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Nota de aplicación

# Trace Level Analysis of Perfluoroalkyl Substances in Solid Cosmetics Following Methanol Extraction

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## Abstract

In the recent years, studies have identified per- and polyfluoroalkyl substances (PFAS) present in popular in cosmetic products (CP). Since these products are directly applied on the skin, the presence of PFAS causes concern due to adverse health effects associated with this group of compounds. Eye shadow and powdered foundation as examples of solid, wax-free CP were sourced for a targeted analysis of a known panel of PFAS compounds. Prior to high sensitivity liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis, the solid samples underwent a stringent work-up procedure to remove interfering ingredients. This approach proved to be sensitive for a range of 30 PFAS compounds of varying chemistry classes following methanol extraction.

## Benefits

- A simple extraction method that can be utilized for a large suite of PFAS from a variety of food matrices
- Sensitive analysis on the Waters Xevo TQ-S micro Mass Spectrometer to detect, quantify, and confirm PFAS at sub-ng/g levels

- Utilization of the PFAS Kit for LC modification to minimize possible system and solvent contaminants to assure accurate results

## Introduction

Per- and polyfluoralkyl substances (PFAS) comprise a large and diverse group of synthetic chemicals which have been produced since the 1950s and have been consistently used since then. PFOS and PFOA are the most studied PFAS compounds, as they were the first to garner publicity with their use in the manufacturing process for Teflon.[1] Due to the strong electronegativity, the perfluoroalkyl moiety ($-C_nF_{2n+1}$) imparts unique properties to molecules including high surface activity, chemical and thermal stability, and water and oil-repellency. The properties of PFAS make them useful low molecular weight surfactants and polymeric material in an array of industrial applications and in the production of consumer products such as non-stick and water-resistant coatings, in firefighting foams and even in cosmetic products (CP).[2] Examples of fluorinated ingredients that have been found in CP include per/polyfluorinated acrylate polymers, naphthalenes, alkanes/alkenes, alcohols, siloxanes, silanes, sulfonamides, ethers, esters, phosphate esters, and acids.[3]

While these properties make PFAS useful for various industries, they have been linked to a variety of potential health concerns including elevated cholesterol, reproductive impacts, and are considered potentially carcinogenic. According to the European Commission's database on cosmetic ingredients (CosIng), these substances are used as emulsifiers, anti-statics, stabilizers, surfactants, film formers, viscosity regulators, and solvents.

Consequently, several regulatory measures have been implemented over the last decade with the aim of reducing environmental emissions and human exposure to PFAS, but regulatory guidance has not yet been proposed for cosmetics. However, the Food and Drug Administration (FDA) will be looking into bringing on methodologies for testing consumer products for PFAS in the future. This will pose an important step to safeguard end-users but will bear a burden on the industry as the sample preparation is challenging and time consuming and overall analyses including instrumentation is expensive. Due to the large variety PFAS, sample preparation workflows have to be adjusted to the sample and/or matrix. Depending on the sample matrix and its complexity, different techniques for analysis are utilized for example gas chromatography/mass spectrometry (GC-MS) would be used for analysis of volatile PFAS whereas non-volatile PFAS compounds would be

characterized by liquid chromatography with mass spectrometric detection (LC-MS). Since the amount of potential PFAS in any samples is very low, the instrumentation must be selective and sensitive for trace level analysis.

Featuring methanol extraction as a new approach as sample preparation, the focus of this study with was to show that trace level concentrations of PFAS can be detected in solid, wax-free cosmetics (Figure 1A–B) using an ACQUITY UPLC I-Class PLUS coupled to a tandem quadrupole mass spectrometer (LC-MS/MS) following a simplified, solid-phase extraction (SPE)-free workflow.

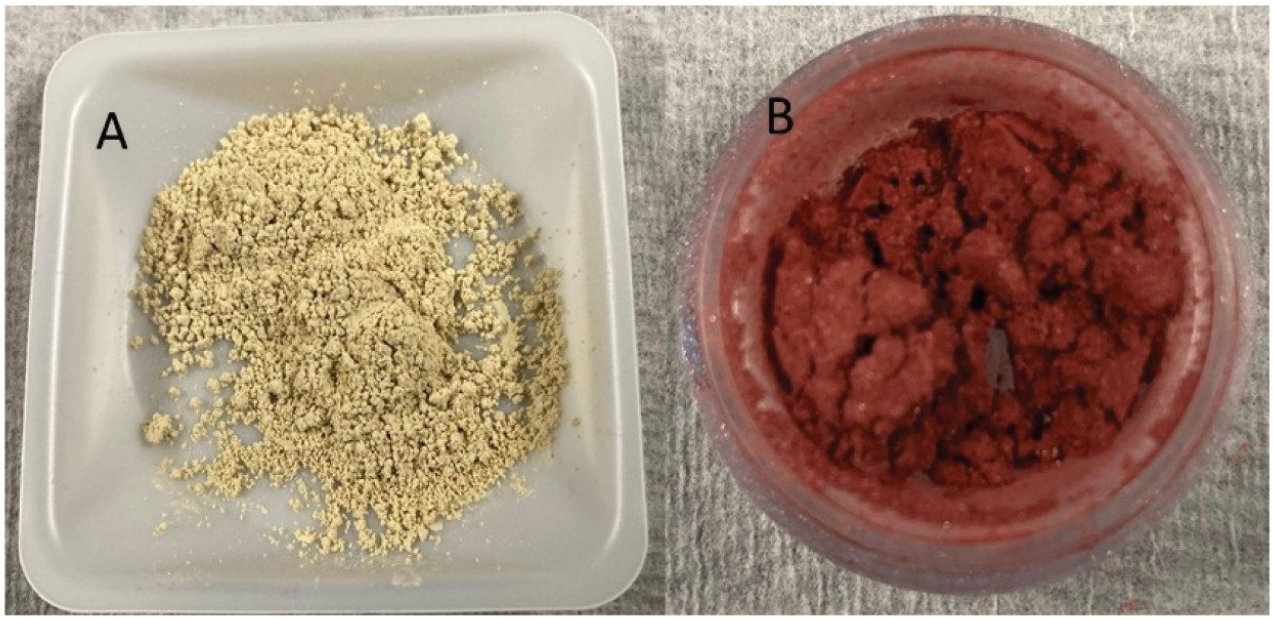


*Figure 1. Solid cosmetics used in this study.  A. foundation; B. eye shadow.*

## Experimental

All standards were obtained from Wellington Laboratories. The method contained a total of 30 PFAS including the following compounds: Carboxylates: C4-C14; Sulfonates: C4-C10; Ethers: GenX, ADONA, 9Cl-PF3ONS, 11Cl-PF3OUdS; Precursors: FBSA, FHxSA, FOSA, NMeFOSAA, NEtFOSAA, 4:2 FTS, 6:2 FTS, 8:2 FTS. Isotope labelled

extraction (MPFAC-24ES) and injection standards (MPFAC-C-IS) were used during extraction and analysis to perform isotope dilution calculations. The extraction standard was spiked in the samples prior to sample preparation and used to correct the native compounds for recovery and matrix effects. The injection standard was added to the sample after clean-up when the sample was reconstituted and used to correct the extraction standards for reconstitution variations, matrix effects, and injection variation.

Since PFAS are ubiquitous in many common laboratory products, care must be taken to reduce risks of contamination during the sample analysis workflow. It is important to use suitable laboratory products and solvents that have been evaluated for PFAS contamination prior to use. The steps of the sample work-up procedure are outlined below. Some of the steps are visualized in Figures 2A–C.

- 1 g sample
- Spike extraction standards (2.5 ng/g)
- Add 10 mL of methanol
- Sonicate for 30 minutes
- Filter: 1) GMF filter 2) 0.22 µm GHP syringe filter
- Dilute 5 mL extract 1:1 with 2 mM ammonium acetate
- Centrifuge 70 minutes at 3900 rpm
- Spike injection standards (5 ng/g)
- Transfer to vial

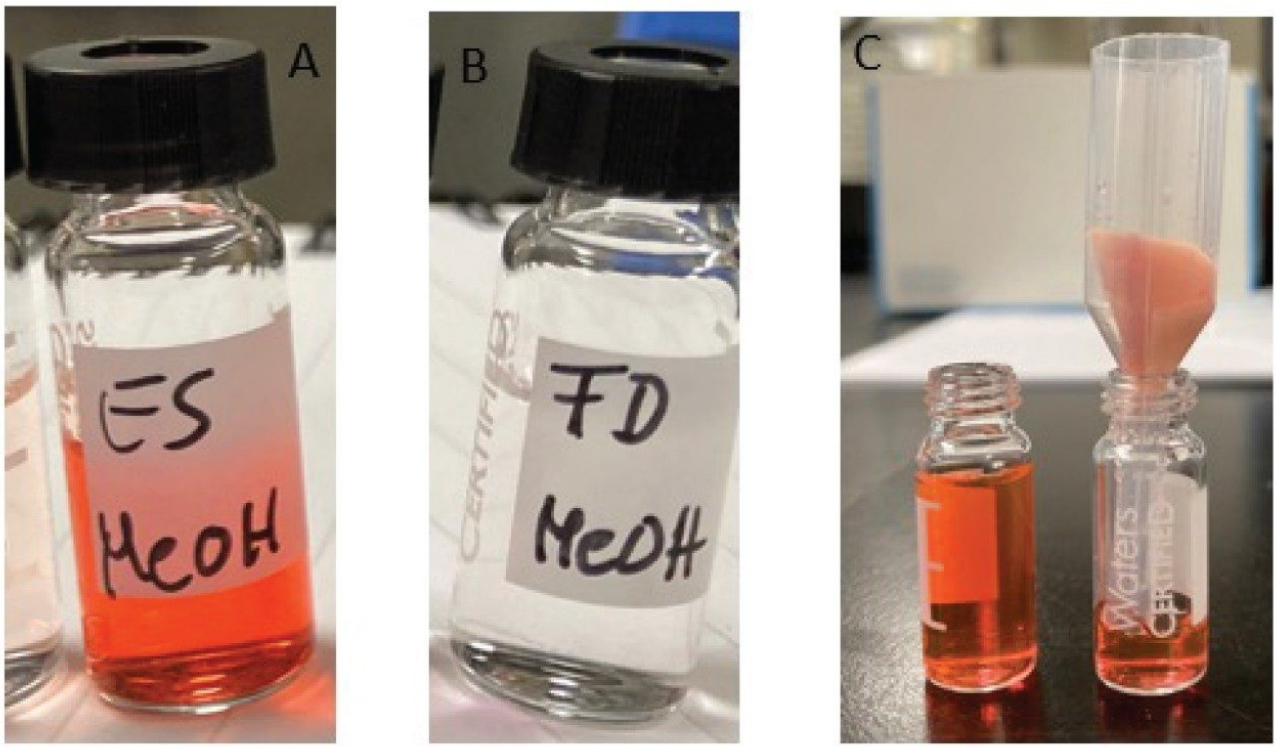


*Figure 2A. methanol extracts of eye shadow (ES); B. foundation (FD); C. filtration (GMF filter) set-up for solid removal.*

## MS Parameters

| | |
|---|---|
| Instrument: | Xevo TQ-S micro Tandem Quadrupole Mass Spectrometer |
| Ion mode: | ESI |
| Capillary voltage: | 0.5 kV |
| Desolvation temp.: | 350 °C |
| Desolvation flow: | 900 L/hr |

| | |
|---|---|
| Cone flow: | 150 L/hr |
| Source temp.: | 100 °C |

## LC Parameters

| | |
|---|---|
| Instrument: | ACQUITY UPLC I-Class PLUS with PFAS Kit |
| Column: | ACQUITY BEH $C_{18}$ 2.1 mm x 100 mm, 1.7 µm |
| Column temp.: | 35 °C |
| Mobile phase A: | Water + 2 mM ammonium acetate |
| Mobile phase B: | Methanol + 2 mM ammonium acetate |
| Injection volume: | 10 µL |
| Gradient: | See Table 1 |

### Gradient settings

| Time (min) | Flow (mL/min) | %A | %B |
|---|---|---|---|
| 0 | 0.3 | 95 | 5 |
| 1 | 0.3 | 75 | 25 |
| 6 | 0.3 | 50 | 50 |
| 13 | 0.3 | 15 | 85 |
| 14 | 0.3 | 5 | 95 |
| 17 | 0.3 | 5 | 95 |
| 18 | 0.3 | 95 | 5 |
| 22 | 0.3 | 95 | 5 |

## Results and Discussion

The variation in chemical structure and properties across the entire suite of thousands of PFAS makes extraction and analysis challenging. This targeted study focused on a panel of about 30 PFAS covering a wide variety of PFAS chemistries with different alkyl chain lengths. Isotopically labeled standards were spiked into the samples both prior to extraction (extraction standard) and after extraction (injection standard). Extraction standards were used to determine recovery of the method. Table 2 lists the PFAS standard panel as well as the injection standards (IS) and extraction standards (ExS) utilized.

| PFAS | Type of standard | PFAS | Type of standard |
|---|---|---|---|
| PFBA | ExS, IS | N-MeFOSAA | ExS |
| PFPeA | ExS | N-EtFOSAA | ExS |
| PFHeA | ExS | PFBS | ExS |
| PFHpA | ExS | PFPeS | |
| PFOA | ExS, IS | PFHxS | ExS |
| PFNA | ExS | PFHpS | |
| PFDA | ExS, IS | PFOS | ExS, IS |
| PFUdA | ExS | PFNS | |
| PFDoA | ExS | PFDS | |
| PFTrDA | | 4:2FTS | ExS |
| PFTeDA | ExS | 6:2FTS | ExS |
| FBSA | | 8:2FTS | ExS |
| FHxSA | | NaDONA | |
| FOSA | ExS | 9Cl-PF3ONS | |
| HFPO-DA (GenX) | ExS | 11Cl-PF3OUdS | |

*Table 2. PFAS standards used in analysis.*

Direct analysis is not feasible with this instrumental set-up, because of the complexity of the sample matrix. Recent publications use various methods for the extraction of PFAS from cosmetics samples.[1,4,5] Since the focus of this study was only on solid cosmetics, a simple direct extraction using methanol was evaluated. Methanol is a suitable solvent for PFAS extraction and takes advantage of the non-polar C-F alkyl chain present in every PFAS structure. Furthermore, the wetting behavior of methanol was superior to water, which would aid the extraction.

Figure 3 illustrates the recovery trend for the spiked extraction and injection standard panel. It ranges from 65 to 100% that can be considered good. The isotope labelled extraction standards were used to evaluate method recovery due to the lack of cosmetics standards free of PFAS.

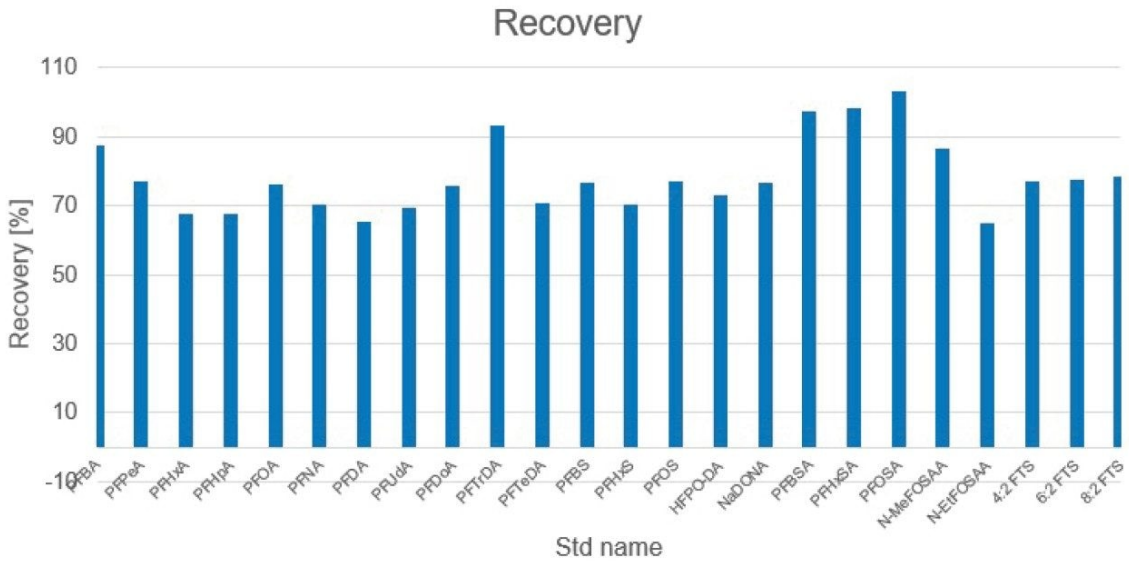


*Figure 3. Recovery plot of spiked extraction standards at 2.5 ng/g.*

The sensitivity of the method can be demonstrated in the following examples of sodium dodecafluoro-3H-4,8-dioxa-nonane-1-sulfonate (NaDONA). Figure 4A demonstrates the calibration response in the range of 0.01–50 ng/mL. Triplicate injections were made of each concentration, demonstrating excellent linearity and reproducibility. Figure 4B shows the chromatograms of the two MRM transitions monitored for NaDONA in a 0.05 ng/mL solvent standard with the ion ratio highlighted in green. Figure 4C exhibits NaDONA spiked into a blank sample prior to extraction, also denoting the ion ratio. The ion ratio was used to confirm detected PFAS in the samples. All PFAS exhibited ion ratios with the generally accepted 20% limit (as compared to the average values derived from the calibration standards) at low and high levels.

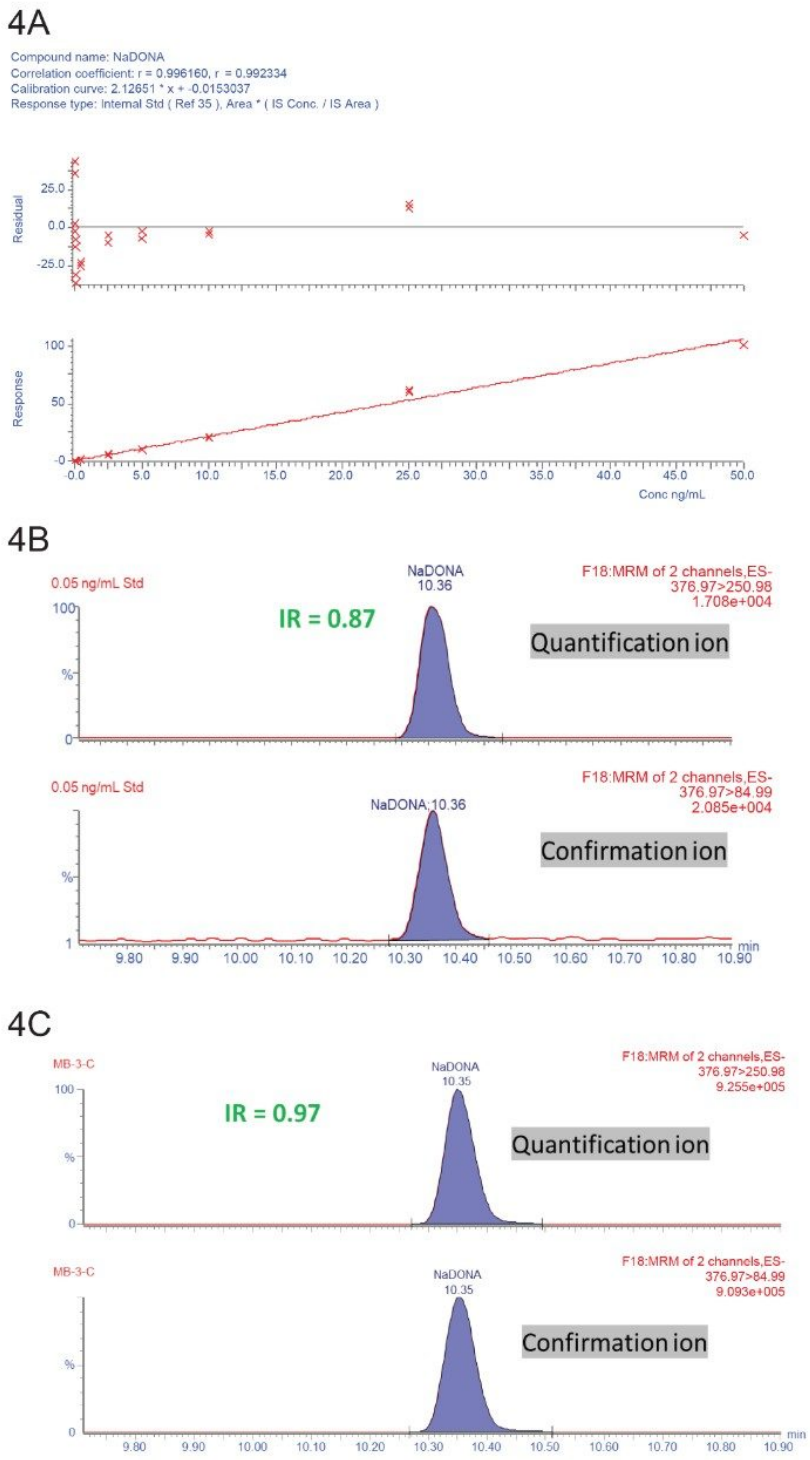


*Figure 4. A. calibration range with residuals plot; B and C. ion ratio calculation for two characteristic*

*MRM transitions of NaDONA.*

The extracted ion chromatograms (EIC) of quantification ions of two non-standard PFAS compounds detected in a blank sample of eye shadow and confirmation ions from extraction standards were used to demonstrate a successful extraction procedure as is depicted in Figures 5 and 6. Retention times of quantification ion and confirmation ion correlate well. Peak areas of sample and solution standard EIC differ due to different spiking levels. Detected PFAS concentrations ranged from 0.2–2.9 ng/g in the samples tested. Perfluorobutanoic acid (PFBA), perfluoro-n-octanoic acid (PFOA), and perfluoro-n-tetradecanoic acid (PFTeDA) were detected in both eye shadow and foundation, whereas perfluoro-n-hexanoic acid (PFHxA) and perfluoro-1-butane-sulfonate (PFBS) were also detected in eye shadow. Concentrations of the detected PFAS compounds in selected eye shadow and powdered foundation samples are reported in Table 3.

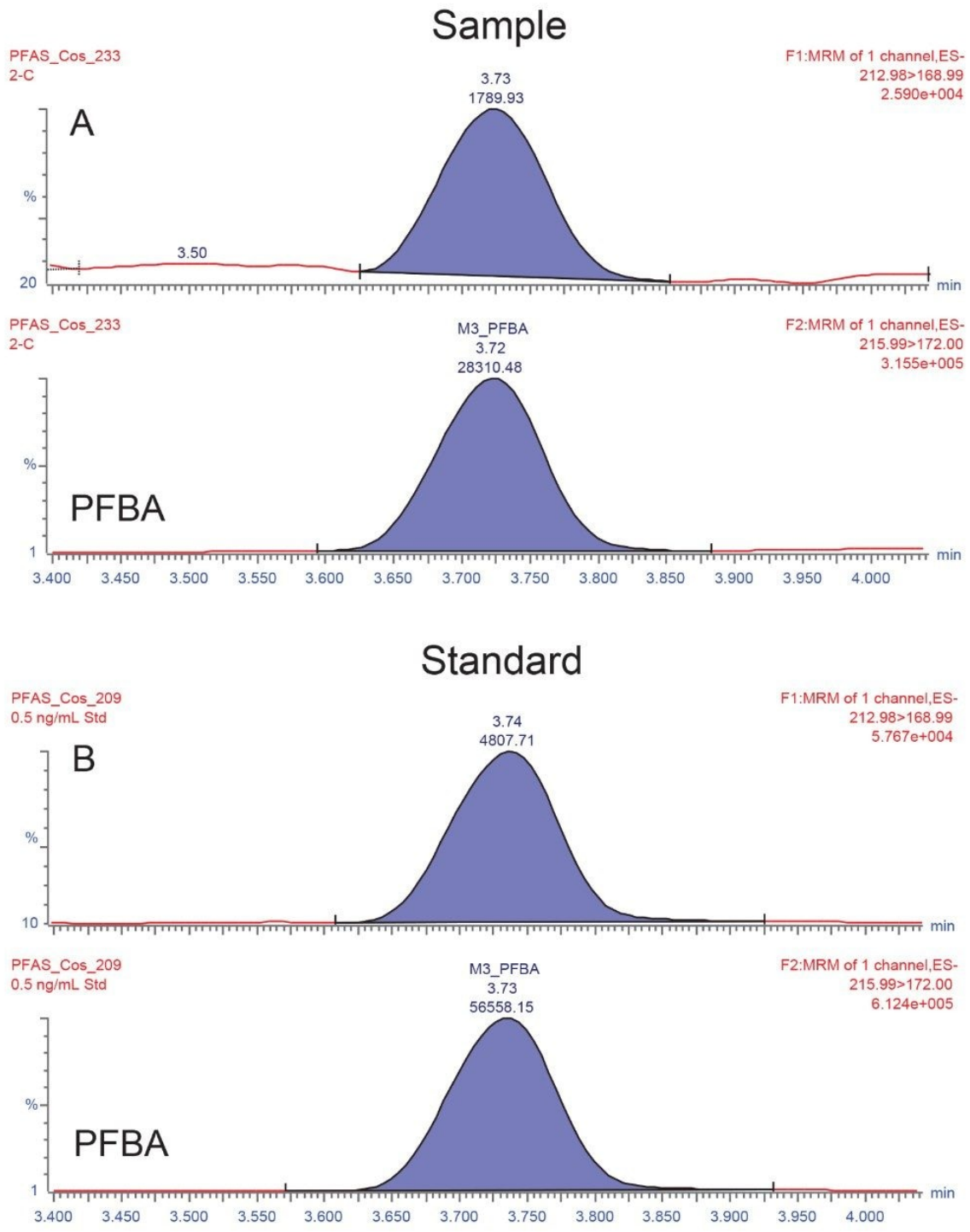


*Figure 5. A. Extracted ion chromatogram of the quantitation ion for PFBA detected in the blank eye*

*shadow sample; B. MRM confirmation transition for PFBA in a 0.5 ng/mL standard solution.*

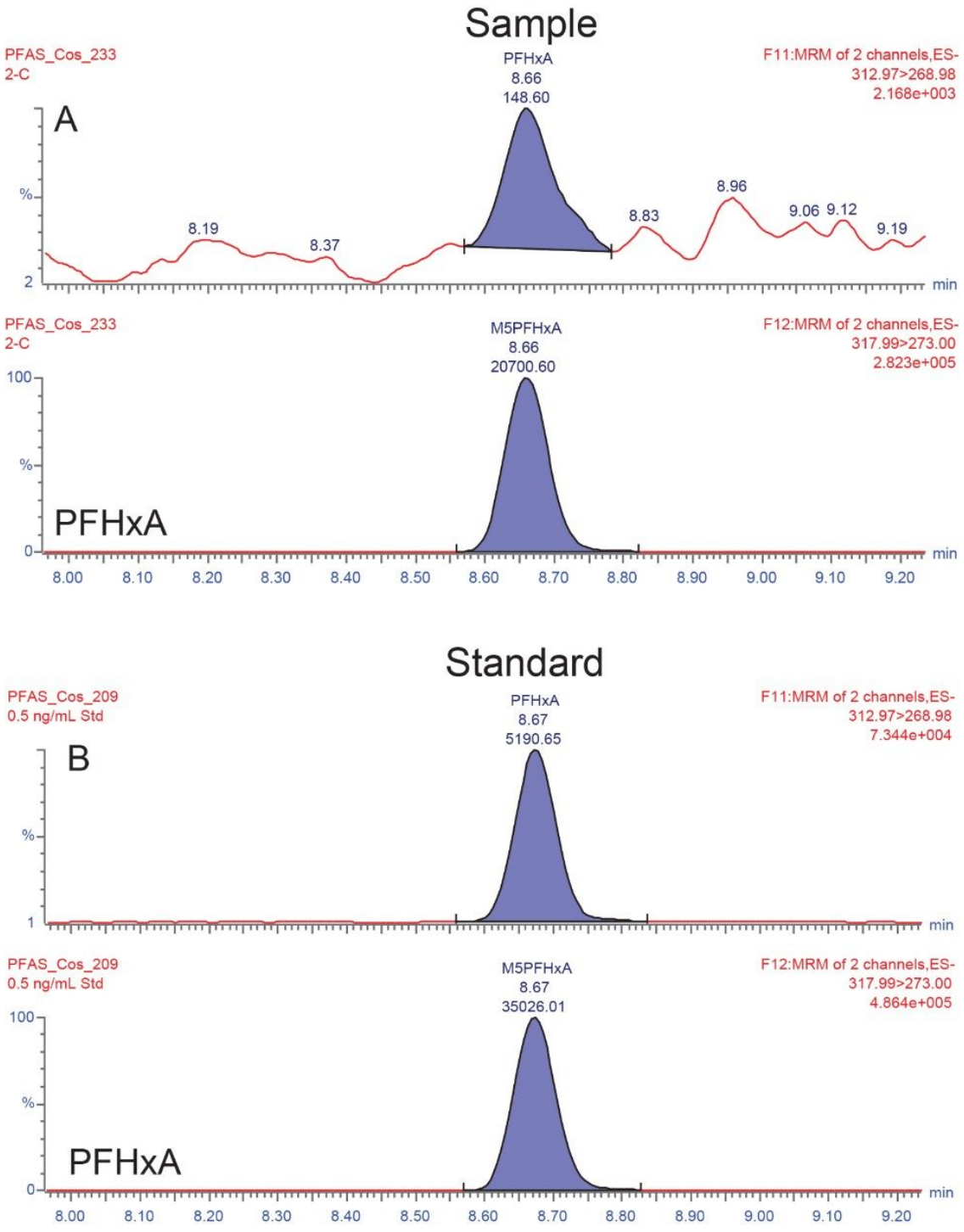


*Figure 6. A. Extracted ion chromatogram of the quantitation ion for PFHxA detected in the blank eye*

*shadow sample; B. MRM confirmation transition for PFHxA in a 0.5 ng/mL standard solution.*

| Sample | Compound | Conc [ng/mL] | Conc [ng/g] |
|---|---|---|---|
| ES | PFBA | 0.29 | 2.9 |
| ES | PFHxA | 0.02 | 0.2 |
| ES | PFOA | 0.12 | 1.2 |
| ES | PFTeDA | 0.02 | 0.2 |
| ES | PFBS | 0.02 | 0.2 |
| FD | PFBA | 0.18 | 1.8 |
| FD | PFOA | 0.01 | 0.1 |
| FD | PFTeDA | 0.02 | 0.2 |

*Table 3. Concentrations of PFAS compounds present in the eye shadow and powdered foundation samples.*

## Conclusion

This preliminary study demonstrates that a simplified methanol extraction procedure is sufficient for example solid cosmetics products.

- Trace level, non-standard PFAS compounds were extracted and detected in eye shadow and powdered

foundation.

- The selectivity and sensitivity of the Xevo TQ-S micro Tandem Quadrupole Mass Spectrometer allowed for the detection, quantification, and confirmation of PFAS at trace levels.
- The installed PFAS kit on the ACQUITY UPLC I-Class PLUS System helped to eliminate instrument related PFAS contamination and streamlines the analysis of this challenging class of chemical contaminants in routine laboratories.

## References


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