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Method of Test for Perfluoroalkyl Substances in Cosmetics

1. Scope

This method is applicable to the determination of 4 perfluoroalkyl substances (PFAS), including perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), nonadecafluorodecanoic acid (PFDA) and perfluorononan-1-oic acid (PFNA) in cosmetics.

2. Method

After extraction, analytes are determined by liquid chromatography/ tandem mass spectrometer (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: electrospray ionization, ESI.

2.1.1.2. Delay Column: Isolator Column, 3.5 μm, 2.1 mm i.d. × 5 cm, or an equivalent product. The delay column was installed between the pump and the autosampler.

2.1.2.3. Column: Poroshell 120 EC-C18, 2.7 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.2. Ultrasonicator.

2.1.3. Vortex mixer.

2.1.4. Centrifuge: centrifugal force > 3800 ×g.

2.2. Chemicals

Methanol and ammonium acetate, LC-MS grade;

Glacial acetic acid, reagent grade;

Deionized water, resistivity ≥ 18 MΩ・cm (at 25℃);

PFOS, PFOA, PFDA and PFNA, all are 100 μg/mL in methanol, reference standards;

Perfluoro[$^{13}C_8$]octane sulfonic acid ($^{13}C_8$-PFOS), perfluoro[$^{13}C_8$]octanoic acid ($^{13}C_8$-PFOA), perfluoro[1,2,3,4,5,6,7,8,9-$^{13}C_9$]decanoic acid ($^{13}C_9$-PFDA) and perfluoro[$^{13}C_9$]nonanoic acid ($^{13}C_9$-PFNA),all are 50 μg/mL in methanol, isotope-labelled internal standards.

2.3. Apparatus and materials

2.3.1. Volumetric flask: 10 mL, Pyrex.

2.3.2. Centrifuge tube: 15 mL, PP.

2.3.3. Membrane filter: 0.22 μm, Nylon.

2.4. Mobile phase

2.4.1. Solvent A:

Dissolve and dilute 0.385 g ammonium acetate with deionized water to 1000 mL. Adjust pH to 4.8 with glacial acetic acid, and filter with a membrane filter.

2.4.2. Solvent B: methanol.

2.5. Internal standard solution preparation

Mix appropriate amount of $^{13}C_8$-PFOS, $^{13}C_8$-PFOA, $^{13}C_9$-PFDA and $^{13}C_9$-PFNA isotope-labelled internal standards, dilute with methanol to 1000 ng/mL as the internal standard solution.

2.6. Standard solution preparation

Mix appropriate amount of PFOS, PFOA, PFDA and PFNA reference standards and the internal standard solution, dilute with methanol to 1-20 ng/mL (containing 10 ng/mL internal standard) as the standard solutions.

2.7. Sample solution preparation

Transfer about 0.4 g of the well-mixed sample accurately weighed into a 10 mL volumetric flask, and add 100 μL of internal standard solution. Add 8 mL of methanol, and ultrasonicate for 30 min. Add methanol to the volume and transfer the solution to a 15 mL centrifuge tube. Centrifuge at 3800 ×g for 5 minutes and filter the supernatant with a membrane filter, and take the filtrate as the sample solution.

2.8. Standard curve establishment

Accurately inject 3 μL of the standard solutions into LC-MS/MS separately, and operate according to the following conditions. Establish the standard curve of each PFAS by the ratio of the peak area of each PFAS to that of the internal standard vs. the concentrations.

LC-MS/MS operating conditions (note):

Delay column: Isolator Column, 3.5 μm, 2.1 mm i.d. × 5 cm.

Column: Poroshell 120 EC-C18, 2.7 μm, 2.1 mm i.d. × 10 cm.

Column temperature: 40℃.

Injection volume: 3 μL.

Mobile phase: a gradient program of solvent A and solvent B is as follows.

Time (min)	A (%)	B (%)
0.0 → 0.5	95 → 95	5 → 5
0.5 → 9.0	95 → 0	5 → 100
9.0 → 12.0	0 → 0	100 → 100
12.0 → 12.5	0 → 95	100 → 5
12.5 → 16.0	95 → 95	5 → 5

Flow rate: 0.3 mL/min.

Sample temperature: 15℃.

Ionization mode: ESI^-.

Capillary voltage: 2.0 kV.

Ion source temperature: 150℃.

Desolvation temperature: 450℃.

Cone gas flow rate: 50 L/hr.

Desolvation gas flow rate: 800 L/hr.

Detection mode: multiple reaction monitoring (MRM). Selected ion pair, cone voltage (CV) and collision energy (CE) are as follows.

Analyte	Ion pair Precursor ion (*m/z*) > product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
PFOS	499 > 80*	30	42
	499 > 99	30	42
PFOA	413 > 369*	15	10
	413 > 169	15	18
PFDA	513.0 > 469*	20	10
	513.0 > 269	20	15
PFNA	463 > 419*	15	10
	463 > 219	15	16
$^{13}C_8$-PFOS (I.S.)	507.0 > 80	30	40
$^{13}C_8$-PFOA (I.S.)	421 > 376	15	10
$^{13}C_9$-PFDA (I.S.)	522.0 > 477	20	12
$^{13}C_9$-PFNA (I.S.)	472 > 427	15	10

*Quantitative ion pair

2.9. Identification and quantification

Accurately inject 3 μL of the sample solution and the standard solutions into LC-MS/MS separately, and operate according to the conditions in section 2.8. Identify each PFAS based on the retention time and the relative ion intensities(note). Calculate the amount of each PFAS in the sample by the following formula:

$$\text{The amount of each PFAS in the sample (ng/g)} = \frac{C \times V}{M}$$

Where,

C: the concentration of each PFAS in the sample solution calculated by the standard curve (ng/mL)

V: the final make-up volume of the sample (mL)

M: the weight of sample (g)

Note 1: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions ($\leq$100%). Maximum permitted tolerances of relative ion intensities by LC-MS/MS are as follows.

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20-50	± 25
> 10-20	± 30
≤ 10	± 50

Note 2: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

Remark

1. Limits of quantitation (LOQs) for PFOS, PFOA, PFDA and PFNA are all 25 ng/g.
2. Further validation should be performed when interfering compounds are found in samples

References

1. Whitehead, H. D., Venier, M., Wu, Y., Eastman, E., Urbanik, S., Diamond, M. L., Shalin, A., Schwartz-Narbonne, H., Bruton, T. A., Blum, A., Wang, Z., Green, M., Tighe, M., Wilkinson, J. T., McGuinness, S. and Peaslee, G. F. 2021. Fluorinated compounds in North American

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2. Harris, K. J., Munoz, G., Woo, V., Sauvé, S. and Rand, A. A. 2022. Targeted and suspect screening of per- and polyfluoroalkyl substances in cosmetics and personal care products. Environ. Sci. Technol. 56: 14594-14604.

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Reference chromatogram

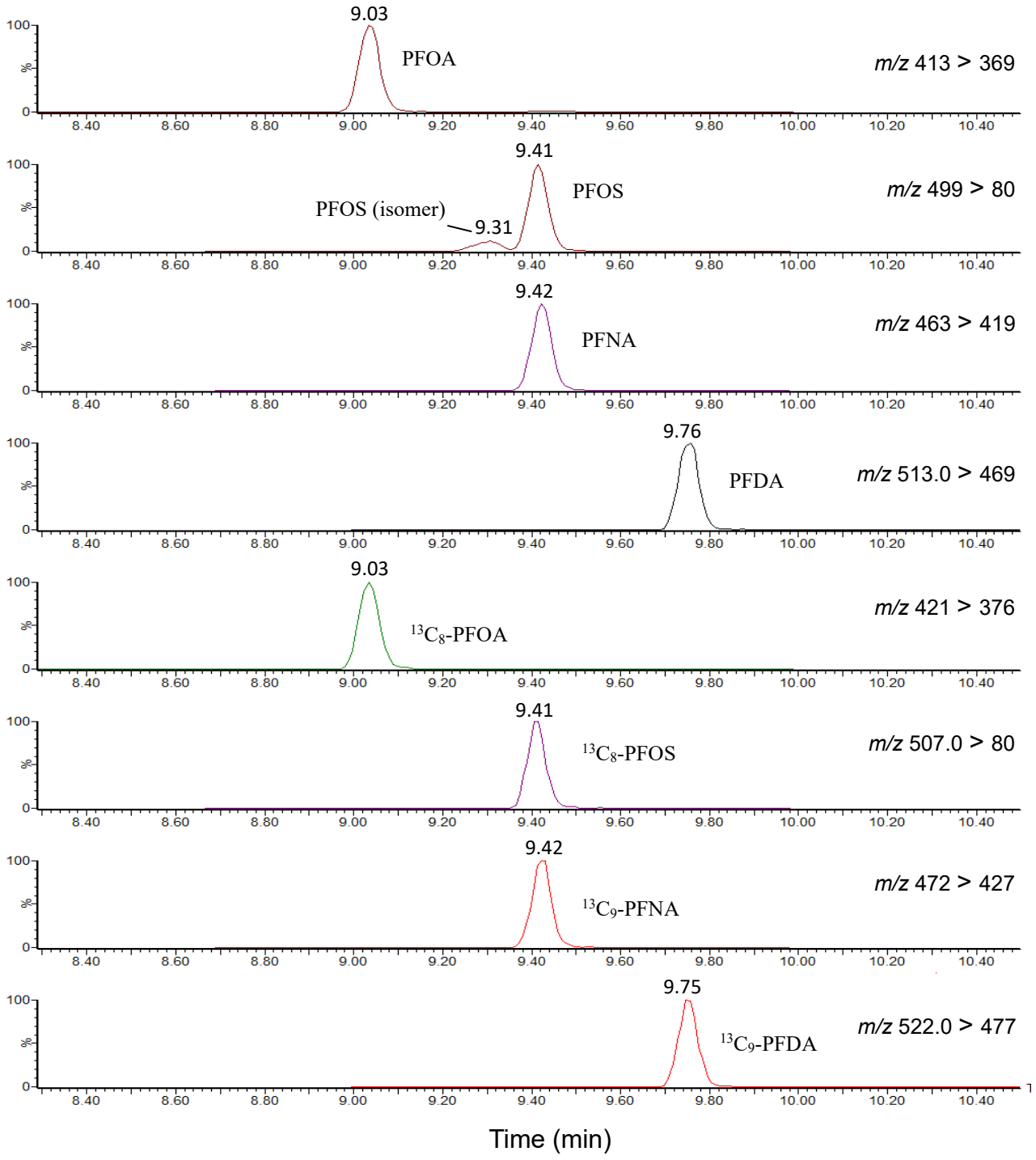

Figure. MRM chromatograms of PFOS, PFOA, PFDA, PFNA and isotope labelled internal standards analyzed by LC-MS/MS.