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Simultaneous Determination of 20 Polyfluoroalkane Substances in Dietary Milk by QuEChERs

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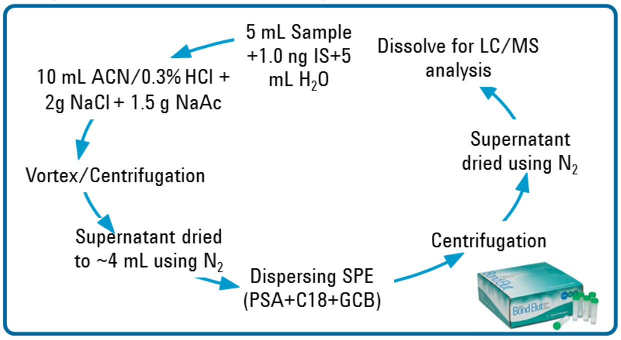
Introduction

Perfluorinated alkaylated substances (PFASs) are one class of emerging organic contaminants, which are featured by unique structural characteristics with hydrophobic polyfluorinated alkane chain in one end and the hydrophilic functional group in the other such as carboxyl and sulfonate groups. PFASs are highly stable and transportable over long distance, and they are extremely difficult to biodegrade over time in the environment[1]. The ubiquitous presence of PFASs in the natural environment has raised increasing concern for their long-term impacts to human health and natural living beings[2-3]. This study aims to establish a highly sensitive and reliable method to simultaneously monitor the levels of PFASs in food, particularly in milk[4].

Sample Extraction and Cleanup



5 mL Sample +1.0 ng IS+5 mL H_2O

10 mL ACN/0.3% HCl + 2g NaCl + 1.5 g NaAc

Vortex/Centrifugation

Supernatant dried to ~4 mL using N_2

Dispersing SPE (PSA+C18+GCB)

Centrifugation

Supernatant dried using N_2

Dissolve for LC/MS analysis

LC Conditions

Agilent Infinity 1290 UHPLC with built-in degasser
Autosampler with temperature control
Column temperature control compartment.
Pre-Column: Zorbax Eclipse C18, 50 × 4.6 mm, 5.0 µm;
Column: Poroshell 120 Eclipse C18, 100 × 2.1 mm, 2.7 µm
Column Temperature: 30°C
Mobile Phase:
Solvent A–5 mM NH_4Ac in H_2O;
Solvent B– 5 mM NH_4Ac in MeOH;
Flow rate: 0.2 mL/min; Injection volume: 5.0 µL
Needle backflush: 5 sec with pure methanol
Gradient elution profile:

Time	Sol. A (%)	Sol. B (%)
0	90	10
3	30	70
13	0	100
14	90	10
20	90	10

Experimental

ESI-QQQ MS Conditions

Agilent 6460 QQQ with JetStream ESI;
Polarity: negative;
Scanning: multiple reactions monitoring (MRM);
Drying gas (N_2) temperature: 300°C;
Drying gas flow rate: 6 L·min^{-1};
Nebulizer gas (N_2) pressure: 45 psi;
Sheath gas temperature: 260°C
Sheath gas flow rate: 11 L·min^{-1};
Capillary voltage: 3500 V;
Nozzle voltage: 500 V;
Software: MassHunter B.05

Table 1. MRM Parameters for MS Detection (Partial)

Analyte	Parent ion *m/z*	Product ion *m/z*	CE/V	Frag. Vol./V
PFBA	213	169*/-	1*	70
PFPA	263	219*/-	1*	70
PFHxA	313	269*/119	1*/9	70
PFHpA	363	319*/169	1*/8	90
PFOA	413	369*/169	2*/9	90
PFNA	463	419*/219	3*/9	110
PFDA	513	469*/269	2*/10	120
PFUnDA	563	519*/319	2*/11	120
PFDoDA	613	569*/169	3*/15	140
PFTrDA	663	619*/169	3*/14	140
PFTeDA	713	669*/269	4*/18	140
PFHxDA	813	769*/169	4*/19	150
PFOcDA	913	869*/219	6*/23	150

*: Quantitative MRM transition

Results and Discussion

Method Optimization

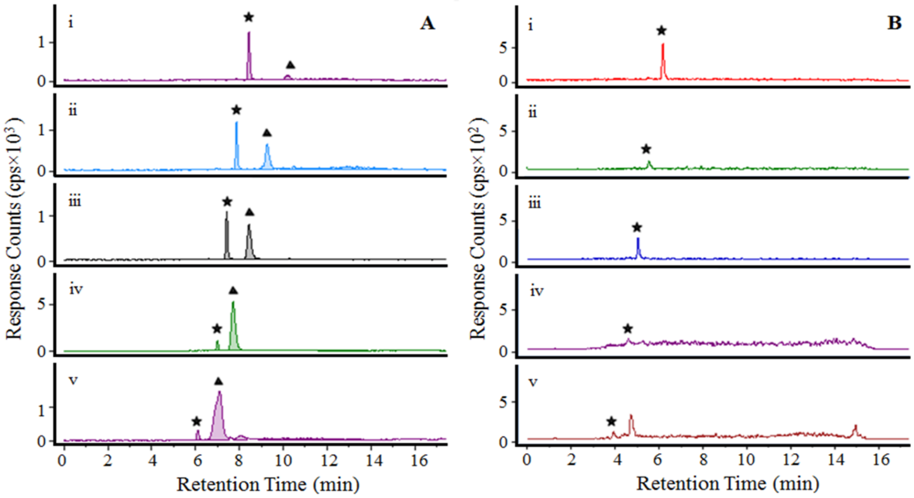


Fig 1. The MRM chromatograms demonstrating the separation of target compounds from the background interference.

A.With trapping column
B.Without trapping column

★ target compound
▲ interference
i. PFOA
ii. PFHpA
iii. PFHxA
iv. PFPA
v. PFBA

Separation and Detection. Interference from LC system can decrease the accuracy of PFASs measurement. To remove the interference from the LC system, a short column was inserted between solvent mixer and the autosampler to trap the interference. As shown in Fig 1A, with the selected trapping column, the interference from the LC system was delayed to elute out of column, and the analytes can be detected with high sensitivity. In comparison, without the trapping column, the same amount of analytes showed very low response (Fig 1B). With such a trapping column, a typical chromatogram for separation of 20 PFASs can be achieved as shown in Fig 2.

Extraction and Cleanup. QuEChERS procedure was applied for milk sample extraction and cleanup. Initially, three solvents including pure ACN, acidified ACN with HCl or formic acid were selected for extraction, and 0.3% HCl acidified ACN showed overall highest recovery for all PFASs (Fig 3). Furthermore, various amount of PSA and C18 sorbents were examined for cleanup. As shown in Fig 4A and 4B, 60 mg PSA and 40 mg C18 showed the highest recovery for most PFASs, respectively, and were thus combined together for cleanup. In addition, 10 mg GCB was added to the sorbents to remove the slight pigment.

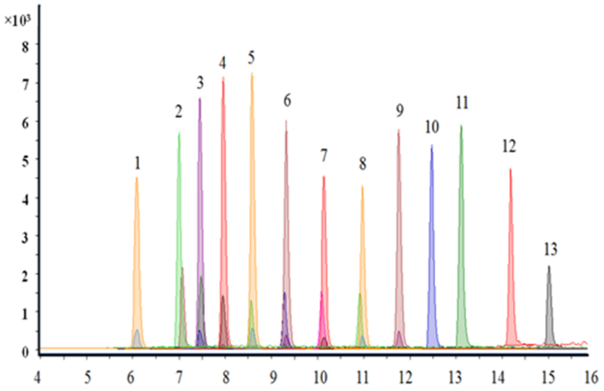


Fig 2. The overlapped MRM chromatograms for the 20 PFASs under the optimized LC-MS/MS conditions.

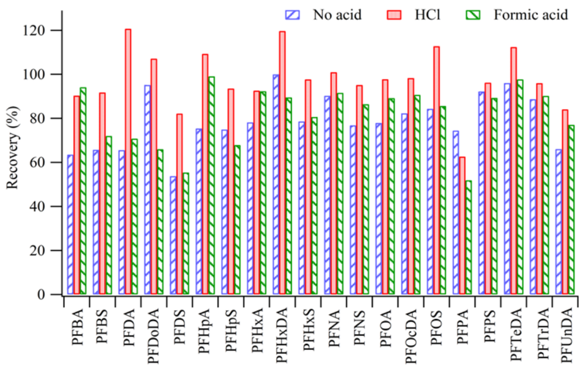


Fig 3. Extraction efficiency using different extraction solvents

Results and Discussion

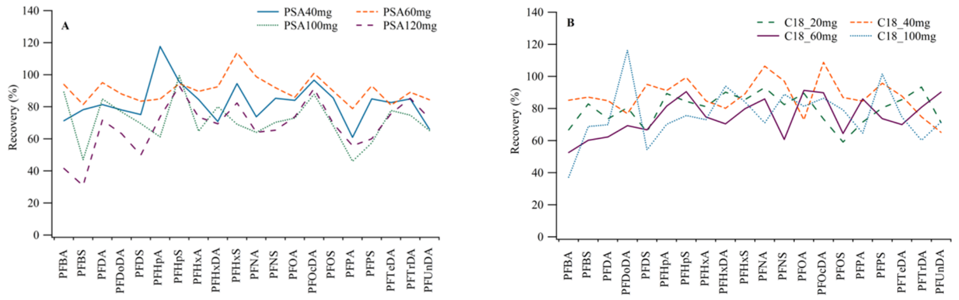


Fig. 4. Cleanup efficiency using various amounts of sorbents.

A. PSA
B. C18

Method Linearity and Sensitivity. Twenty PFASs showed excellent linearity, with $\gamma^2 \geq 0.997$ (Table 1). The LODs and LOQs for 13 PFCAs were 0.0030 $\mu g \cdot L^{-1}$ and 0.010 $\mu g \cdot L^{-1}$, respectively; and those for 7 PFSAs were 0.010 $\mu g \cdot L^{-1}$ and 0.050 $\mu g \cdot L^{-1}$, respectively (Table 2).

Accuracy and Precision. At three spiking levels including LOQs, the average recoveries for PFCAs were within 78.5%-111.1% with RSD within 1.2-13.1% (n=6); and those for PFSAs were 72.8-104.9% with RSD <15% (n=6). The distribution of recoveries for 20 PFASs were shown in Fig 5.

Table 2. Linearity and sensitivity of 20 PFASs using the developed method

CPD	Linear equation	r^2	LOD ($\mu g \cdot L^{-1}$)	LOQ ($\mu g \cdot L^{-1}$)	CPD	Linear equation	r^2	LOD ($\mu g \cdot L^{-1}$)	LOQ ($\mu g \cdot L^{-1}$)
PFBA	y=1.0832x+0.0840	0.9992	0.0030	0.010	**PFTeDA**	y=2.2169x-0.0877	0.9984	0.0030	0.010
PFPA	y=1.1292x+0.1905	0.9995	0.0030	0.010	**PFHxDA**	y=1.3530x+0.0682	0.9991	0.0030	0.010
PFHxA	y=1.3487x+0.1783	0.9996	0.0030	0.010	**PFOcDA**	y=0.7745x+0.0331	0.9983	0.0030	0.010
PFHpA	y=1.4805x+0.1522	0.9991	0.0030	0.010	**PFBS**	y=1.3518x-0.0647	0.9975	0.0030	0.010
PFOA	y=1.7161x+0.0583	0.9997	0.0030	0.010	**PFPS**	y=1.3764x+0.0438	0.9994	0.010	0.050
PFNA	y=1.7989x+0.1209	0.9989	0.0030	0.010	**L-PFHxS**	y=1.0978x+0.2054	0.9980	0.010	0.050
PFDA	y=9.8660x+1.8285	0.9994	0.0030	0.010	**L-PFHpS**	y=1.1167x-0.0016	0.9999	0.010	0.050
PFUnDA	y=1.6208x+0.0636	0.9999	0.0030	0.010	**L-PFOS**	y=1.5211x+0.0652	0.9996	0.010	0.050
PFDoDA	y=1.5565x+0.0906	0.9989	0.0030	0.010	**L-PFNS**	y=1.2969x+1.3288	0.9994	0.010	0.050
PFTrDA	y=1.5234x+0.0611	0.9996	0.0030	0.010	**L-PFDS**	y=1.2589x+0.0561	0.9997	0.010	0.050

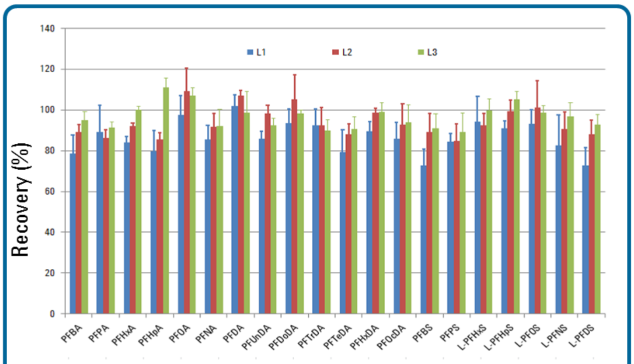


Fig 5. Spiking recoveries at three different levels in milk.

Conclusions

- QuEChERS combined with online interference trapping LC-MS/MS method was developed;
- The developed method has the advantages of low background, high sensitivity, high accuracy and precision, and wide dynamic linear range.
- The method can be applied for real milk sample analysis.

References:

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