Identification and Quantification of PFAS in Food Contact Materials using MRM^{HR} Workflow on X500R QTOF System

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In comparison to other surfactants, perfluorinated alkyl substances (PFAS) have stable physiochemical structures with hydrophobic and oleophobic properties. They are widely used in industrial and consumer products like plastic packaging materials for food and as coating in non-stick pans. Due to their chemical stability and low reactivity, PFAS are highly resistant to degradation even in living organisms and can therefore be accumulated in the food chain. Human exposure to PFAS residues has been implicated in incidences of cancer, obesity, endocrine system disruption and other adverse health effects. [1]

With the rapid growth in the food delivery industry in China (and globally) in the past two years, one-time-use plastic packaging materials are widely used by merchants due to their low cost and high durability [2]. One-time-use plastic has become a source of public concern and environmental pollution. Given the tremendous persistence of PFAS in the environment and the adverse effect on human health, monitoring of PFAS residue has gained traction in China and elsewhere.

In China, the level of PFOS and PFOA in food contact materials and products is regulated according to the latest National Food Safety Standard (GB 31604.35-2016). The detection limit is set at 1.0ng/g while the quantification limit is set at 2.0ng/g. In 2006, the European Union (EU) has set a regulation that the level of PFOS in finished products should not exceed 0.005% of the product mass.

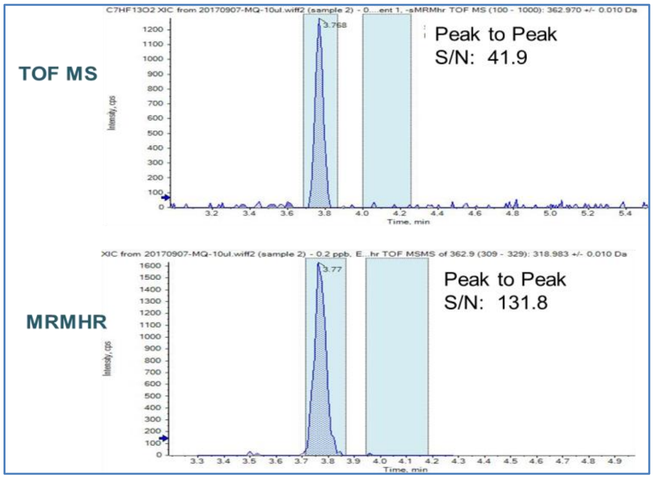


Figure 1. Signal-to-Noise Comparison of PFHpA using TOF-MS and MRM^{HR} Data Using a Post Spiked 0.2 ppb Matrix Blank. Monitoring the transition and the high resolution fragment ion results in greater specificity and reduced baseline, so signal-to-noise demonstrates marked improvement and method sensitivity is maximized.

The X500R QTOF system has the industry's fastest scanning speed, allowing for the implementation of the unique MRM^{HR} acquisition mode to provide excellent quantitative performance using high-resolution MS/MS data. This approach to quantitation with LC-QTOF-MS/MS minimizes matrix interferences and the patented Turbo V ion source with curtain gas interface, twin sprayer technology and built-in automatic calibration system help to improve and maintain instrument robustness and maintain high mass accuracy results. The high resolution MS/MS spectra can also be used for qualitative analysis by calculating the ion ratio for confirmation, thus reducing false positives by taking advantage of the data acquired on the LC-QTOF platform.

Key Workflow Advantages

- PFAS quantitation using an easily established method and minimal method development
- 10-minute run time using a Phenomenex Kinetex® C18 column demonstrates separation of PFAS targets
- MRM^{HR} workflow using MS/MS for selectivity vs high resolution TOF MS mode provides improved signal-to-noise
- QTOF technology can be utilized for quantitative analysis of PFAS suite without compromising method performance (excellent sensitivity, linearity demonstrated)

Methods

Sample Preparation: The food packaging material to be tested is cut into small pieces. For coating sample, scrape it with a small knife. The sample preparation procedure was adapted from National Standard of China (document number GB 31604.35-2016) which is implemented on 19 April 2017 (Figure 2).

A total of eight samples were collected as test samples which include disposable meal box, plastic bag, beverage bottle, coating of non-stick pan, etc. Packaging materials in the collected samples were mainly polyethylene, polystyrene and polytetrafluoroethylene.

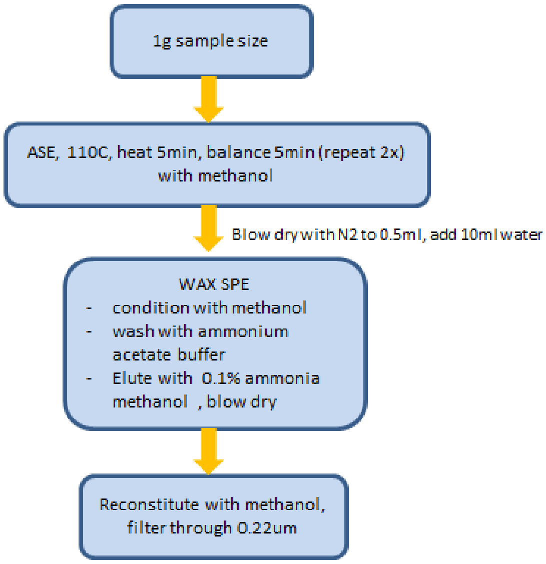


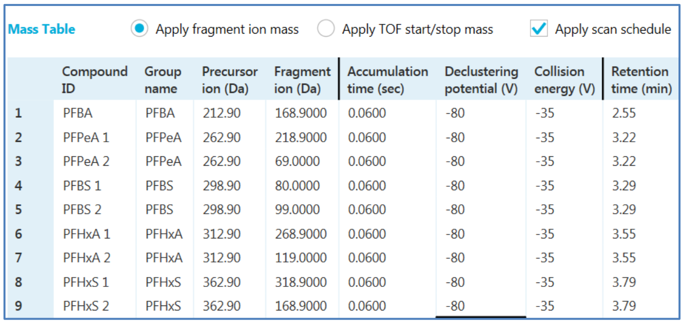
Figure 2. Extraction and Clean-up Process Flow Diagram.

Chromatography: Using the SCIEX ExionLC™ AD System with a Phenomenex Kinetex, 2.6µm C18, 100 X 2.0 mm, compounds were separated using a gradient elution with mobile phase A of 5mM NH_4AC in water and mobile phase B of 5mM NH_4AC in methanol (flow rate of 0.3 mL/min, column temperature 40 ºC).

Mass Spectrometry: The SCIEX X500R QTOF System was used analyse the compounds operating in negative ion polarity using the *Scheduled* MRM^{HR} acquisition mode (Table 1). Source conditions were as follows: CUR of 30psi; CAD of 7; IS of -4500V; Temp 500 ºC; GS1 of 50psi; GS2 of 55psi.

Data Processing: All data was processed with SCIEX OS Software.

Table 1. *Scheduled* MRM^{HR} Method Setup in SCIEX OS. Unique RTs can be defined for each transition for each analyte.



Mass Table ● Apply fragment ion mass ○ Apply TOF start/stop mass ☑ Apply scan schedule

	Compound ID	Group name	Precursor ion (Da)	Fragment ion (Da)	Accumulation time (sec)	Declustering potential (V)	Collision energy (V)	Retention time (min)
1	PFBA	PFBA	212.90	168.9000	0.0600	-80	-35	2.55
2	PFPeA 1	PFPeA	262.90	218.9000	0.0600	-80	-35	3.22
3	PFPeA 2	PFPeA	262.90	69.0000	0.0600	-80	-35	3.22
4	PFBS 1	PFBS	298.90	80.0000	0.0600	-80	-35	3.29
5	PFBS 2	PFBS	298.90	99.0000	0.0600	-80	-35	3.29
6	PFHxA 1	PFHxA	312.90	268.9000	0.0600	-80	-35	3.55
7	PFHxA 2	PFHxA	312.90	119.0000	0.0600	-80	-35	3.55
8	PFHxS 1	PFHxS	362.90	318.9000	0.0600	-80	-35	3.79
9	PFHxS 2	PFHxS	362.90	168.9000	0.0600	-80	-35	3.79

Establishing the *Scheduled* MRM^{HR} Quantitative Method

The SCIEX OS software is fully automated with a user-friendly interface, greatly reducing the time to establish the acquisition method. The MRM parameters can be set up easily in two different ways. For compounds which are in MS/MS spectral library, fragment ions can be imported easily from the library to build the MRM^{HR} method list. Up to 5 fragment ions can be imported at the same time using a single click. For compounds not found in the spectral library, spectra can be added easily to the library using TOF MS-IDA-MS/MS data acquired for standards of the desired targets.

MRM parameters like retention time, declustering potential (DP) and collision energy (CE) from an existing triple quadrupole method are fully transferrable.

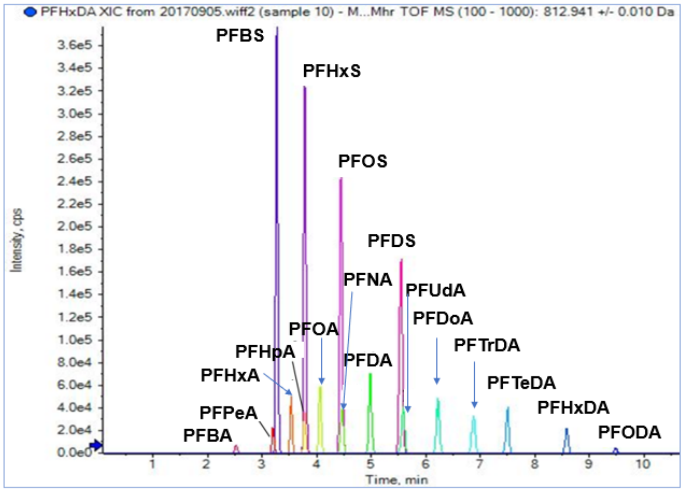


Figure 3. TOF MS Extracted Ion Chromatogram of 17 PFAS. Good separation was achieved for most of the 17 PFAS compounds analysed.

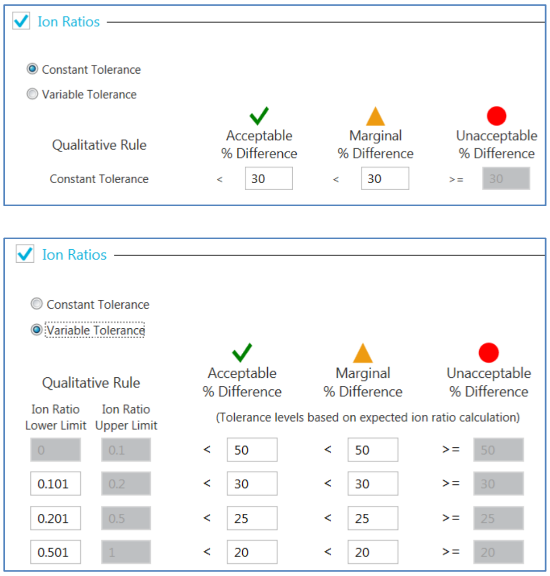
MRM^{HR} Quantitation of PFAS

Chromatogram of 17 PFAS utilizing extracted precursor ion data from TOF-MS scan are shown (Figure 3).

High Selectivity Data: Comparing 0.2 ppb post spiked in matrix blank, PFHpA show higher selectivity in MRM^{HR} mode as compared to TOF-MS mode for quantification (Figure 1). Monitoring the high resolution fragment ion from the full scan MS/MS data collected provides greater specificity and reduced baseline, so signal-to-noise demonstrates marked improvement and method sensitivity is maximized.

Linearity and Accuracy: The 17 monitored PFAS demonstrate good linearity and accuracy (Figure 4) with the correlation coefficients above 0.99. Accuracy values are within the permissible deviation range for LOD and LOQ according to the national standards.

Ion Ratio Calculations: Ion ratios can be easily calculated using the SCIEX OS software. Ion ratio confirmation can be visually displayed in the chromatogram and result table. Depending on the requirement, the confirmation tolerance can be defined using either constant tolerance or variable tolerance as shown in Figure 5.



☑ Ion Ratios

● Constant Tolerance
○ Variable Tolerance

Qualitative Rule	Acceptable % Difference	Marginal % Difference	Unacceptable % Difference
Constant Tolerance	< 30	< 30	>= 30

☑ Ion Ratios

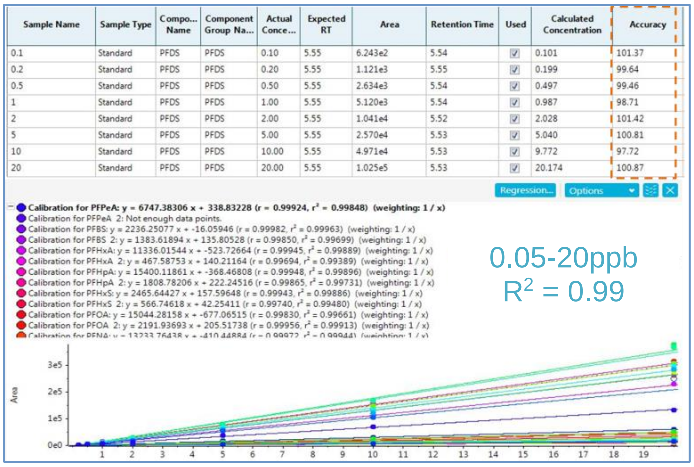
○ Constant Tolerance
● Variable Tolerance

Qualitative Rule

(Tolerance levels based on expected ion ratio calculation)

Ion Ratio Lower Limit	Ion Ratio Upper Limit	Acceptable % Difference	Marginal % Difference	Unacceptable % Difference
0	0.1	< 50	< 50	>= 50
0.101	0.2	< 30	< 30	>= 30
0.201	0.5	< 25	< 25	>= 25
0.501	1	< 20	< 20	>= 20

Figure 5. Setting up Tolerance for Ion Ratios Confirmation. Constant tolerance (same percent difference from measured standard ion ratio) or variable tolerance (varying percent difference dependant on concentration level) can be utilized when determining whether an unknown same meets the criteria for qualitative analyte identification by ion ratio confirmation. Different levels of percent difference can be defined by the user to be flagged as within “Acceptable,” “Marginal,” or “Unacceptable.”



Sample Name	Sample Type	Compo... Name	Component Group Na...	Actual Conce...	Expected RT	Area	Retention Time	Used	Calculated Concentration	Accuracy
0.1	Standard	PFDS	PFDS	0.10	5.55	6.243e2	5.54	☑	0.101	101.37
0.2	Standard	PFDS	PFDS	0.20	5.55	1.121e3	5.55	☑	0.199	99.64
0.5	Standard	PFDS	PFDS	0.50	5.55	2.634e3	5.54	☑	0.497	99.46
1	Standard	PFDS	PFDS	1.00	5.55	5.120e3	5.54	☑	0.987	98.71
2	Standard	PFDS	PFDS	2.00	5.55	1.041e4	5.52	☑	2.028	101.42
5	Standard	PFDS	PFDS	5.00	5.55	2.570e4	5.53	☑	5.040	100.81
10	Standard	PFDS	PFDS	10.00	5.55	4.971e4	5.53	☑	9.772	97.72
20	Standard	PFDS	PFDS	20.00	5.55	1.025e5	5.53	☑	20.174	100.87

Calibration for PFPeA: y = 6747.38306 x + 338.83228 (r = 0.99924, r² = 0.99848) (weighting: 1 / x)
Calibration for PFPeA 2: Not enough data points.
Calibration for PFBS: y = 2236.25077 x + -16.05946 (r = 0.99982, r² = 0.99963) (weighting: 1 / x)
Calibration for PFBS 2: y = 1383.61894 x + 135.80528 (r = 0.99850, r² = 0.99699) (weighting: 1 / x)
Calibration for PFHxA: y = 11336.01544 x + -523.72664 (r = 0.99945, r² = 0.99889) (weighting: 1 / x)
Calibration for PFHxA 2: y = 467.58753 x + 140.21164 (r = 0.99694, r² = 0.99389) (weighting: 1 / x)
Calibration for PFHpA: y = 15400.11861 x + -368.46808 (r = 0.99948, r² = 0.99896) (weighting: 1 / x)
Calibration for PFHpA 2: y = 1808.78206 x + 222.24516 (r = 0.99865, r² = 0.99731) (weighting: 1 / x)
Calibration for PFHxS: y = 2465.64427 x + 157.59648 (r = 0.99943, r² = 0.99886) (weighting: 1 / x)
Calibration for PFHxS 2: y = 566.74618 x + 42.25411 (r = 0.99740, r² = 0.99480) (weighting: 1 / x)
Calibration for PFOA: y = 15044.28158 x + -677.06515 (r = 0.99830, r² = 0.99661) (weighting: 1 / x)
Calibration for PFOA 2: y = 2191.93693 x + 205.51738 (r = 0.99956, r² = 0.99913) (weighting: 1 / x)
Calibration for PFNA: y = 13233.76438 x + -410.44884 (r = 0.99972, r² = 0.99944) (weighting: 1 / x)

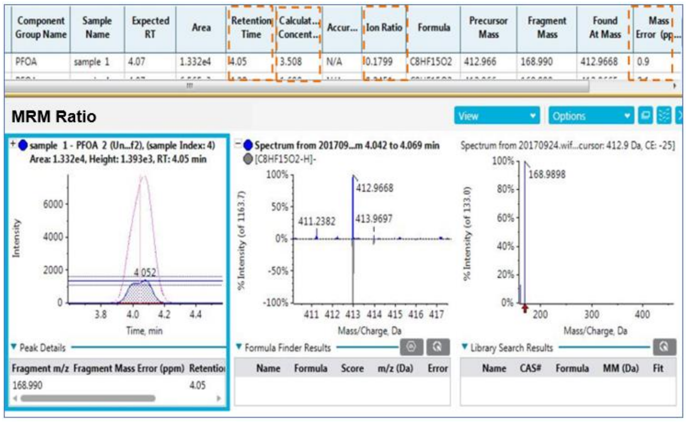
0.05-20ppb
$R^2 = 0.99$

Figure 4. Calibration Curve of 17 PFAS with Acceptable Accuracy and Linear Response.

Detection of PFAS in Food Contact Materials

SCIEX OS software combines both qualitative and quantitative results in one single interface (Figure 6). The result table show the retention time, concentration, peak area, ion ratio confirmation and the mass error of 0.9ppm for a sample tested positive with PFOA.

Among the eight samples, eight types of PFAS were detected as shown in Table 2. Two out of eight samples have levels which exceeded regulated level of 1ng/g by national standard. Most of the detected PFAS are the acid derivatives of PFOA and primarily found in non-stick pan coating and disposable meal boxes. The number of actual samples collected in this test is rather small; hence statistically it does not imply that all related products are unsafe for consumers.



Component Group Name	Sample Name	Expected RT	Area	Retention Time	Calculat... Concent...	Accur...	Ion Ratio	Formula	Precursor Mass	Fragment Mass	Found At Mass	Mass Error (pp...
PFOA	sample 1	4.07	1.332e4	4.05	3.508	N/A	0.1799	C8HF15O2	412.966	168.990	412.9668	0.9

MRM Ratio

sample 1 - PFOA 2 (Un...f2), (sample Index: 4)
Area: 1.332e4, Height: 1.393e3, RT: 4.05 min

Spectrum from 201709...m 4.042 to 4.069 min
[C8HF15O2-H]-

Spectrum from 20170924.wif...cursor: 412.9 Da, CE: -25]

Fragment m/z	Fragment Mass Error (ppm)	Retentio
168.990		4.05

Figure 6. PFOA Results in Actual Sample.

Table 2. PFAS Content in Different Food Contact Samples.

	Detected Amount (ng/g)							
	PFHxA	PFHpA	PFOA	PFDA	PFuDA	PFDoA	PFTrDA	PFTeDA
Meal box 1	*0.14*	*0.16*	*3.15*	-	-	-	-	-
Meal box 2	-	-	*3.12*	-	-	-	-	-
Plastic bag 1	-	-	-	-	-	-	-	-
Plastic bag 2	-	-	-	-	-	-	-	-
Drink bottle 1	-	-	-	-	-	-	-	-
Drink bottle 2	-	-	-	-	-	-	-	-
Non-stick pan 1	-	-	-	*0.11*	*0.15*	*0.13*	*0.15*	-
Non-stick pan 2	-	-	-	-	-	-	-	*0.17*

- Falls below the detection level of this method.

Summary

The SCIEX X500R QTOF system and SCIEX OS software brings powerful performance capabilities for routine testing of PFAS. The unique MRM^{HR} quantification method enables high selectivity even in real sample with matrix interference. This improves the detection and quantification of PFAS which can meet the EU regulation and national standards in China.

Although the concentration of PFAS in most of the test samples falls below the regulated level, the detection rate of perfluorinated alkyl substances is relatively high indicating that the quality of food contact/packaging materials may pose potential risks on consumer's health.

References

1. Xu R, Tan H, Yang H B, *et al*. (2014) *Food and Fermentation Industries*, **40(10)**: 205.
2. Kannan K, Tao L, Sinclair E, *et al.* (2005) *Arch Environ Con Tox*, **48**: 559.
3. GB 31604.35-2016, National Food Safety Standard - Food contact materials and products - Determination of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA).