

Determination of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) in paper-based food packaging samples

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Abstract

In this study, a method for simultaneous determination of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in paper-based food packaging was validated. The paper samples were extracted with ethanol/water (1 : 1, v/v) mixture at 70°C for 2 h by using an incubator shaker. The extracts were passed through 0.2 µm filters before LC-MS/MS analysis. The method detection limit and method quantification limit of both PFOA and PFOS were 0.1 and 0.3 ng/g, respectively. Correlation coefficients > 0.99 were obtained over concentration ranges from 0.3 to 10 ng/mL. The method recovery ranged from 100 - 106% with good repeatability (RSD < 5%). The method was applied to analyze 23 paper samples, however, PFOA and PFOS were not detected in any of these samples.

Keywords: PFOA, PFOS, paper-based food packaging, LC-MS/MS.

1. INTRODUCTION

PFOA and PFOS belong to perfluoroalkyl substances (PFASs), which have been used in many industrial fields as stainproof furniture, carpets, clothing, firefighting foam, coating materials, and cosmetics, because of their oil-resistant and waterproof properties [1-3]. Due to features of PFASs such as extreme resistance to degradation even at high temperatures and resistance to water and oil, these compounds have been widely used in food contact materials such as non-stick kitchen utensils and food-contact materials [4]. PFOA and PFOS are very stable in the environment and bio-accumulative, so they are classified as persistent

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organic pollutants (POPs) under the Stockholm Convention [3]. PFOA and PFOS can damage livers and other organs, and cause immune disruption, endocrine effects, and reproductive harm in animal studies. Moreover, PFOA can cause liver, pancreatic, testicular, and mammary gland tumors in laboratory animals. In 2006, the US Environmental Protection Agency (US EPA) classified PFOA as a potential human carcinogen [5]. In 2020, the European Union published the Commission Delegation Regulation (EU) 2020/784 amending Annex I to Regulation (EU) 2019/1021 of the European Parliament and of the Council as regards the listing of PFOA, its salts, and PFOA-related compounds. This regulation sets concentrations of PFOA or any of its salts equal to or below 0.025 mg/kg; PFOA-related compounds or a combination of PFOA-related compounds equal to or below 1 mg/kg in substances, mixtures, or articles [6].

PFOA and PFOS have been detected in many kinds of food and beverages such as fish, meat, offal, egg, cracker, chip, cake, chocolate, vegetable, milk, and juice [5]. These compounds have also been found in food-contact materials such as popcorn bags [7], oil-resistant food packaging [1], ice cream cups, fast food wrappers for sandwiches, non-stick baking papers, muffin cups, aluminum foil bags [3, 8]. The occurrence of these pollutants in foods and food-contact materials implies considerable exposure risk in humans. In Vietnam, PFOS, PFOA, and other PFASs have been detected in fish samples [9-10]. However, to our knowledge, there is no study investigating the presence of PFASs in food-contact materials. In this work, an analytical method for quantification of PFOA and PFOS in paper-based food packaging using liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed. The validated method was then applied to analyze PFOA and PFOS concentrations in several paper packaging samples, providing preliminary and updated information about the occurrence of these concerning chemicals in food packaging materials in Vietnam.

2. MATERIALS AND METHOD

2.1. Chemicals and reagents

Perfluorooctanoic acid (PFOA) was purchased from Sigma-Aldrich and Perfluorooctane sulfonic acid (PFOS) was obtained from Dr Ehenstorfer. Ethanol, formic acid, methanol, acetonitrile, and ammonium formate were obtained from Merck (Germany). Minisart[®] syringe filters were purchased from Sartorius (Germany).

The stock solutions of PFOA and PFOS (1 mg/mL each compound) were prepared in methanol from solid standards. The stock solutions were diluted to 50 ng/mL to prepare a working standard solution. A five-point calibration curve of PFOA and PFOS were prepared in ethanol/water (1:1, v/v) at concentration 0.3, 0.50, 1.0, 5.0, and 10.0 ng/mL.

2.2. Sample preparation

Twenty-three paper packaging samples were collected from different markets in Cau Giay District, Hanoi, including 5 cupcake papers, 5 fast-food containers, and 13 oil proof

papers. All samples were new packages and had not contacted food products. Before analysis, the samples were cut to size approximately 1 cm² with scissors. After cutting, sample pieces with printed surface were discarded, and only plain pieces were subjected for analysis.

Each sample was weighed about 1.00 ± 0.1 g into a 15 mL polypropylene centrifuge tube, and 10 mL of ethanol/water (50 : 50, v/v) mixture was added. The sample tube was placed in a GyromaxTM incubator shaker (Amerex Instruments, Inc., USA) and shaken at 70 °C for 2 h. Then, the tube was left to cool down at room temperature. The extract solution was further filtered through a 0.2 µm syringe filter, transferred into an auto-injector vial, and analyzed by LC-MS/MS.

2.3. Instrumental analysis

In this study, PFOA and PFOS were analyzed on a SCIEX 6500 Triple Quad instrument using negative electrospray ionization (ESI) at multiple reaction monitoring mode (MRM). The analytes were separated on an Agilent Eclipse plus C18 column (150 × 2.1 mm, 3.5 µm). The mobile phase included methanol (A) and 1% formic acid in ultrapure water (B). The chromatographic gradient conditions were: initial 30% B for 1.5 min, increased from 30 to 100% A in 3 min, maintained for 3 min; then decreased to 30% B in 2 min and maintained for 2 min. The injection volume was 2 µL and the flow rate was set at 0.5 mL/min. The MS/MS conditions: capillary voltage 4,500 V, nitrogen curtain gas at 35 psi, nitrogen collision gas at 8 psi, and capillary temperature at 350°C. The mass transitions for identification and quantification of PFOS and PFOA were 499 → 98.9, 499 → 79.9 and 413 → 369, 413 → 169, respectively.

3. RESULTS AND DISCUSSION

3.1. Optimizing of chromatography conditions

An Agilent Eclipse Plus C18 (150 × 2.1 mm, 3.5 µm) column was used to separate PFOS and PFOA. Some elution programs were tested to optimize the mobile phase, including (1) acetonitrile and 0.1% formic acid; (2) methanol and 0.1% formic acid, 10 mM ammonium formate; and (3) methanol and 0.1% formic acid. Peak areas of PFOA and PFOS at 10 ng/mL PFOA and PFOS standard solutions obtained by different mobile phases were presented in Table 1.

Table 1. Chromatography area of PFOS, PFOA with different mobile phases

<i>Mobile phase</i>	<i>PFOS</i>	<i>PFOA</i>
<i>Acetonitrile: 0.1% formic acid</i>	No peak	314000
<i>Methanol: 0.1% formic acid, 10 mM ammonium formate</i>	75700	652000
<i>Methanol: 0.1% formic acid</i>	2680000	2220000

As shown in Table 1, signals of PFOS were not observed with mobile phase comprising acetonitrile and 0.1% formic acid. The mobile phase of methanol and 0.1% formic acid provided much greater peak areas of both PFOS and PFOA as compared to those obtained by similar mobile phase with addition of 10 mM ammonium formate. Therefore, the mixture of methanol and 0.1% formic acid was selected for further experiments.

3.2. Method validation

The analytical method of PFOA and PFOS was validated for specificity, linearity, limits of detection and quantification, repeatability, and recovery.

3.2.1. Specificity

The blank sample, spiked sample, and standard solution were analyzed to evaluate method specificity (Figure 1). Each compound was identified by 1 parent and 2 daughter ions, corresponding to identification points (IPs) of 5.

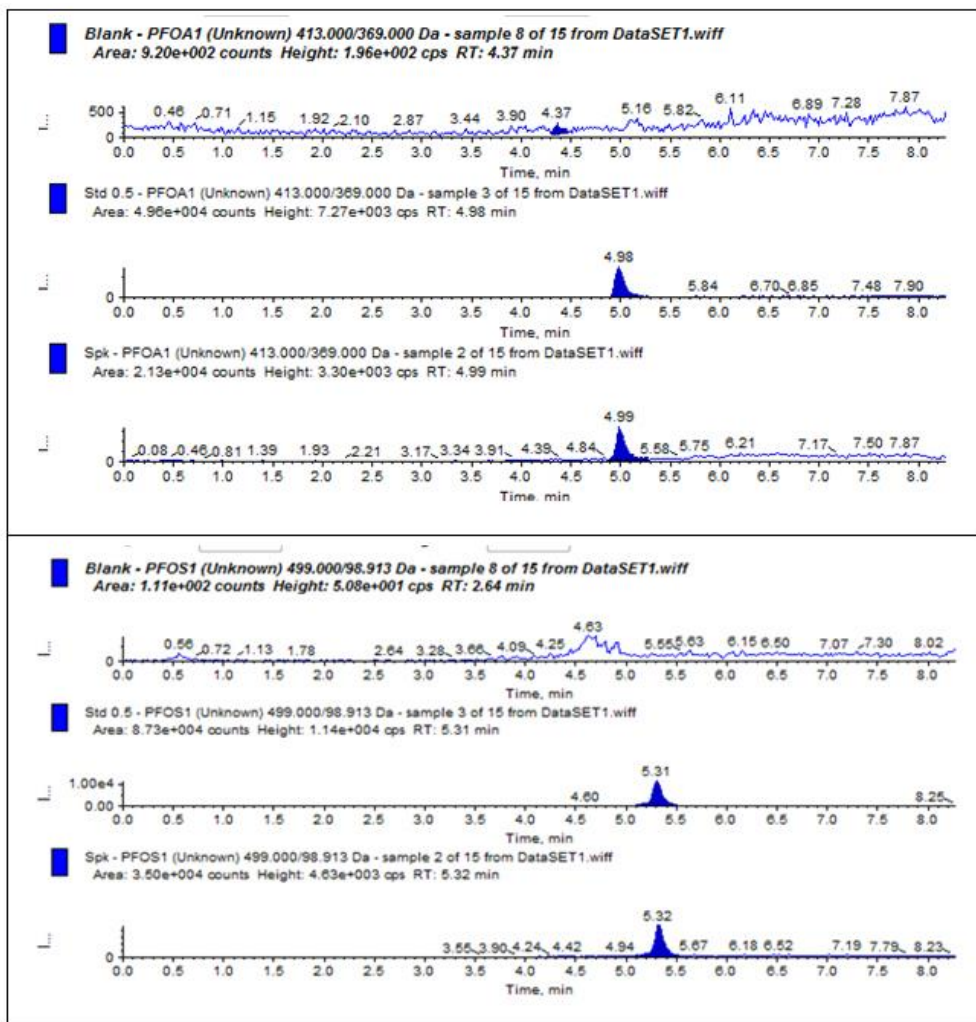


Figure 1. Chromatogram of a blank sample, a spiked sample, and a standard solution of PFOA and PFOS

Figure 1 shown the chromatograms of a blank sample, a spiked sample, and a standard solution of PFOA. One peak was detected at the retention time of around 4.98 min in the chromatograms of the spiked sample and the standard solution. Meanwhile, the chromatogram of the blank sample did not show any peak at that time. Similar results were also observed for PFOS at retention time of around 5.23 min. These observations indicated that our LC-MS/MS method had adequate specificity for PFOA and PFOS analysis.

3.2.2. Linearity, limit of detection, limit of quantitation

The linearity of the chromatographic response was tested using six standard solutions in concentration range of 0.3 to 10 ng/mL. Good correlation coefficients ($R = 0.999$) were achieved for both PFOS and PFOA (Table 2 and Figure 2), indicating linear relationships between concentrations and peak areas of the target compounds.

Table 2. LOD, LOQ, Linearity, Regression equations and correlation coefficients of PFOS and PFOA calibration curves ($Y = \text{peak area}$, $X = \text{concentration in ng/mL}$)

Compound	LOD (ng/g)	LOQ (ng/g)	Linearity (ng/mL)	Regression equation	Correlation coefficient (R)
PFOS	0.1	0.3	0.3 - 10	$Y = 333000 X + 18800$	0.999
PFOA	0.1	0.3	0.3 - 10	$Y = 203000 X + 21800$	0.999

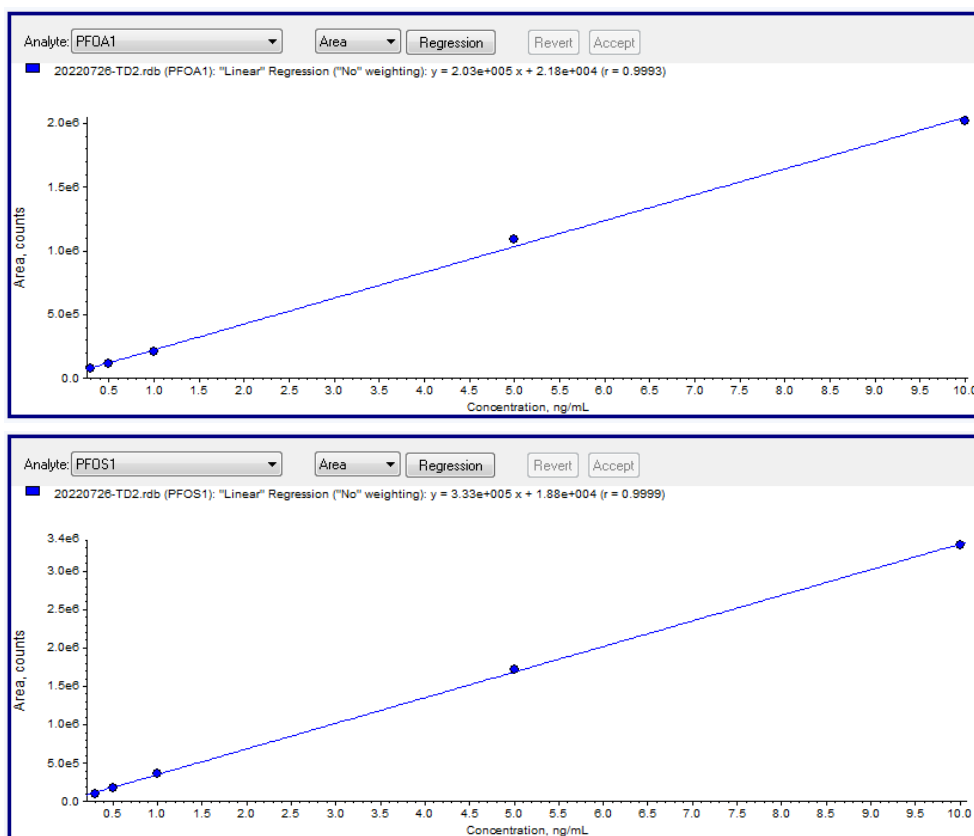


Figure 2. Calibration curves of PFOA and PFOS

Limits of quantitation were evaluated by spiking the lowest concentrations of PFOS and PFOA into the blank samples. The signal-to-noise ratios of PFOA and PFOS peaks after sample treatment at the low spiked concentration should be less than 10. Accordingly, the LOQs of PFOS and PFOA were 0.3 ng/g. The LODs were estimated at 0.1 ng/g for both compounds.

3.2.3. Repeatability and recovery

The blank samples of oil-proof paper were spiked with standard mixture of PFOA and PFOS at three concentration levels of 0.3, 1.5, and 3.0 ng/g. Each spiked sample was placed in a 15-mL centrifuge tube and stored at ambient temperature in 3 days before analysis. The repeatability and recovery tests ($n = 6$) were performed to verify the precision and trueness of the method. The recoveries and relative standard deviations (RSD) of PFOA and PFOS are shown in Table 3.

Table 3. Within-day precisions and recoveries of PFOA and PFOS

<i>Compounds</i>	<i>Spiking amount (ng/g)</i>	<i>Within-day precision (RSD%)</i>	<i>Mean recovery (%)</i>
<i>PFOS</i>	0.3	2.77	106
	1.5	4.61	104
	3.0	4.67	105
<i>PFOA</i>	0.3	1.70	100
	1.5	4.04	104
	3.0	2.05	105

The RSD values of the determination of PFOS, PFOA ranged from 1.70 to 4.67%, indicating acceptable precision of the analytical method over the three concentration levels. The mean recoveries ranged from 100 to 106%, showing high accuracy. The recovery rates and relative standard deviations of PFOA and PFOS obtained by our method satisfied requirement of the AOAC International for trace analysis at ppb levels.

3.3. Application to real paper packaging samples

In this study, twenty-three of paper-based food packaging samples were analyzed using the validated method. These samples include 5 cupcake papers, 5 fast-food containers, and 13 oil-proof papers. PFOA and PFOS were not detected in all the samples, indicating that these substances have not been added into our analyzed samples. Analytical results of PFOA and PFOS in paper-based food packaging samples collected from different locations in the world are tabulated in Table 4.

Table 4. Concentrations of PFOA and PFOS found in paper-based food packaging samples

Country	Samples	Concentrations	Reference
Vietnam	Cupcake paper, fast-food container, oil-proof paper	PFOA and PFOS: not detected	This study
Taiwan	To-go box, burger paper, fried paper box, chip paper bag, microwave popcorn paper bag, oil-proof paper	PFOS: not detected; PFOA: found in two microwave popcorn papers (13.2 and 223 ng/g), one oil-proof paper (103 ng/g)	[1]
Thailand	Instant food cup, microwave popcorn bag, beverage cup, ice cream cup, fast food container, dessert container, baking paper	PFOS: not detected - 92.48 ng/dm ² PFOA: not detected - 16.91 ng/dm ²	[3]
Greek	Beverage cup, ice cream cup, fast food paper box, fast food wrapper, microwave baking paper, aluminum foil bag	PFOA and PFOS: not detected	[8]

As shown in Table 4, the detection of PFOA and PFOS varied greatly between studies. These two chemicals were not detected in any sample of our study as well as those collected from Greek [8]. PFOS was also not detected in many food-packaging samples collected from Taiwan [1]. PFOA was only measured in two Taiwanese microwave popcorn papers (13.2 and 223 ng/g) and one oil-proof paper (103 ng/g) [1]. Meanwhile, both PFOA and PFOS were frequently found in paper samples (e.g., instant food cup, microwave popcorn bag, beverage cup, ice cream cup, fast food container, dessert container, baking paper) collected from Bangkok, Thailand at concentrations as high as 16.91 and 92.48 ng/dm², respectively [3]. These observations may relate to the differences in application rates and usage patterns of PFOA, PFOS, and products containing them between investigated countries and sampling periods.

4. CONCLUSION

In the present study, a liquid chromatography–tandem mass spectrometry method was validated for the simultaneous analysis of PFOA and PFOS in several paper-based food packaging samples. The method was demonstrated to have adequate specificity, linearity, sensitivity, and accuracy, meeting the requirements of AOAC International for trace analysis at ppb levels. The method was successfully applied to determine PFOA and PFOS in 23 paper samples randomly collected from local markets in Hanoi, Vietnam. These two compounds were not detected in all the samples of this study. However, further

investigations on the occurrence of PFASs (other than PFOA and PFOS) and other types of food-contact materials should be performed.

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Xác định perfluorooctanoic acid (PFOA) và perfluorooctane sulfonic acid (PFOS) trong bao bì thực phẩm bằng giấy

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Tóm tắt

Trong nghiên cứu này, phương pháp phân tích đồng thời perfluorooctanoic acid (PFOA) và perfluorooctane sulfonic acid (PFOS) bằng thiết bị sắc ký lỏng khối phổ hai lần (LC-MS/MS) trong bao bì thực phẩm giấy đã được thẩm định. Các mẫu bao bì giấy được chiết với hỗn hợp ethanol : nước (1 : 1, v/v) ở nhiệt độ 70°C trong 2 h. Dịch chiết được lọc qua màng lọc 0,2 µm trước khi phân tích trên thiết bị LC-MS/MS. Giới hạn phát hiện và giới hạn định lượng của phương pháp lần lượt là 0,1 và 0,3 ng/g đối với 2 chất phân tích. Hệ số tương quan tuyến tính đạt $R > 0,99$ trong khoảng nồng độ 0,3 đến 10 ng/mL. Độ thu hồi của phương pháp dao động trong khoảng từ 100 - 106% với độ lặp lại RSD < 5%. Phương pháp đã được ứng dụng để phân tích 23 mẫu bao bì thực phẩm bằng giấy nhưng không mẫu nào phát hiện thấy PFOS và PFOA.

Từ khóa: PFOA, PFOS, bao bì thực phẩm bằng giấy, LC-MS/MS.