Application Note

Clinical Research

Analysis of Perfluoroalkyl Substances in Hair by Agilent Bond Elut ENV Solid Phase Extraction and LC/Q-TOF

A rapid biomonitoring screening method using the Agilent 1290 Infinity II LC and the Agilent 6546 Q-TOF

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Abstract

This application note describes a rapid analytical method for the detection of 20 perfluoroalkyl substances (PFAS) in human hair within 10 minutes. Solid phase extraction (SPE) using the Agilent Bond Elut ENV cartridge was used to extract the sample. The extract was then separated using liquid-chromatography (Agilent 1290 Infinity II LC) coupled to a quadrupole time-of-flight mass spectrometer (Agilent 6545 LC/Q-TOF) for accurate mass measurement of PFAS in hair. The limits of quantitation (LOQ) were in the range of 0.07 to 0.5 ng/g, demonstrating the sensitivity of the method. Linearity was 0.1 (or 0.2 or 0.5) to 10 ng/g, and relative standard deviations (RSDs) were 1 to 16%. To verify the applicability of the method for the determination of PFAS in hair, 11 samples obtained from adults in the general population were tested.

Introduction

Per- and poly-fluoroalkyl substances (PFAS) are a group of fluorinated compounds that are judged to be persistent organic pollutants (POPs) by the Stockholm Convention.[1] PFAS can be released to the environment during manufacturing, or through use and disposal of PFAS-containing products, such as surfactants, carpets, fire-retardants, and food packaging. The short chain perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are especially persistent in the environment. These compounds are the most widely studied compounds, according to the US Environmental Protection Agency (EPA).[2]

Hair is gaining popularity in biomonitoring assessments of human exposure to organic pollutants. Compared with biological fluids, hair is easy to collect. Also, the stability of both the compounds and the matrix has contributed to its use in chronic consumption/exposure studies.[3,4] However, there are relatively few papers in the literature on the determination of PFAS in hair compared to other biological fluids.[5–8] The high sensitivity required to detect contaminants in hair, in the order of ng/g, and the relative novelty of biomonitoring for PFAS, may have limited research in this area. All the methods in the literature used sample purification by SPE using weak anion exchange (WAX) or carbon surface (CS) sorbents followed by analysis using triple quadrupole LC/MS.

The aim of this study was to develop and validate a routine LC/MS method for the determination of PFAS in hair, based on accurate mass measurements using Q-TOF. In addition to identification of PFAS with accurate mass measurements, Q-TOF can be used for retrospective data analysis—a useful way to detect potential direct and indirect biomarkers of PFAS exposure. As a proof-of-concept of the method, it was applied to the determination of 20 PFAS compounds in 11 hair samples provided by volunteers.

Experimental

Materials and reagents

PFAS compounds (>98% purity) and internal standards (IS) were obtained from Wellington Laboratories (Guelph, Ontario, Canada). The compounds included:

- 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)
- 9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)
- perfluoro-*n*-butanoic acid (PFBA)
- perfluoro-1-butanesulfonic acid (PFBS)
- perfluoro-*n*-decanoic acid (PFDA)
- perfluoro-*n*-dodecanoic acid (PFDoA)
- perfluoro(2-ethoxyethane)sulfonic acid (PFEESA)
- perfluoro-1-heptanesulfonic acid (PFHpS)
- perfluoro-*n*-heptanoic acid (PFHpA)
- perfluoro-1-hexanesulfonic acid (PFHxS)
- perfluoro-*n*-hexanoic acid (PFHxA)
- perfluoro-3-methoxypropanoic acid (PFMPA)
- perfluoro-4-methoxybutanoic acid (PFMBA)
- perfluoro-*n*-nonanoic acid (PFNA)
- perfluoro-1-octanesulfonic acid (PFOS)
- perfluoro-*n*-octanoic acid (PFOA)
- perfluoro-*n*-pentanoic acid (PFPeA)
- perfluoro-1-pentanesulfonic acid (PFPeS)
- perfluoroundecanoic acid (PFUnA)
- dodecafluoro-3H-4,8-dioxanonanoic acid (ADONA)

Mass-labeled (^{13}C) perfluoroalkylcarboxylic acid (M2PFOA) and mass-labeled (^{13}C) perfluoroalkylsulfonate (MPFOS) were used as the IS.

An Agilent API-TOF reference mass solution was used for reference mass correction (part number G1969-85003). Working standard solutions at a concentration of 10 ng/mL were prepared by diluting the perfluoroalkyl compounds and mass labeled analogs in methanol. Solutions were stored at –20 °C and left at room temperature for at least 2 hours to equilibrate before use. LC/MS grade solvents such as methanol and acetonitrile that were used for the mobile phases and purification steps were bought from Merck (Darmstadt, Germany). Formic acid (98 to 100% for LC/MS) was also bought from Merck. Water for the mobile phase was obtained using an Arium mini ultrapure water system (Sartorius, Goettingen, Germany). Ammonium acetate was acquired from Sigma-Aldrich (Saint Louis, MO, USA).

For the solid-phase extractions of the PFAS compounds, 200 mg, 6 mL Agilent Bond Elut ENV SPE cartridges (part number 12255014) were used. The cartridges use a modified styrene-divinylbenzene (PS-DVB) polymer that is designed for the extraction of polar organic residues. Each cartridge contains 125 µm spherical particles with a high degree of cross-linking, useful for high-volume, fast-flowthrough applications.

Hair sample preparation

Hair samples were obtained from 11 adult-volunteers from the general population. The samples were rinsed with 10 mL of water and then double rinsed with 10 mL of acetone. The hair samples were left to dry at room temperature and then cut into small pieces. 100 mg of each hair sample was weighed into a polypropylene vial and 10 µL of the mass labeled IS was added. Acetonitrile (2 mL) was added before extraction in an ultrasound bath at 45 °C for 45 minutes. The extracts were collected in polypropylene vials and the extraction procedure was repeated a second time. SPE using Bond Elut ENV cartridges was used to clean the extracts. Finally, the extracts were reconstituted in 500 µL water/methanol (90/10 v/v).

Instrumentation

The LC/MS analysis was performed using an Agilent 1290 Infinity II LC coupled to an Agilent 6546 Q-TOF. Separations were carried out using an Agilent InfinityLab Poroshell 120 EC-C18 column, 2.1 × 100 mm, 1.9 µm (p/n 695675-902). An Agilent delay column, C18 4.6 × 30 mm (p/n 5062-8100) was placed after the pump exit to delay any perfluorinated interferents originating from the fluidic system.

The mobile phases consisted of water with 20 mM ammonium acetate (A) and acetonitrile with 0.1% formic acid (B), with a flow rate of 0.3 mL/min. The gradient program was as follows:
3% B at time 0,
25% B at 1 minute,
25 to 85% B from 1 to 9 minutes,
85 to 97% from 9 to 10 minutes,
isocratic 97% B for 2 minutes,
equilibration at 3% B up to 15 minutes.
The injection volume was 20 µL.

The Q-TOF was operated in negative ion mode. Analytes were detected in high accurate mass scan mode in the range 100 to 1,000 *m/z* at a rate of 2 spectra/sec and 3376 transients/spectrum. Reference masses (112.9855 and 980.0163 *m/z*) were acquired throughout the run. Analysis of the collected data was carried out with the Q-TOF MassHunter acquisition software (version B.04.00).

Method validation

Retention times, formulae, and accurate masses of the analyzed compounds are listed in Table 1. The method was assessed using validation parameters including sensitivity, linearity, and accuracy.[9] For verification of selectivity and specificity, three samples containing the internal standard mix, and six samples without the mix were investigated for interfering signals.

Table 1. Summary of the retention time, formula, and accurate masses of each analyzed compound.

Compound Name	RT (min)	Formula	Exact Mass	Exact Mass $[M-H]^-$
PFBA	2.64	$C_4HF_7O_2$	213.9865	212.9792
PFMPA	2.87	$C_4HF_7O_3$	229.9814	228.9741
PFPeA	3.28	$C_5HF_9O_2$	263.9833	262.9760
PFMBA	3.50	$C_5HO_3F_9$	279.9782	278.9709
PFHxA	3.97	$C_6HF_{11}O_2$	313.9801	312.9728
PFBS	4.07	$C_4HF_9O_3S$	299.9503	298.9430
PFEESA	4.40	$C_4HF_9O_4S$	315.9452	314.9379
PFHpA	4.63	$C_7HF_{13}O_2$	363.9769	362.9696
PFPeS	4.80	$C_5HF_{11}O_3S$	349.9471	348.9398
ADONA	4.86	$C_7H_2F_{12}O_4$	377.9762	376.9689
PFOA	5.26	$C_8HF_{15}O_2$	413.9737	412.9664
PFNA	5.35	$C_9HF_{17}O_2$	463.9705	462.9632
PFHxS	5.51	$C_6HF_{13}O_3S$	399.9439	398.9366
PFHpS	6.10	$C_7HF_{15}O_3S$	449.9407	448.9334
PFDA	6.42	$C_{10}HF_{19}O_2$	513.9673	512.9600
PFOS	6.68	$C_8HF_{17}O_3S$	499.9375	498.9302
PFUnA	6.99	$C_{11}HF_{21}O_2$	563.9641	562.9568
9Cl-PF3ONS	7.15	$C_8HClF_{16}O_4S$	531.9029	530.8956
PFDoA	7.54	$C_{12}HF_{23}O_2$	613.9609	612.9537
11Cl-PF3OUdS	8.21	$C_{10}HClF_{20}O_4S$	631.8965	630.8892

Results and discussion

The chromatographic separation for all 20 PFAS was achieved within 10 minutes, with no coeluting peaks.

The sensitivity, expressed as LOD and LOQ[9], was in the range of 0.02 to 0.12 ng/g and 0.08 to 0.5 ng/g, respectively. According to the sensitivity data (using LOQ to define the lowest calibration point), the tested calibration range was 0.1 to 10 ng/g for PFBS, PFEESA, PFPeS, PFOA, PFHxS, PFHpS, PFOS, 9Cl-PF3ONS; 0.2 to 10 ng/g for PFBA, PFPeA, PFNA, and 11Cl-PF3OUdS; and 0.5 to 10 ng/g for PFMPA, PFMBA, PFHxA, PFHpA, ADONA, PFUnA, and PFDoA.

Results for intraday and interday accuracy are summarized in Table 2.[9] All intraday and interday recoveries were within 70 to 130% with RSDs <20%, indicating outstanding measurement stability.

Finally, to investigate the applicability of the proposed method for the biomonitoring of PFAS, the hair samples collected from 11 volunteers were tested. Only four of the 20 PFAS were detected in the samples, namely, PFBA, PFBS, PFOA, and PFOS. PFOA and PFOS are known to be persistent in the environment.[2]

At least one PFAS was detected in each of the 11 hair samples. PFOS was the most prevalent compound, found in seven samples, and PFOA was found in four samples. The measured concentrations for most of the PFAS were at a low level (<LOQ to 0.587 ng/g), which was mostly comparable with the results of other studies.

Table 2. Intra- and inter-day accuracies of the LC/Q-TOF method.

	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)	
Compound	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

Conclusion

The study has shown the suitability of LC/Q-TOF for the determination of PFAS in human hair. The fast and reliable method used Agilent Bond Elut ENV cartridges for SPE to prepare the sample extracts for analysis using an Agilent 6545 LC/Q-TOF. The method was validated for the determination of 20 PFAS, before being applied to the analysis of hair samples. Only four-types of PFAS were found to be present in the 11 hair samples that were tested using this method, with at least one PFAS determined in each sample.

The speed, sensitivity, linearity, and accuracy of the accurate-mass measurement of PFAS using LC/Q-TOF makes it suitable for routine biomonitoring studies. The method could also be used for retrospective screening of samples for new members of the PFAS family, for example the emerging class of branched PFAS.

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