Determination of Perfluorinated Compounds in Milk Tea by High Performance Liquid Chromatography-Tandem Mass Spectrometry

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Abstract [**Objectives**] This study was conducted to establish a detection method for the simultaneous determination of 18 perfluorinated compounds (PFCs) in milk tea by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). [**Methods**] The samples were first subjected to precipitation of proteins by potassium ferrocyanide solution and zinc acetate solution, and then extracted by acetonitrile and detected by LC-MS/MS. Next, sodium chloride was added, and after vortex centrifugation, the acetonitrile layer was dried by blowing with nitrogen. Subsequently, 1 ml of methanol was added to prepare a test solution. An ACQUITY UPLC BEH C18 (2.6 m, 2.1 mm × 100 mm) chromatographic column was used for liquid phase separation, and gradient elution was performed using 10 mmol ammonium acetate solution-acetonitrile as the mobile phase. The MS detection adopted the MRM mode for acquisition, positive and negative ion mode switching for simultaneous determination, and external standard method for quantification. [**Results**] In the linear ranges of the target substances, the correlation coefficients R^2 were all greater than 0.99. The detection limits of the method was in the range of 0.001 – 0.05 μg/kg, and the quantitation limits were in the range of 0.03 – 0.20 ng/L. The recovery values ranged from 72.8% to 110.5%. [**Conclusions**] This method has high sensitivity and good accuracy, and thus strong practical value.

Key words Liquid chromatography-tandem mass spectrometry; Milk tea; Perfluorinated compound

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Perfluorinated compounds (PFCs) are organic compounds in which all hydrogen atoms linked to carbon atoms are replaced by fluorine atoms, including perfluorocarboxylic acids (PFCAs), perfluorosulfonic acids (PFSAs), perfluorosulfonamides (PFASs) and fluorotelomer alcohols (FTOHs), which are widely used in textiles, lubricants, surfactants, food packaging and non-stick pan coatings, electronic products, fir-proof suits, fire foam and other fields[1 – 3]. PFCs have been listed as new persistent organic pollutants because of their persistence and bioaccumulation. PFCs have been listed as new persistent organic pollutants because of their persistence and bioaccumulation. The study on the toxic effects of PFCs on human body is still in the initial stage, but animal experiments have shown that perfluorinated compounds have hepatotoxicity, embryonic toxicity, reproductive toxicity, neurotoxicity and carcinogenicity. They can interfere with endocrine and change animals' instinctive behavior, and may have potential developmental neurotoxicity to human beings, especially young children. In addition, some studies have shown that the exposure of pregnant mothers to PFCs has short-term and long-term harmful effects on the physical and psychological development of newborns and babies. Therefore, it is recommended that women of childbearing age minimize their exposure to disposable paper packaged foods, indoor and outdoor dust, and other related pathways related to PFCs[4 – 6].

At present, there are preliminary reports on perfluorinated compounds. Tang *et al.*[7] studied the residues of PFCs in animal-derived foods by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Liang *et al.*[8] studied the residual amount of perfluorinated and polyfluorinated compounds in cosmetics by UHPLC-MS/MS. Chen[9] studied the PFCs in infant formula milk powder. Wang *et al.*[10] investigated PFCs in food contact materials by HPLC-MS/MS. In this study, PFCs in milk tea were investigated scientifically by HPLC-MS/MS, aiming to establish a rapid and accurate detection method.

Materials and Methods

Experimental apparatuses and reagents

Apparatuses: Ultra-high performance liquid chromatograph-tandem mass spectrometer (AB 4500, AB Company, USA); MFV-24 nitrogen blowing concentrator (Guangzhou Detelogy Technology Co., Ltd.); Milli-Q ultrapure water instrument (Millipore Company, USA); TG16W high-speed centrifuge (Changsha Pingfan Instrument Co., Ltd.); AS30600BT ultrasonic oscillator (Tianjin Automatic Science Instrument Co., Ltd.); 0.22 μm microfiltration membrane.

Reagents: Standard samples, all from GRG Metrology & Test Group Co., Ltd., as shown in Table 1; potassium ferrocyanide (analytically pure), zinc acetate (analytically pure), sodium chloride (analytically pure) and ammonium acetate (analytically pure), all from Sinopharm Group Co., Ltd.; methanol (chromatographic purity) and acetonitrile (chromatographic purity), all from Merck, Germany.

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Table 1 Data of standard solutions of 18 PFCs

Perfluorinated compound	Abbreviation	CAS number	Concentration // μg/ml
N-Ethylperfluorooctane sulfonamidoacetic acid	N-EtFOSAA	1691-99-2	100
Perfluoropentanoic acid	PFPeA	2706-90-3	100
Potassium perfluorobutanesulfonate	PFBS	29420-49-3	100
Perfluorobutyric acid	PFBA	375-22-4	100
N-Methylperfluorooctane sulfonamidoacetic acid	N-MeFOSAA	2355-31-9	100
Perfluorooctanoic acid	PFOA	375-85-9	100
Perfluoroundecanoic acid	PFUdA	2058-94-8	100
Perfluorodecane sulfonic acid	PFDS	335-77-3	100
Perfluorooctane sulfonic acid	PFOS	1763-23-1	100
Sodium perfluoro-1-nonanesulfonate	PFNS	98789-57-2	5
Perfluorononanoic acid	PFNA	375-95-1	100
Perfluorododecanoic acid	PFDoA	307-55-1	100
Sodium perfluoro-1-dodecanesulfonate	PFDoS	108026-35-3	5
Perfluorooctadecanoic acid	PFODA	16517-11-6	100
N-Methyl-perfluoroctanesulfonamide	N-MeFOSA-M	31506-32-8	100
N-Ethyl-N-2-hydroxyethyl perfluorooctansulfulfonamide	EtFOSE	2991-50-6	100
Perfluorohexanoic acid	PFHxA	307-24-4	100
Sodium perfluorohexanesulfonate	PFHxS	82382-12-5	100
Perfluoroheptanoic acid	PFHPA	375-85-9	100
Perfluoroheptanesulfonic acid	PFHps	21934-50-9	100
Perfluorodecanoic acid	PFDA	335-76-2	100

Preparation of solutions

Preparation of standard stock solution: Target liquid standard substances with different concentrations were transferred into 10 ml brown glass bottles, respectively. Standard stock solutions with the concentration of 1 μg/ml were then prepared with methanol. The prepared solutions were sealed and stored in a refrigerator at −18 ℃ for later use.

Preparation of standard working solution: Different volumes of standard stock solutions were accurately transferred to different 10 ml volumetric bottles. The obtained solutions were prepared into mixed standard solutions with different concentrations by diluting with methanol.

Sample treatment

A 10 g of sample was weighed into a 50 ml centrifuge tube, and added with 2 ml of potassium ferrocyanide solution and 2 ml of zinc acetate solution in turn. After vortex mixing, 10 ml of acetonitrile was added, and ultrasonic extraction was performed for 20 min. Next, 2 g of sodium chloride was added, and after vortex-mixing and standing for 10 min, centrifugation was performed at 8 000 r/min for 5 min. Subsequently, 5 ml of solution was taken from the organic layer and dried by the blowing with nitrogen in a water bath at 40 ℃. Finally, 1 ml of methanol was added, and the obtained solution was filtered with a 0.25 μm filter membrane for later determination.

Instrument condition

Chromatographic conditions: An ACQUITY UPLC BEH C18 (2.6 μm, 2.1 mm × 100 mm) column was used to separate target substances. The injection volume was 3 μl, and HPLC separation was performed with a column temperature at 35 ℃ and a flow rate at 0.3 ml/min all the time. The mobile phase A was 10 mmol/L ammonium acetate solution. The mobile phase B was acetonitrile. The conditions of gradient elution are shown in Table 2.

Table 2 Mobile phase gradient elution procedure

Time	Flow rate // ml/min	A	B
0.0	0.3	95.0	5.0
2.0	0.3	70.0	30.0
4.0	0.3	10.0	90.0
6.0	0.3	10.0	90.0
6.1	0.3	95.0	5.0
7.5	0.3	95.0	5.0

Mass spectrometry conditions: The time window was divided by the MRM mode to collect negative ions. An electrosprayion source (ESI) was used. The temperature of desolventizing gas was 350 ℃. The ion source temperature was 150 ℃. The flow rate of carrier gas was 650 L/h, and the flow rate of collider was 50 L/h. The specific parameters are shown in Table 3.

Results and Analysis

Optimization of chromatographic conditions

In this study, different mobile phases were compared, and the mobile phase compositions of water:methanol, 5 mmol/L ammonium acetate solution: acetonitrile and 10 mmol/L ammonium acetate solution:acetonitrile were analyzed respectively. When ammonium acetate and acetonitrile were selected, a better effect was achieved. The composition of methanol and water produced wider peaks , and the shapes of peaks were ugly. When ammonium acetate was used as the mobile phase, the shapes of peaks were better, and the peaks were narrower. Its concentration was adjusted, and it was found with the concentration of ammonium acetate increasing, the targets with a good peak type increased. Finally, 10 mmol/L ammonium acetate solution: acetonitrile was selected as the mobile phase, which realized the chromatogram of 18 PFCs with good peak type, strong signal and good separation effect. The total ion chromatogram of 18 PFCs is shown in Fig. 1.

Table 3 MS Parameters for 18 PFCs

Compound	Parent ion m/z	Daughter ion m/z	Declustering voltag ev	Collision energy ev	Capillary voltage kv	Ionization mode
Perfluorobutyric acid (PFBA)	213.1	169.1	15	8	1.5	ESI$^-$
	213.1	169.0*	15	8		
Potassium perfluorobutanesulfonate (PFBS)	299.2	98.9	15	25	1.5	ESI$^-$
	299.2	79.9*	15	40		
Perfluoropentanoic acid (PFPeA)	263.2	218.9*	16	8	1.5	ESI$^-$
	263.2	68.7	16	38		
Perfluorohexanoic acid (PFHxA)	313.2	268.9*	12	8	1.5	ESI$^-$
	313.2	119.0	12	18		
Sodium perfluorohexanesulfonate (PFHxS)	399.2	79.8	18	40	1.5	ESI$^-$
	399.2	98.7*	18	38		
Perfluoroheptanoic acid (PFHpA)	363.2	168.8	15	15	1.5	ESI$^-$
	363.2	319.1*	15	10		
Perfluoroheptanesulfonic acid (PFHpS)	449.2	79.7*	18	42	1.5	ESI$^-$
	449.2	98.7	18	45		
Perfluorooctanoic acid (PFOA)	413.1	168.8	18	15	1.5	ESI$^-$
	413.1	369.1*	18	10		
Perfluorooctane sulfonic acid (PFOS)	499.2	79.7*	18	42	1.5	ESI$^-$
	499.2	99.0	18	45		
Perfluorononanoic acid (PFNA)	463.2	218.9	14	12	1.5	ESI$^-$
	463.2	419.1*	14	10		
Sodium perfluoro-1-nonanesulfonate (PFNS)	549.1	98.9	20	42	1.5	ESI$^-$
	549.1	79.7*	20	45		
Perfluorodecanoic acid (PFDA)	513.2	468.9*	25	10	1.5	ESI$^-$
	513.2	219.0	25	15		
Perfluorodecane sulfonic acid (PFDS)	599.1	98.9	25	40	1.5	ESI$^-$
	599.1	79.9*	25	45		
Perfluoroundecanoic acid (PFUdA)	563.2	518.9*	22	42	1.5	ESI$^-$
	563.2	219.0	22	14		
Perfluorododecanoic acid (PFDoA)	613.1	568.9*	25	10	1.5	ESI$^-$
	613.1	169.1	25	22		
Perfluorooctadecanoic acid (PFODA)	913.1	168.7	25	35	1.5	ESI$^-$
	913.1	869.1*	25	15		
N-Methylperfluorooctane sulfonamidoacetic acid (MeFOSA)	512.0	169.0*	25	22	1.5	ESI$^-$
	512.0	169.1	25	22		
N-ethylperfluorooctane sulfonamidoacetic acid (EtFOSE)	630.0	59.1	25	13	1.5	ESI$^+$
	630.0	59.0*	25	13		

The mark * indicates a quantitative ion.

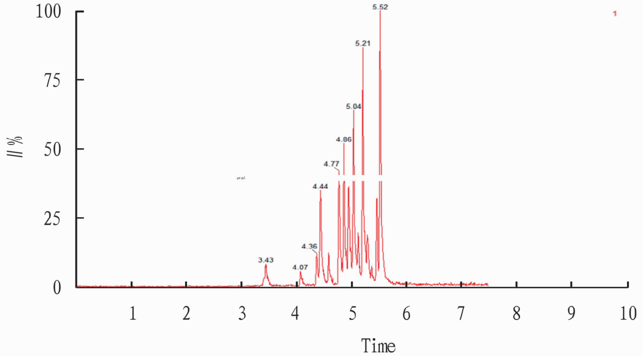

Fig. 1 Total ion chromatogram of 18 PFCs

Linear equations and detection limits

Different volumes were transferred from the standard stock solutions of the 18 targets into 10 ml volumetric flasks, and the solutions were diluted to constant volume with methanol, obtaining solutions with following standard curve concentrations: 2.00, 6.00, 10.00, 20.00, 40.00, 60.00, 80.00 and 100.00 ng/ml. Working curves were drawn taking the MS peak area of quantitative ion of each PFC as the y-axis and mass concentration as the x-axis. The detection limits were calculated with 3 times signal-to-noise ratio, and the quantitation limits were calculated with 10 times signal-to-noise ratio. The linear equations, regression coefficients, detection limits and quantitation limits are shown in Table 4. As can be seen from the table, the peak areas of the 18 target substances had a good linear relationship with their mass concentrations, and the linear correlation coefficient R^2 of each substance was higher than 0.99.

Table 4 Linear equations and detection limits of 18 PFCs

Compound	Retention time//min	Linear equation	Correlation coefficient R^2	Detection limit//mg/kg	Quantitation limit//mg/kg
Perfluorobutyric acid (PFBA)	3.34	$y = 106.298 \times x + 45.733 5$	0.996	0.05	0.20
Potassium perfluorobutanesulfonate (PFBS)	4.35	$y = 346.428 \times x + 247.33$	0.995	0.005	0.020
Perfluoropentanoic acid (PFPeA)	4.00	$y = 94.133 8 \times x + 23.881 2$	0.995	0.01	0.03
Perfluorohexanoic acid(PFHxA)	4.30	$y = 176.482 \times x - 4.573 9$	0.996	0.01	0.03
Sodium perfluorohexanesulfonate (PFHxS)	4.77	$y = 165.41 \times x + 106.802$	0.997	0.01	0.03
Perfluoroheptanoic acid (PFHpA)	4.51	$y = 95.350 9 \times x - 24.837 1$	0.992	0.01	0.03
Perfluoroheptanesulfonic acid (PFHpS)	4.93	$y = 492.05 \times x + 100 3.66$	0.994	0.005	0.020
Perfluorooctanoic acid (PFOA)	4.68	$y = 237.629 \times x + 97.214 6$	0.994	0.005	0.020
Perfluorooctane sulfonic acid (PFOS)	5.08	$y = 703.799 \times x + 639.586$	0.995	0.01	0.03
Perfluorononanoic acid (PFNA)	4.85	$y = 184.514 \times x + 132.857$	0.995	0.05	0.20
Sodium perfluoro-1-nonanesulfonate (PFNS)	5.23	$y = 48.535 8 \times x - 22.615 8$	0.995	0.001	0.003
Perfluorodecanoic acid (PFDA)	5.01	$y = 70.433 9 \times x - 25.367 7$	0.995	0.001	0.003
Perfluorodecane sulfonic acid (PFDS)	5.37	$y = 756.554 \times x + 317.726$	0.996	0.01	0.03
Perfluoroundecanoic acid (PFUdA)	5.17	$y = 95.998 1 \times x + 88.843$	0.995	0.05	0.20
Perfluorododecanoic acid (PFDoA)	5.31	$y = 202.007 \times x + 52.592 1$	0.993	0.01	0.03
Perfluorooctadecanoic acid (PFODA)	6.03	$y = 254.162 \times x + 313.36$	0.994	0.01	0.03
N-Methylperfluorooctane sulfonamidoacetic acid (MeFOSA)	6.07	$y = 376.976 \times x - 114.317$	0.997	0.01	0.03
N-ethylperfluorooctane sulfonamidoacetic acid (EtFOSE)	6.20	$y = 1 473.62 \times x - 492.467$	0.996	0.005	0.020

Accuracy of method

The mixed standard solution of the 18 kinds of PFCs was accurately added to the blank milk tea sample for standard recovery test, and the recovery and precision of the method were investigated. The spiked concentrations were divided into three levels: low (2 μg/kg), medium (10 μg/kg) and high (20 μg/kg), and the recovery values were between 72.8% and 110.5%. The recovery data are shown in Table 5.

Table 5 Recovery data of 18 PFCs

Compound	Spiked concentration 0.5 mg/kg	Spiked concentration 2.0 mg/kg	Spiked concentration 5.0 mg/kg
Perfluorobutyric acid (PFBA)	88.1	81.0	110.5
Potassium perfluorobutanesulfonate (PFBS)	89.8	90.3	89.7
Perfluoropentanoic acid (PFPeA)	72.8	91.4	80.5
Perfluorohexanoic acid (PFHxA)	89.5	84.5	86.7
Sodium perfluorohexanesulfonate (PFHxS)	99.8	88.6	90.6
Perfluoroheptanoic acid (PFHpA)	83.6	105.1	88.0
Perfluoroheptanesulfonic acid (PFHpS)	86.4	88.9	99.1
Perfluorooctanoic acid (PFOA)	82.0	99.5	101.1
Perfluorooctane sulfonic acid (PFOS)	88.3	102.8	86.7
Perfluorononanoic acid (PFNA)	85.9	95.6	88.0
Sodium perfluoro-1-nonanesulfonate (PFNS)	84.8	93.9	89.8
Perfluorodecanoic acid (PFDA)	99.3	94.0	89.2
Perfluorodecane sulfonic acid (PFDS)	99.0	86.9	92.4
Perfluoroundecanoic acid (PFUdA)	91.4	87.8	92.3
Perfluorododecanoic acid (PFDoA)	91.8	96.2	94.9
Perfluorooctadecanoic acid (PFODA)	85.5	99.4	104.9
N-Methylperfluorooctane sulfonamidoacetic acid (MeFOSA)	86.8	90.6	85.0
N-ethylperfluorooctane sulfonamidoacetic acid (EtFOSE)	99.0	86.3	87.9

Analysis of actual samples

Three kinds of milk tea samples of different brands were randomly purchased, and the PFCs were detected by the established mature detection method. The results showed that the 18 perfluorinated and polyfluorinated compounds were all not detected.

Conclusions and Discussion

In this study, an LC-MS/MS method was established for the simultaneous analysis of 18 PFCs in milk. The samples were first subjected to precipitation of proteins by potassium ferrocyanide solution and zinc acetate solution, and then extracted by acetonitrile for the detection by LC-MS/MS. The detection limits of the method were in the range of 0.001 – 0.05 μg/kg, and the quantitation limits were in the range of 0.03 – 0.20 ng/L. The recovery values ranged from 72.8% to 110.5%. The results showed that the method has high sensitivity and accuracy. This study provides a rapid, accurate and reliable analysis method for the determination of PFCs in milk tea, which has strong practical value.

References

[1] LIU Y, GAO XH, ZHOU Y, *et al.* Review on pollution characteristics and removal technologies of perfluorinated and polyfluorinated compounds in China[J]. Journal of Qinghai Environment, 2023(4): 033. (in Chinese).

[2] ZHANG Q, WANG JJ, LIN JZ, *et al.* Pollution level and health risk assessment of perfluorinated compounds in water and four kinds of foods [J]. Studies of Trace Elements and Health, 2024, 41(3): 45 –47. (in Chinese).

[3] SONG YM, ZHOU LN, HAO WL, *et al.* Pollution status and related research progress of perfluorinated compounds[J]. Environmental Engineering, 2017, 35(10): 5. (in Chinese).

[4] ZHANG M, LOU QT, SHAO QW, *et al.* Research progress of perfluorinated compounds pollution status and risk assessment[J]. Asian Journal of Ecotoxicolog, 2019, 14(3): 24. (in Chinese).

[5] WANG ZX, YAO Q, QIN KL, *et al.* Research progress of perfluorinated compounds pollution status and risk assessment[J]. Journal of Environmental and Occupational Medicine, 2020(11): 037. (in Chinese).

[6] WU SH, ZHAO N, CAO SY, *et al.* Per- and polyfluorinated compounds-analytical methodssample pretreatmentdeterminationenvironmental media [J]. Environmental Protection and Circular Economy, 2023, 43(9): 66 –71. (in Chinese).

[7] TANG YX, BAI YM, LIU LL, *et al.* Simultaneous determination of 21 perfluorinated compounds in animal-derived food by UPLC-MS/MS[J]. Food & Machinery, 2023, 39(7): 29 –39. (in Chinese).

[8] LIANG ZY, ZHANG QY, ZHOU X, *et al.* Determination of perfluorinated and polyfluoroalkyl substances in cosmetics by ultra high performance liquid chromatography-tandem mass spectrometry[J]. Journal of Instrumental Analysis, 2023, 42(11): 1413 –1423. (in Chinese).

[9] CHEN X. Determination of two perfluorinated compounds in infant formula milk powder by LC-MS/MS[J]. The Food Industry, 2021(7): 320 – 322. (in Chinese).

[10] WANG J, ZHANG ZH, MAI XX, *et al.* Determination of the migration of 27 perfluorinated compounds in food contact materials by high performance liquid chromatography-tandem mass spectrometry[J]. Journal of Instrumental Analysis, 2018, 37(5): 6. (in Chinese).

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[5] ANASTASSIAEDS M, LEHOTAY SJ, STAJNBAHER D, *et al.* Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in product [J]. J AOAC Int, 2003, 86(2): 412 –431.

[6] DENG HL, CHEN Y, XU SL, *et al.* Research progress on the status and detection methods of pesticide residues in grapes and their products[J]. Chinese Journal of Health Laboratory Technology, 2022, 32(9): 1039 – 1042. (in Chinese).

[7] LUO X. Establishment of analytical method for multiple pesticide residues in Chinese chives and study on matrix effects[D]. Zibo: Shandong University of Technology, 2022. (in Chinese).

[8] LIU YW. Application of QuEChERS in analysis of multiple pesticide residues in medicinal plants[D]. Beijing: Chinese Academy of Agricultural Sciences, 2011. (in Chinese).

[9] LI YL. Detection of pesticide residues in fruits and vegetables by improved QuEChERS-GCMS/MS method combined with magnetic nanoparticles[M]. Tai'an: Shandong Agricultural University, 2015. (in Chinese).

[10] DONG J, PAN YX, ZHU LP, *et al.* Improvements and applications of the QuEChERS method in multi-residue analysis of 54 pesticides in vegetables and fruits[J]. Journal of Instrumental Analysis, 2008, 27(1): 66 –69. (in Chinese).

[11] SHAO H, LIU S, YANG M, *et al.* Determination of pesticide residues in vegetables by graphitized carbon black dispersion solid phase extraction-gas chromatography-mass spectrometry[J]. Agricultural Quality & Standards, 2008(3): 43 –45. (in Chinese).

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU