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Using Agilent Hybrid Multisampler with Triple Quadrupole Mass Spectrometer to Solve Solvent Effect in PFCAs and its Precursor FTOHs Analysis

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Introduction

PFAS (per- and polyfluoroalkyl substances) are a group of synthetic compounds characterized by a carbon chain with fluorine atoms bonded. Within PFAS, perfluoro carboxylic acids (PFCAs) are a key subclass, which feature a fully fluorinated carbon chain terminated by a carboxyl group, described by the general formula $CF_3(CF_2)_nCOOH$ (n≥0). This unique architecture endows PFCAs with exceptional surface-active properties, robust hydrophobicity, and oleophobicity, driving their extensive use in industrial applications—particularly as water- and stain-resistant agents in textile and leather manufacturing. As precursors of PFCAs, fluorotelomer alcohols (FTOHs) often serve as indirect sources of PFCAs.

In recent years, with growing global attention to PFCAs and their precursors FTOHs, analytical method standards for these substances have been successively introduced. According to the pre-treatment process recommended in BS EN ISO 23702 - 1: 2023 <Leather — Per- and polyfluoroalkyl substances Part 1: Determination of non-volatile compounds by extraction method using liquid chromatography>, methanol was ultimately used as the main diluent for the sample solution. However, in the data analysis process, significant solvent effect issues often arise when methanol diluent is applied to the sample, especially as injection volumes increase.

To address these solvent effect issues, Feed Injection technology of Agilent Hybrid Multisampler was applied in this study. By applying Feed Injection mode, sample was continually infused into the mobile phase stream, which mediated strong sample solvent effects and improved peak shape. The Feed Injection mode of Hybrid Multisampler is shown in Figure 1. With a triple quadrupole mass spectrometer employed, this study has established a method for the simultaneous analysis of PFCAs and their precursor FTOHs in consumer products, aiming to meet daily high-throughput detection needs.

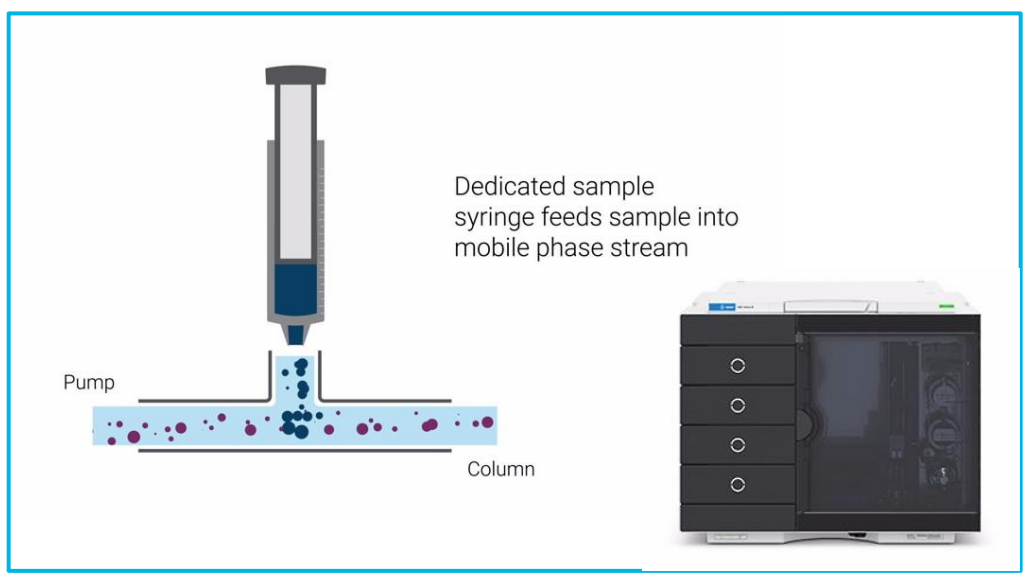

Figure 1: Feed Injection Mode of Hybrid Multisampler.

Experimental

Standard Preparation

In accordance with the final sample dilution solution used in BS EN ISO 23702-1:2023, the standard mixture of PFAS were prepared in methanol.

Method

- LC Conditions

Agilent UHPLC 1290 System		
Column:	Agilent ZORBAX RRHD Eclipse Plus C18, 3.0 x 100 mm, 1.8 µm (p/n: 959758-302)	
Column Temperature:	40 ℃	
Injection Volume:	20 µL	
Flow Rate:	0.4 mL/min	
Mobile Phase A:	5 mM ammonium acetate in water	
Mobile Phase B:	Acetonitrile	
Gradient:	Time 0.0 0.5 2.0 7.0 12.0 14.0 17.0 17.1 19.0	B% 10 10 30 45 75 100 100 10 10
Stop time:	19 min	
Feed injection:	- Feed speed: 5% of flow (adaptive) - Flush out: automatic - Feed solvent: mobile phase A - Inner/outer wash solvent: mobile phase B - Reconditioning: mobile phase A	

- MS Conditions

Agilent 6475 triple quadrupole mass spectrometr	
Ion mode:	AJS ESI Negative Mode
Gas Temp:	150 ℃
Gas Flow:	9 L/min
Nebulizer:	55 psi
Sheath gas Temp:	250 ℃
Sheath gas Flow:	12 L/min
Capillary Voltage:	3500 V
Nozzle Voltage:	1500 V

Results and Discussion

UHPLC Separation and MRM Overlay Chromatogram

The UHPLC-MS/MS method for simultaneous determination of 50 PFAS (including 16 PFCAs, 5 FTOHs and 29 other PFAS) has been established in this study. Figure 2 showed the MRM (multiple reaction monitoring) overlay chromatograms for the compounds, with FTOH concentration at 5 ng/mL and FTCA as well as other PFAS concentrations at 0.1 ng/mL.

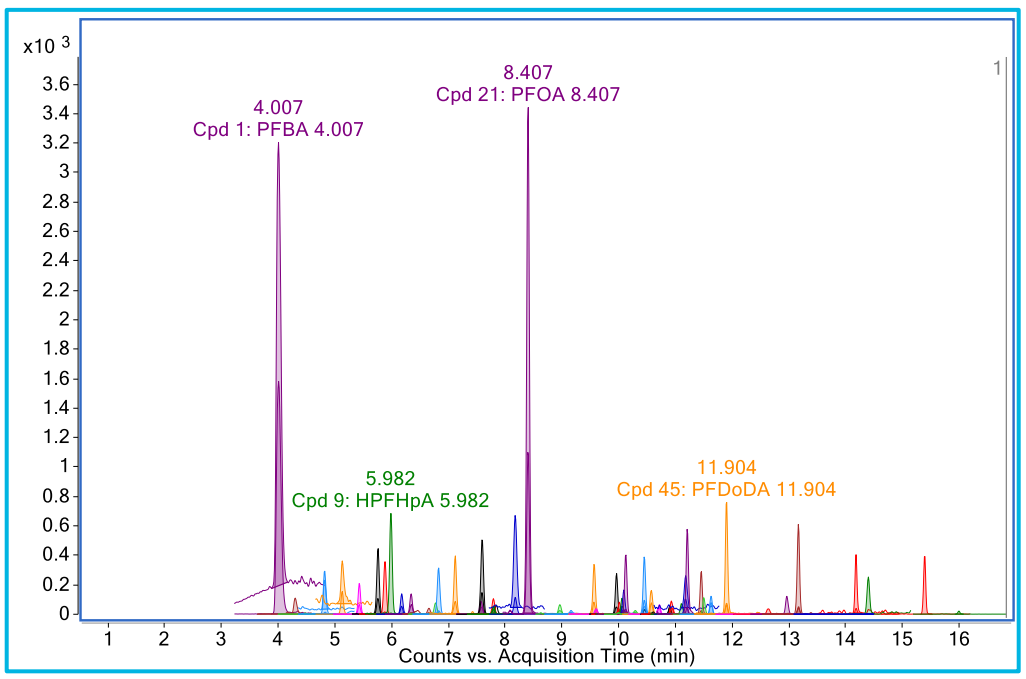

Figure 2: The MRM Overlay Chromatogram for Each PFAS

Optimization of Feed Injection Parameter

Among these target PFAS, PFBA (Perfluorobutanoic acid) was characterized by its high polarity, and exhibited weak retention on a C18 column, which resulted in it being the earliest compound to be eluted among these 50 compounds. Consequently, it made PFBA susceptible to solvent effect during the analytical process, particularly when the sample diluent was a strong solvent, such as methanol. In view of this characteristic of PFBA, the following discussion on the Feed Injection of Hybrid Multisampler for solving solvent effect, take the changes in peak shape of PFBA as a representative example.

Pump Flow Optimization

To optimize the parameter for feed speed, 20 µL of the standard sample was injected at different percentages of pump flow (5%, 10%, 20% and 40%) to assess the peak performance. The influence of different pump flow on the peak shape of PFBA is shown in Figure 3. As can be seen from the figure, with the decrease of pump flow, the peak shape of PFBA became closer to normal distribution, and the solvent effect was significantly decreased. Conversely, the elution time of PFBA is slightly delayed. At 5% of pump flow, PFBA obtained a good peak sharp. Therefore, 5% of pump flow was chosen in this study.

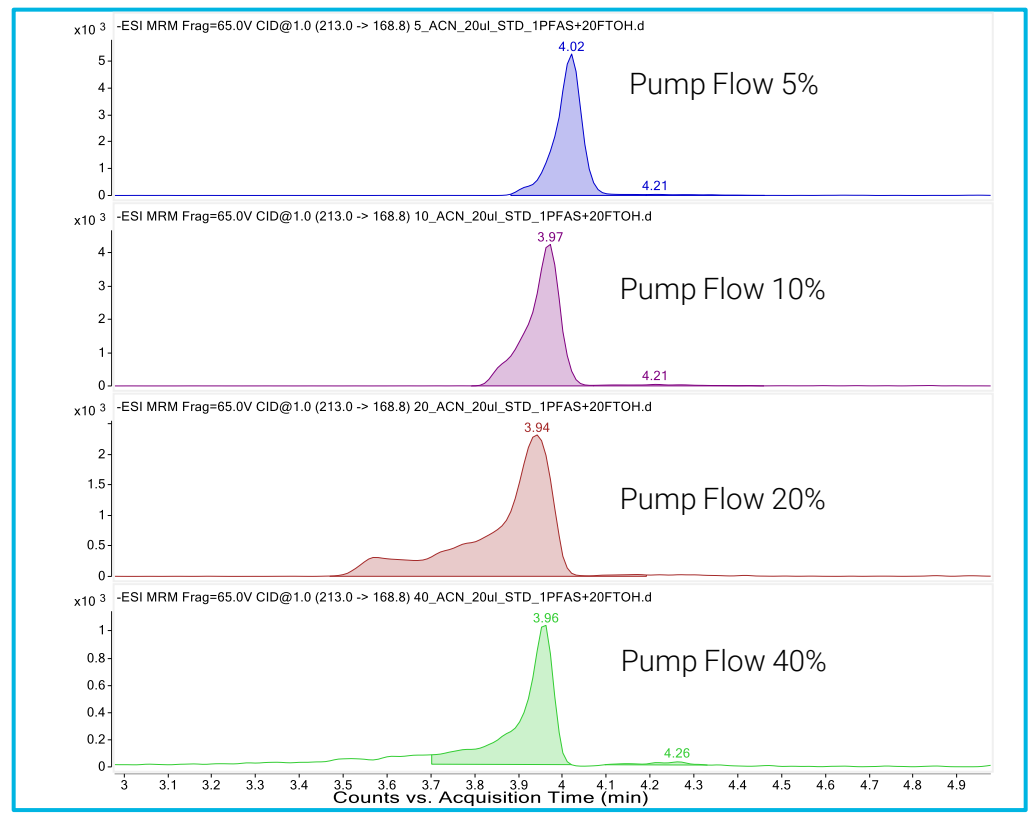

Figure 3: The Peak Shape of PFBA Using Different Pump Flow

Injection Volume Optimization

The injection volume was operated at different injection volumes of 10, 20 and 40 µL for optimization. The resulting comparison chromatogram is shown in Figure 4. It showed that the peak shape of PFBA remains relatively ideal when the injection volume is 10 µL and 20 µL. However, when the injection volume reaches 40 µL, the peak shape of PFBA is more significantly affected by solvent effect. Based on the above observation, an injection volume of 20 µL was chosen for this experiment.

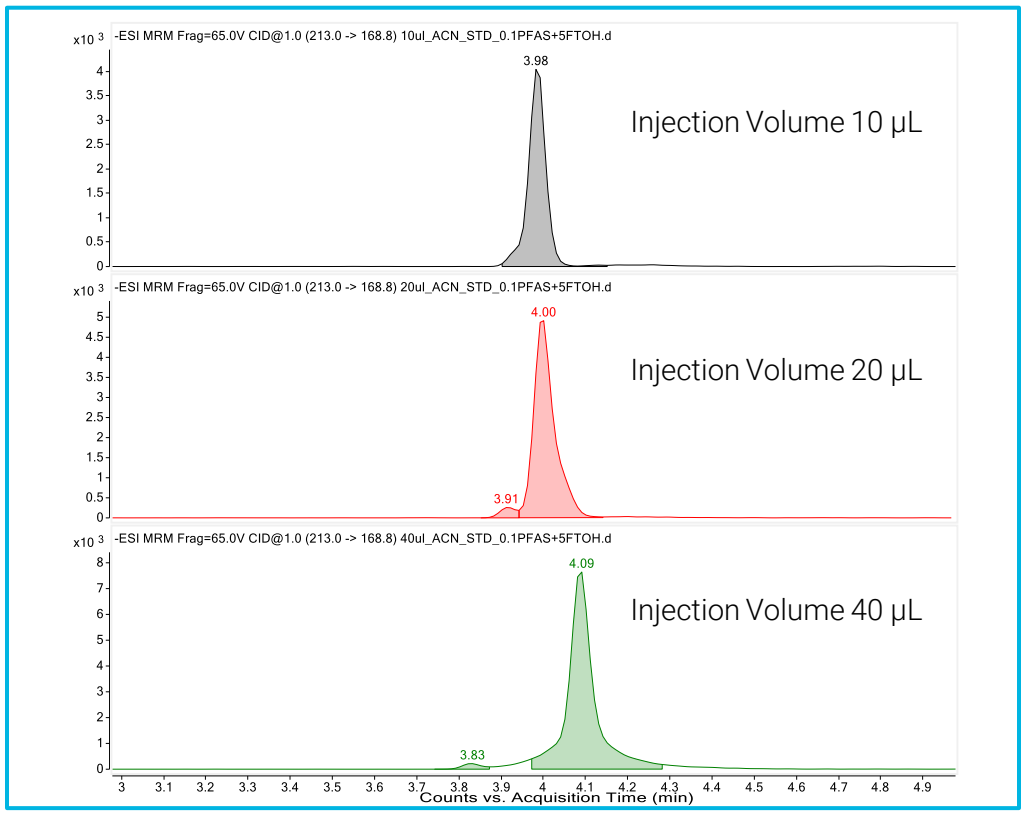

Figure 4: The Peak Shape of PFBA at Different Injection Volumes

Results and Discussion

Calibration Curve of PFCAs and FTOHs

Matrix solvent blank was processed according to the pre-treatment process in BS EN ISO 23702-1: 2023 and spiked with PFAS standards at a series of concentrations to attain matrix-matched calibration curve solutions. For PFCAs, the concentrations were 0.1, 0.5, 1, 5, and 10 µg/L. For FTOHs, the concentrations were 5, 10, 20, 50, and 100 µg/L. In examples for PFBA and 4:2 FTOH, figure 5 showed they achieved good linearity, and the linear correlation coefficients r^2 were 0.998 and 0.999 respectively. For all 50 target PFAS, linearity was $r^2 \geq 0.99$, indicating that this method could be accurately used for the quantitative analysis of PFAS including PFCAs and FTOHs.

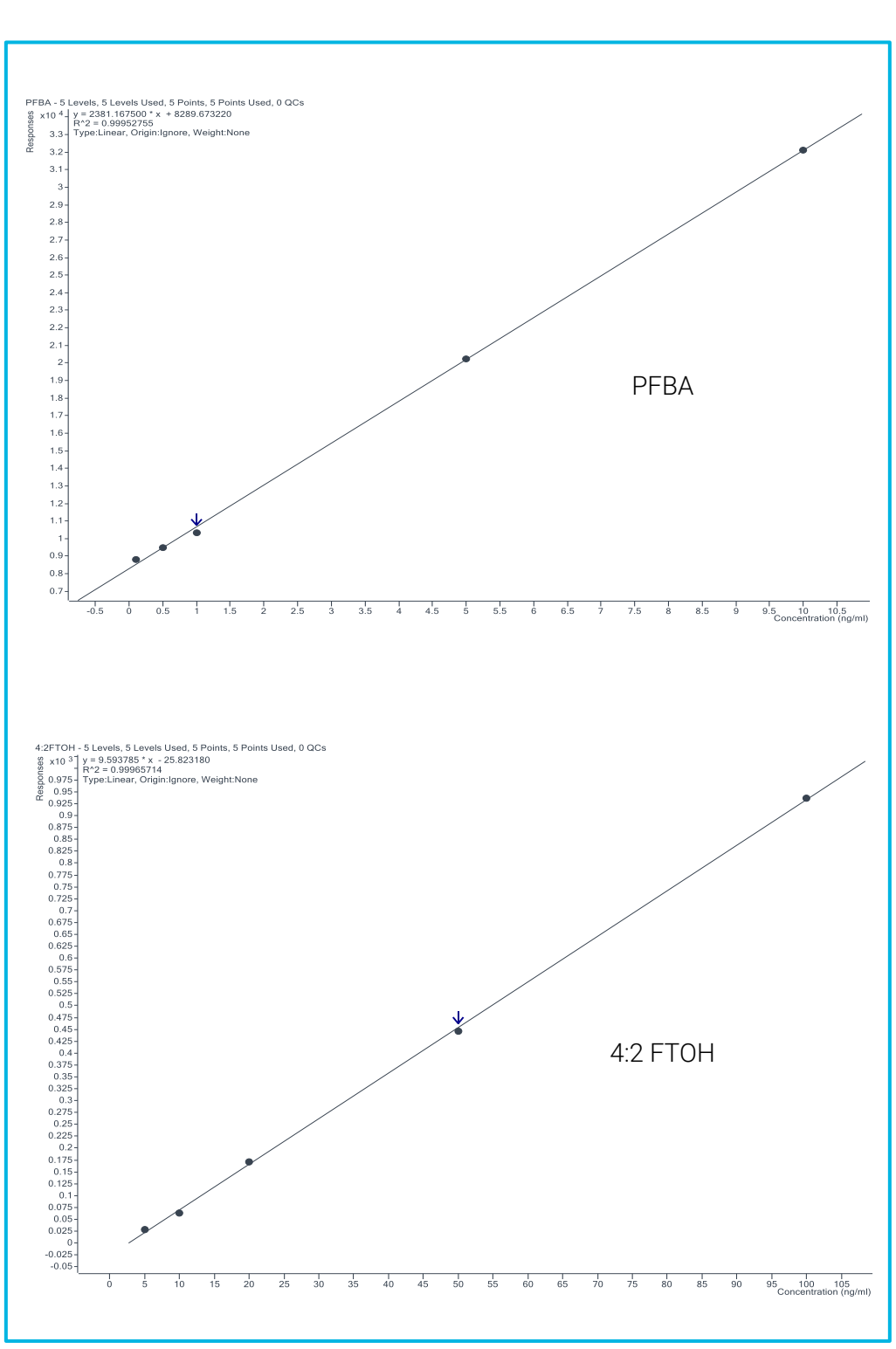

Figure 5: The Calibration Curve of PFBA and 4:2 FTOH

Repeatability of PFCAs and FTOHs

In this study, the repeatability of retention time (RT) and peak area of all target compounds were investigated. The results indicated that with six replicate injections of matrix-spiked sample, the relative standard deviation (RSD) of the retention time and peak area of all PFAS compounds were less than 0.1% and 10.0%, respectively.

Compound	Spiked	RSD	
		RT	peak area
PFCAs (16)	0.1 µg/L	0.01% ~ 0.04%	2.1% ~ 7.1%
FTOHs (5)	5 µg/L	0.02% ~ 0.1%	5.4% ~10.0%
Others (29)	0.1 µg/L	0.01% ~ 0.05%	3.4% ~ 8.5%

Conclusions

By applying Feed Injection mode of Hybrid Multisampler, a new method was developed to solve the solvent effect without decreasing injection volume or re-diluting sample solvent. Using Feed Injection mode, the sensitivity of the method to analyze PFCAs and FTOHs simultaneously was improved by increasing injection volume to 20 µL and the early-eluting compounds, such as PFBA, achieved good peak shape. The separation of 50 PFAS can be rapidly achieved within 19 min at the optimized UHPLC condition. For linearity and repeatability, all compounds achieved good performances (linear correlation coefficients r^2 >0.99; RSD of retention times <0.1%, n=6; RSD of peak area <10.0%, n=6).

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