

LC-MS/MS Analysis of Emerging Food Contaminants

Detection of Perfluoroalkyl Acids in Food and Food Packaging using the SCIEX QTRAP® 6500+ System

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Overview

The note describes an LC-MS/MS method using the SCIEX QTRAP® 6500+ LC-MS/MS system for the analysis of perfluoroalkyl acids (PFAA) in food and food packaging material. Limits of detection between 0.5 and 1 ng/L (parts-per-trillion, ppt) were achieved for different PFAA. Quantitation was performed with good linearity ($r > 0.99$) and repeatability (%CV < 10%). MRM ratios and QTRAP® MS/MS spectra were used for confident compound identification.

The developed method was successfully applied to the analysis of food and food packaging samples. Short chain perfluorocarboxylic acids ($C < 8$) were detected in different samples. We also observed the migration of PFBA from a cupcake wrapper into a cupcake.

Introduction

PFAA are useful anthropogenic chemicals that are widely used in consumer and industrial products. This class of compounds includes thousands of chemicals, including perfluorocarboxylic acids, such as perfluorooctanoic acid (PFOA), perfluorosulfonic acids, such as perfluorooctanesulfonic acid (PFOS), and perfluorophosphonic acids. Many of these compounds are toxic, resistant to degradation, bioaccumulate in the food chain and they are regularly found in the blood of animals and humans worldwide. Due to their persistence the production of PFOA and PFOS was phased out in 2000 and other substances with similar chemical properties, like short chain PFAA, are used instead.¹⁻³

PFAA are used to coat the surface of cookware and food packaging (non-stick coating) and can migrate into food, representing a potential source of human exposure.⁴⁻⁶

Another potential source for PFAA exposure to humans is the use of reclaimed water for irrigation of food crops. The dual hydrophobic/lipophobic nature of PFAA enables the compounds to reside in significant quantities in both the aqueous and sludge effluent streams of wastewater treatment plants. The aqueous effluent stream can be used for agricultural purposes. However, concerns have been raised regarding the presence of chemicals of emerging concern in reclaimed water. Recent studies showed that PFAA bioaccumulate in fruits and vegetables. Especially



short-chain perfluorocarboxylic acids were shown to accumulate in the edible portion of plants.⁷

In this paper we present a method using LC-MS/MS for the analysis in food and food packaging material to study migration. Samples were extracted using simple solvent extraction and analyzed using the SCIEX QTRAP® 6500+ system in negative polarity electrospray ionization (ESI).

Experimental

Standards

Standards of PFAA (#PFAC-MXB) and internal standards (#MPFAC-MXA) were obtained from Wellington Laboratories (Guelph, ON, Canada).

Food and food packaging material samples were obtained from local supermarkets.

Sample preparation

Homogenized food samples (2 g) were extracted with 10 or 20 mL methanol. Packaging material (10 cm²) was cut into small random pieces and extracted with 10 mL methanol. The extracts were centrifuged and diluted with water to minimize potential ion suppression.

LC Separation

LC separation was performed using a SCIEX ExionLC™ AC system with a Restek Raptor C18 (30 x 2 mm, 2.7 μm) column and a mobile phase gradient consisting of water and methanol with 5 mM ammonium formate (Table 1).

The injection volume was 10 μL.

Table 1. LC gradient conditions

Time (min)	Flow Rate (mL/min)	A (%)	B (%)
0.0	1.0	70	30
0.5	1.0	70	30
1.5	1.0	25	75
6.0	1.0	5	95
7.0	1.0	5	95
7.1	1.0	70	30
10.0	1.0	70	30

A second Restek Raptor C18 column of identical dimension was installed between the LC mixer and the injector valve of the autosampler to trap PFAA background from the LC system.

MS/MS Detection

The SCIEX QTRAP® 6500+ system with IonDrive™ source was operated using an ESI probe in negative polarity. The MRM transitions monitored are listed in Table 2.

The *Scheduled* MRM™ Pro algorithm was activated to automatically optimize dwell times for each transition to achieve best data quality. A target cycle time of 300 msec and compound-dependent detection windows were used.

At least two transitions were monitored for each compound (except for PFBA and PFPeA). Identification was achieved using the ratio of quantifier and qualifier ion. In addition, information dependent acquisition (IDA) was used to acquire MS/MS spectra for mass spectral library searching.

Ion source parameters were set to the following values: CUR = 30 psi; Gas1 = 50 psi; Gas2 = 60 psi; TEM = 400°C; and IS = -4500 V. The collision gas (CAD) was set to 'medium'.

Table 2. Retention times (RT) and MRM transitions of perfluorocarboxylic acids (PFBA to PFOcDA) and perfluorosulfonic acids (PFBS to PFDS)

Compound	#C	RT (min)	Q1	Q3
PFBA	4	0.35	213	169
PFPeA	5	0.92	263	219
PFHxA	6	1.69	313	269, 119
PFHpA	7	1.90	363	319, 169, 119
PFOA	8	2.04	413	369, 169, 219
PFNA	9	2.15	463	419, 219, 169
PFDA	10	2.24	513	469, 219, 269
PFUnDA	11	2.36	563	519, 269, 219
PFDoDA	12	2.52	613	569, 269, 319
PFTTrDA	13	2.75	663	619, 219, 169
PFTeDA	14	3.05	713	669, 219, 169
PFHxDA	16	3.70	813	769, 269, 219
PFOcDA	18	4.38	913	869, 219, 319
PFBS	4	1.26	299	80, 99
PFHxS	6	1.91	399	80, 99
PFOS	8	2.14	499	80, 99
PFDS	10	2.34	599	80, 99

Results and Discussion

An example chromatogram is shown in Figure 1.

Low chemical background was achieved by using a trap column installed between LC pumps and autosampler.

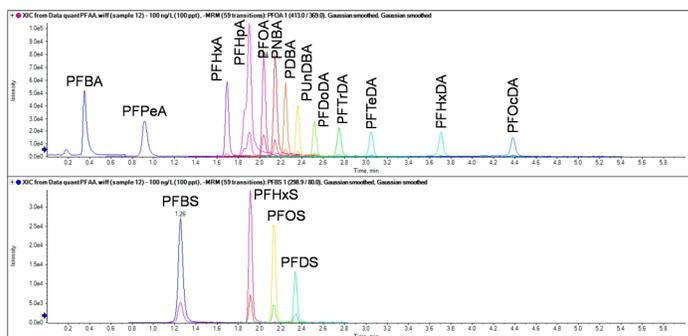


Figure 1. Example chromatogram of a 0.1 ng/mL (100 ppt) standard, perfluorinated carboxylic acids (top) and perfluorosulfonic acids (bottom)

Limits of detection (LOD) between 0.5 and 2 ng/L (ppt) were achieved. Good linearity was observed for all compounds with coefficient of regression >0.99 and accuracy between 80 and 120%.

Triplicate injections were performed at 1, 10, 100, and 1000 ng/L. Coefficient of variation (%CV) was typically below 10%. Compounds with available internal standard showed slight better repeatability and had a wider linear dynamic range for quantitation.

Compound identification was achieved using the ratio of quantifier and qualifier MRM transition. MultiQuant™ software (version 3.0.2) automatically calculates ion ratios, displays tolerance levels and flags outliers in the result table. An example for the 1 ng/L standard of PFOA and PFOS is shown in Figure 2.

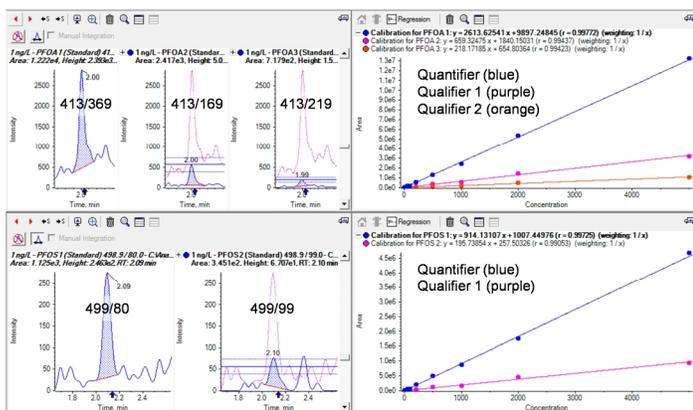


Figure 2. Peak review of 1 ng/L with MRM ratio tolerances of 30% for PFOA (top) and PFOS (bottom) and calibration lines from 1 to 5000 ng/L with good regression of > 0.99 for all MRM transitions

The QTRAP® 6500+ system also allows the acquisition of full scan MS/MS spectra through information dependent acquisition (IDA). In such an IDA method MS/MS spectra are automatically acquired once an MRM in the survey scan exceeds a user-defined threshold. Dynamic background subtraction (DBS) further helps to trigger MS/MS spectra in case of co-eluting compounds. For the MS/MS scan standardized collision energies of CE = -35 V with a spread (CES) of 15 V were used. MS/MS library searching to enhance confidence in compound identification can automatically be performed in MasterView™ software version 1.1 (Figure 3).

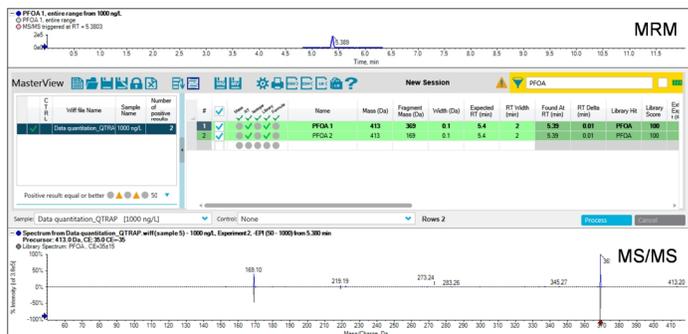


Figure 3. Identification of PFOA based on retention time matching and MS/MS library searching in MasterView™ software

Application of the Developed Method to Samples of Food and Food Packaging

The method was applied to the analysis of PFAA in food and food packaging samples using a slower (10 min) gradient and a Restek Raptor C18 (50 x 2 mm) column.

Only perfluorocarboxylic acids shorter than C8 were detected. The example presented below shows the findings of PFBA, PFPeA, PFHxA, PFHpA in packaging material, coated paper bag of corn chips (Figure 4a). We also analyzed the cupcake wrappers and the cupcake baked in it. The wrapper contained 0.42 ng/cm² and the cupcake 2.65 ng/kg PFBA.

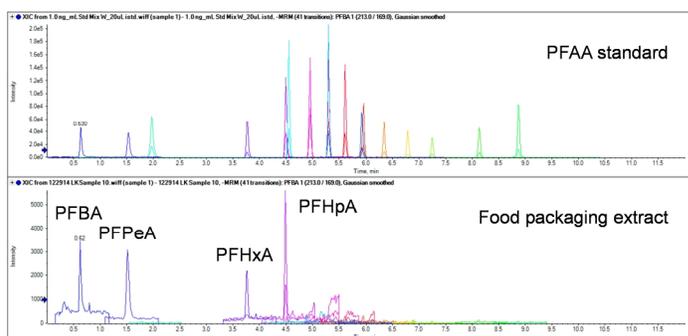


Figure 4a. Results of PFAA findings packaging material of corn chips (bottom) in comparison to a PFAA standard using the longer LC gradient (top)

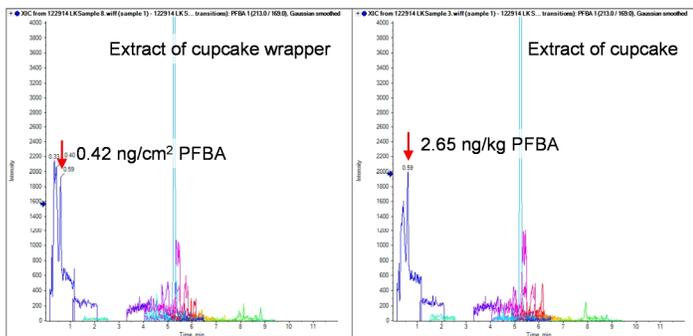


Figure 4b. Migration of PFBA from a cupcake wrapper (left) into a home-baked cupcakes (right)

Summary

In this note we presented a highly sensitive LC-MS/MS method for the analysis of PFAA in food and food packaging material to study migration. Samples were extracted using simple solvent extraction and analyzed using the SCIEX QTRAP® 6500+ system. The MS/MS was operated in MRM mode, enabling limits of quantitation at low or sub 1 ng/L (parts-per-trillion, ppt) Good accuracy, repeatability, and linearity for quantitation were achieved over 3 orders of magnitude.

Short-chain PFAA were detected in different food and food packaging samples. We also observed the migration of PFBA.

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