, 3.0 x 50 mm 1.8 μm) was placed after pump exit to delay any perfluorinated interferents originating from fluidic system. The mobile phase A consisted of a solution of 0.1 % formic acid/20 mM ammonium acetate in water (v/v) and mobile phase B of a solution of 0.1% formic acid in acetonitrile (v/v). Flow rate was 0.4 ml/min. The gradient was as follows: A-B, 97%-3% at time 0, A-B, 75%-25% at 1 min, gradient to A-B 15%-85% from 1 to 9 min, gradient to A-B 3%-97% from 9 to 10, isocratic A-B, 3%-97% for 2 min, equilibration at 3% B up to 15 minutes. The volume of injection was optimized and the final result was 20 μl. The Q-TOF instrument was operated in negative ion mode and source parameters were set as follow: capillary at 3500 V, gas temperature at 320°C, sheat gas temperature at 350 °C, drying gas at 8 l/min, nebulizer 35 psi, sheat gas flow at 12 l/min. All source parameters were optimized under LC conditions. Analytes were detected in high accurate mass scan in the range 100-1000 m/z at a rate of 2 spectra/sec and 3376 transients/spectrum. References masses were acquired throughout the run and were 112.9855 and 980.0163 m/z. Identification of each compound in matrix was by accurate mass (≤ 5 ppm) of the [M-H]⁻ measurement, isotopic pattern distribution (isotope abundance and isotope spacing match) and retention time compared to standards. Tune parameters: 10 GHz, negative mode, m/z range 3200, high resolution. Analysis of the collected data was carried out with the Masshunter software (version B.04.00), Agilent Technologies, Santa Clara, CA).

2.4 Method validation

Retention times, formulae, M and [M-H]$^-$ accurate masses of the analysed compounds are listed in table 2. The assessed validation parameters were sensitivity, linearity, accuracy, matrix effect and stability of processed samples. Since there is no certified reference material available for PFAS analysis in hair, a hair sample with the lowest content of PFAS was used to prepare matrix-matched calibrators and quality controls (QCs). Calculations were by subtracting the amount of PFAS in blank sample. For verification of selectivity and specificity, three samples with addition of the internal standards as well as six samples without addition of the internal standard mix were investigated for interfering signals. A matrix-matched validation is of pivotal importance to minimize analytical variability in chromatographic analyses, not always completely balanced by using internal standards, and the improvement in accuracy is well balanced with the extra effort in subtracting endogenous values. For this reason, the adopted strategy was to employ a validation in hair matrix for the presented method.

2.4.1 Calibration model and method sensitivity

The calibration model was tested in spiked hair matrix at 7 calibration levels (0.1, 0.2, 0.5, 1, 2, 5, 10 ng/g), 3 replicates each level, by least-squares regression procedure estimation. Origin was not included and a weight factor of 1/x was applied. Sensitivity described as limit of detection (LOD) and limit of quantification (LOQ) were calculated as follow. LOD was defined according to the U.S. Environmental Protection Agency (EPA) Method Detection Limit (MDL) procedure found in Title 40 Code of Federal Regulations Part 136 (40 CFR 136, Appendix B, revision 1.11) [25] as the lowest concentration level that can be determined to be statistically different from a blank (99% confidence). It was determined by analysing seven replicates of the lowest point of calibration and by calculating the T-Students confidence at 99% interval. The LOQ was mathematically defined as equal to 10 times the standard deviation of the results for seven replicates at

lowest concentration used to determine a justifiable limit of detection [26]. Both LOD and LOQ concentrations were experimentally verified in spiked hair matrix.

2.4.2 Accuracy, matrix effect and stability

Intra- and interday accuracy of the method was evaluated by measuring five replicates of QC samples at two concentration levels (0.7 and 1.5 ng/g) on three non-consecutive days. To obtain an average evaluation of the matrix effect over the entire quantification range, expressed as bias %, the slopes of the calibration curves prepared in water and hair matrix were used for each compound, instead of the traditional evaluation by single points [27]. Stability of processed hair sample was tested over 48 hours at room temperature by comparing results with fresh prepared hair extracts.

3 Results

The adjusted chromatographic separation for all 20 PFAS was achieved within 10 minutes (Fig. 1), with no coeluting peaks (for retention times see table 2). Sample preparation was optimized by selection of appropriate solvent for sample extraction between acetonitrile and ethyl acetate. Acetonitrile provided the highest recovery for all the compounds and was then selected over ethyl acetate. Sample clean-up followed the procedure of Ruan *et al*., 2019 with a modification of sample extraction according to previous testing [24]. The sensitivity expressed as LOD and LOQ were in the range 0.02-0.12 ng/g and 0.08- 0.5 ng/g, respectively. According to sensitivity data, the tested calibration range was 0.1–10 ng/g for PFBS, PFEESA, PFPeS, PFOA, PFHxS, PFHpS, PFOS, 9Cl-PF3ONS; 0.2-10 ng/g for PFBA, PFPeA, PFNA and 11Cl-PF3OUdS; 0.5-10 ng/g for PFMPA, PFMBA, PFHxA, PFHpA, ADONA, PFUnA and PFDoA. Results for intraday and interday accuracy at the two QC levels were in the range 1-16% and 2-16% for all PFAS, respectively (all data summarized in table 3). In table 4 results of matrix-effect and stability are

summarized. The calculated matrix-related effect was comprised from 52% to 119%; stability of processed samples, tested while maintaining the samples in the autosampler at room temperature for 48h, were in the range of 45-100%. Finally, to investigate the applicability of the proposed method for the biomonitoring of PFAS, 11 hair samples collected from the general population were tested. The results are summarized in table 5. Briefly, four out the 20 PFAS were detected in the analysed samples, namely, PFBA, PFBS, PFOA and PFOS. All samples revealed the presence of at least one compound of the family of PFAS, with prevalence of PFOS (7/11) and PFOA (4/11). The measured concentrations were at a low level for most of the PFAS (< LOQ- 0.587 ng/g), mostly comparable with the results of other studies, with the only exception of sample number 1, who showed a PFBA hair concentration of 14.580 ng/g (Fig.2). This subject, the only to present the concomitant occurrence of PFBA, PFOA and PFOS, lived in a particular region recognised for a widespread environmental contamination of PFAS.

4 Discussion

The introduction of more versatile and user-friendly instrumentation of accurate mass measurement has allowed the routinely application of this technique in all specialized laboratories. The advantages of using this technology for biomonitoring purposes rely on both the possibility to retrospectively screen for new member of PFAS family, for example the new emerging class of branched PFAS, not considered in this study and generally in other studies, but also to set up a metabolomic approach to discover any possible direct or indirect biomarker of PFAS exposure. This approach has not yet been proved and surely represent a further perspective of the present study. The difficulties in PFAS determination rely also in the absence of any commercial reference hair blank matrix, strongly desirable, since the finding of a real "blank" sample is very difficult, as demonstrated in many studies. As advised by some authors [22], a unique hair sample was used in this study for

validating the analytical method to minimize variability concerning hair matrix (color, gender, diet). The criteria for selection of the blank for method validation was the minimum number of PFAS detected at the lowest concentration. Validation testing produced good results in all tested parameters. Sensitivity was mostly comparable to other methods based on LC-triple quad determination and the sometimes-observed minor sensitivity for some PFAS may compensate with the advantages of accurate mass measurement. Accuracy for the low and high QCs were satisfactory in both intra and inter-day values. There are no official guidelines on acceptable recovery ranges of PFAS in hair, however, the EPA provides recovery guidelines for PFAS in drinking water, of 50%-150%. The observed matrix-effect was both as ion-suppression and ion-enhancement result. PFBA, PFNA, PFDA and PFDoA exhibited ion-enhancement (101-119%), while the remaining PFAS produced ion-suppression in the order of 52-95%. Stability performance was variable among the different PFAS: PFPeA, PFMBA, PFBS, PFEESA, PFHpA, PFPeA, ADONA, PFOA, PFNA, PFHxS, PFHpS, PFOS and 9Cl-PF3ONS were stable in processed sample for 48h at room temperature in the range 80-100%, demonstrating their persistent characteristic behaviour. PFBA, PFMPA, PFHxA, PFDA, PFDoA and 11Cl-PF3OUdS were stable in the range 45-74%. It should be kept in mind that all these compounds are considered very stable in the environment and consequently the loss in the observed concentration may not rely on compound degradation itself, but merely on sample masking, due to the possible formation of protein complexes. It is in fact known that some PFAS may form protein-molecule complexes [28-31] and in consideration of the very easy sample clean-up procedure, the presence of leftover of biological material could not be excluded. This data confirmed the necessity to analyse processed samples as soon as possible to avoid undesirable underestimation. The developed methodology was finally applied to 11 real samples from volunteers. The subjects lived in different Italian region, both genders were equally represented, age was in the range 3- 57 years old (data not

shown). All samples revealed the presence of at least one PFAS in detectable amounts, one sample exhibited 3 compounds (PFBA, PFOA and PFOS) and two sample contained 2 compounds (PFBA and PFOA or PFOS). The detection frequency rate was 64% PFOS (7/11), 36 %PFOA (4/11), 27% PFBA (3/11) and 9% PFBS (1/11). PFOS and PFOA are the most detected compounds when PFAS presence is investigated in population, independently from regional settings, while the presence and prevalence of other PFAS may vary with local aspects [21,23].

5 Conclusions

A fast and reliable LC–QTOF method was developed, validated and applied to a set of real samples. The method showed suitable for biomonitoring purposes in hair matrix. As future perspectives, a wider cohort of samples for biomonitoring of the Italian population will be considered, 2) the QTOF acquisition will be exploited to detect possible direct and indirect biomarker of PFAS exposure (metabolomic approach). Considering the paucity of evidence about the toxicokinetics and toxicodynamics of PFAS and the existence, especially in regions with high exposure, of a real and potentially severe risk to health, it is essential to increase our knowledge regarding the biological behaviour of PFAS in humans, also with the hair analysis.

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Tables

Table 1. Overview of the available methods and their <mark>sensitivi</mark>ty for PFAS determination in hair.

Ref	N. of PFAS	Washing/ rinse	Extraction	Sample purification	Technique	LOD (ng/g)	LOQ (ng/g)
(21)	8	Water and acetone	Acetonitrile	Oasis WAX	LC-triple quadrupole	0.026-0.069	0.083-0.22
(22)	15	Water and acetone	Ethylacetate	Dispersive ENVI-Carb	LC-triple quadrupole	0.003-0.013	0.006-0.3
(23)	11	Water and acetone	methanol and acetonitrile (1:1 v/v)	Wax cartridge	LC-triple quadrupole	0.0143-0.0827	0.114-0.796
(24)	22	Water, SDS 0.1%, water	2 X 5% water/95% methanol	ENVI-Carb cartridge	LC-triple quadrupole	0.009-0.05	0.02-0.25

Table 2. Summary of names, retention times, formulae<mark>, M and [M-H]⁻</mark> accurate masses of the analysed compounds.

Compound name	RT (minutes)	Formula	Exact mass M	Exact mass [M-H]⁻
PFBA	2.65	C4HF7O2	213,9865	212,9793
PFMPA	2.87	C4HF7O3	229,9814	228,9741
PFPeA	3.28	C5HF9O2	263,9833	262,976
PFMBA	3.5	C5HO3F9	279,9782	278,9709
PFHxA	3.97	C6HF11O2	313,9801	312,9728
PFBS	4.07	C4HF9O3S	299,9503	298,943
PFEESA	4.40	C4HF9O4S	315,9452	314,9379
PFHpA	4.63	C7HF13O2	363,9769	362,9696
PFPeS	4.80	C5HF11O3S	349,9471	348,9398

ADONA	4.86	C7H2F12O4	377,9762	376,9689
PFOA	5.26	C8HF15O2	413,9737	412,9664
PFNA	5.35	C9HF17O2	463,9705	462,9632
PFHxS	5.51	C6HF13O3S	399,9439	398,9366
PFHpS	6.1	C7HF15O3S	449,9407	448,9334
PFDA	6.42	C10HF19O2	513,9673	512,96
PFOS	6.68	C8HF1703S	499,9375	498,9302
PFUnA	6.99	C11HF21O2	563,9641	562,9568
9Cl-PF3ONS	7.15	C8HClF16O4S	531,9029	530,8956
PFDoA	7.54	C12HF23O2	613,9609	612,9537
11Cl-PF3OUdS	8.21	C10HClF20O4S	631,8965	630,8892

Table 3. <mark>Intraday accuracy and precision</mark> of the presented method**.**

Compound	Intraday accuracy				Interday accuracy			
	QC low (0.7 ng/g)		QC high (1.5 ng/g)		QC low (0.7 ng/g)		QC high (1.5 ng/g)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
PFBA	104	13	118	7	98	13	102	14
PFMPA	102	12	106	9	102	10	97	11
PFPeA	108	4	101	9	102	11	95	11
PFMBA	92	8	99	5	96	10	88	9
PFHxA	109	16	102	6	105	12	93	9
PFBS	84	5	104	5	82	6	100	8
PFEESA	92	7	84	2	87	6	80	5
PFHpA	96	7	92	8	93	6	86	7
PFPeS	83	1	80	2	84	3	80	2
ADONA	106	16	104	15	112	9	98	12
PFOA	104	8	100	9	105	10	94	8
PFNA	107	10	108	8	99	10	97	13
PFHxS	81	3	83	3	83	3	84	2
PFHpS	82	2	84	4	80	5	80	5
PFDA	115	4	81	2	111	7	80	3
PFOS	112	3	112	4	108	7	104	7
PFUnA	100	12	101	10	97	16	102	11
9Cl-PF3ONS	108	8	84	4	100	7	80	5
PFDoA	99	12	102	10	100	13	94	11
11Cl-PF3OUdS	108	10	86	6	110	7	85	5

Table 4. Summary of the results obtained for LOD, LOQ, matrix effect and stability for each compound.

Compound name	LOD (ng/g)	LOQ (ng/g)	Matrix effect (%)	Stability (48h, RT) (%)
PFBA	0.10	0.20	101	59
PFMPA	0.12	0.40	92	61
PFPeA	0.10	0.13	84	80
PFMBA	0.10	0.50	95	100
PFHxA	0.10	0.50	71	74
PFBS	0.02	0.10	65	100
PFEESA	0.02	0.07	61	100
PFHpA	0.1	0.24	78	87
PFPeS	0.02	0.08	59	100
ADONA	0.07	0.24	80	100
PFOA	0.02	0.08	73	85
PFNA	0.05	0.15	105	81
PFHxS	0.02	0.05	59	100
PFHpS	0.02	0.08	54	100
PFDA	0.1	0.50	102	52
PFOS	0.02	0.08	71	94
PFUnA	0.09	0.29	92	74
9Cl-PF3ONS	0.02	0.08	83	100
PFDoA	0.09	0.30	119	45
11Cl-PF3OUdS	0.05	0.15	52	72

RT: room temperature

Table 5. LC-qTOF results of the PFAS determination in 11 real hair samples (S1-S11). All results are expressed in ng/g.

| Compound name	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
PFBA	14.580	0.239	0.587	-	-	-	-	-	-	-	-
PFMPA	-	-	-	-	-	-	-	-	-		
PFPeA	-	-	-	-	-	-	-	-	-	-	-
PFMBA	-	-	-	-	-	-	-	-	-	-	-
PFHxA	-	-	-	-	-	-	-	-	-	-	-
PFBS	-	-	-	-	-	0.496	-	-	-	-	-
PFEESA	-	-	-	-	-	-	-	-	-	-	-
PFHpA	-	-	-	-	-	-	-	-	-	-	-
PFPeS	-	-	-	-	-	-	-	-	-	-	-
ADONA	-	-	-	-	-	-	-	-	-	-	-
PFOA	0.080	-	0.144	-	-	-	-	0.150	0.178	-	-
PFNA	-	-	-	-	-	-	-	-	-	-	-
PFHxS	-	-	-	-	-	-	-	-	-	-	-
PFHpS	-	-	-	-	-	-	-	-	-	-	-
PFDA	-	-	-	-	-	-	-	-	-	-	-
PFOS	0.194	0.123	-	0.147	0.192	-	0.239	-	-	< LOQ	0.095
PFUnA	-	-	-	-	-	-	-	-	-	-	-
9Cl-PF3ONS	-	-	-	-	-	-	-	-	-	-	-
PFDoA	-	-	-	-	-	-	-	-	-	-	-
11Cl-PF3OUdS	-	-	-	-	-	-	-	-	-	-	-

-: not detected.

Figure 1. Extracted ion chromatogram (EIC) of a hair spiked at 0.5 ng/g of PFAS. Retention times are reported in table 2.

1: PFBA; 2: PFMPA; 3: PFPeA; 4: PFMBA; 5: PFHxA; 6: PFBS; 7: PFEESA; 8: PFHpA; 9: PFPeS; 10: ADONA; 11: PFOA; 12: PFHxS; 13: PFNA; 14: PFHpS; 15: PFDA; 16: PFOS; 17: PFUnA; 18: 9Cl-PF3ONS; 19: PFDoA; 20: 11Cl-PF3OUdS.
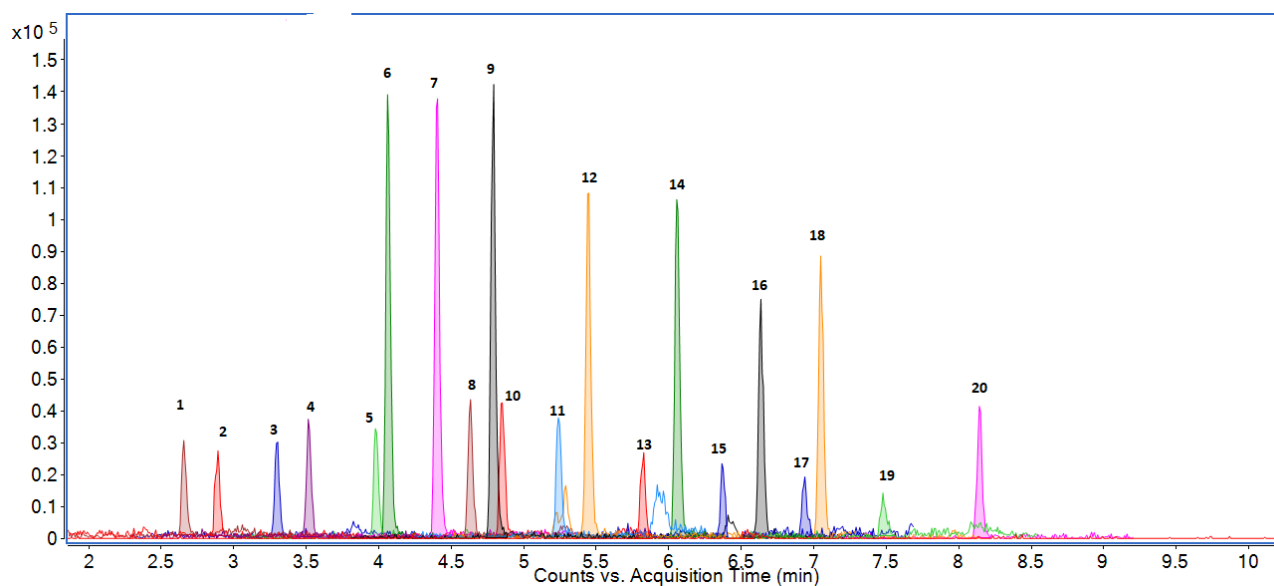
Figure 2. Representative EIC of a real hair sample positive to PFBA (14.580 ng/g). In the window, EIC of the isotopic pattern of PFBA (m/z 212.9793, m/z 213.9826, m/z 214.9838 ± 5 ppm); IS: M$_2$PFOA.