Analysis of PFAS Including Ultra Short-Chain PFAS

Introduction

Per- and polyfluoroalkyl substances, generally known as PFAS are organic compounds that contain fluorine in their structures. PFAS have high stability due to their strong C-F bonds and decompose very slowly in nature. Therefore, many studies are being conducted on their environmental behavior, toxicity evaluation, and removal, disposal, and analysis methods. Regulations have been set in many countries. An example of analysis method is the U.S. Environmental Protection Agency's EPA method 1633, which includes analysis of 40 PFAS compounds. PFAS measurement often requires analysis at low concentration levels, which makes it necessary to use high-performance mass detectors.

The primary challenge of LC condition optimization is to separate target substances. However, even if the eluent achieves good separations of target substances, if it is not suitable for mass spectrometry ionization, the sensitivity will be low. Thus, an in-depth consideration is required for the selection of an eluent. PFAS with carbon number of eight, such as PFOS and PFOA, and PFAS with even longer chains are generally analyzed using reverse-phase columns. However, strong acids and some important raw materials of fluorinated compounds (e.g., trifluoroacetic acid, with which a carbon number of two) are weakly retained on reverse-phase columns, and thus reversed-phase columns are not suitable for their simultaneous analysis.

In this application, we optimized LC separation conditions to achieve both good sensitivity and separation for short-chain PFAS, PFOS and PFOA, using a Shodex HILICpak VT-50 2D column; which is a polymer-based column modified with a quaternary ammonium functional group.

Methods

1. Column

Table 1 lists the specifications of Shodex HILICpak VT-50 2D used in this study.

2. Samples

Table 2 lists PFAS analyzed in this study. All reagents except DFA and PFPrA were mixed and dissolved in a 1:1 mixture of water and methanol (sample solution #1). DFA and PFPrA were dissolved separately from others in a 1:1 mixture of water and methanol (sample solution #2). This is to prevent the influence from MCA and TCA derived peaks which interfere with DFA and PFPrA peaks.

Table 1. Specifications of VT-50 2D

Column Size	2.0 mm I.D. x 150 mm
Particle Size	5 μm
Pore SIze	100 Å
Packing Material	Polyvinyl Alcohol
Functional Group	Quaternary Ammonium
pH Range	2 - 13
Max. Pressure	10 MPa
Max. Fow Rate	0.5 mL/min
Temp. Range	4 - 60 ℃
Housing Material	PEEK
Guard Column	VT-50G 2A

Table 2. Samples analyzed in the study

	Abb.	Formulae	Conc.
C8	PFOS[*1]	$C_8F_{17}SO_3H$	100 ng/mL
C8	PFOA[*1]	$C_7H_{15}COOH$	100 ng/mL
C4	PFBS	$C_4F_9SO_3H$	100 ng/mL
C4	PFBA	C_3F_7COOH	100 ng/mL
C3	PFPrA	C_2F_5COOH	10 μg/mL
C3	HFIP[*2]	$(CF_3)_2CHOH$	10 μg/mL
C2	TFA	CF_3COOH	10 μg/mL
C2	DFA	CHF_2COOH	10 μg/mL
C1	TFMS	CF_3SO_3H	100 ng/mL
C2	TCA[*3]	CCl_3COOH	10 μg/mL
C2	DCA[*3]	$CHCl_2COOH$	10 μg/mL
C2	MCA[*3]	$CH_2ClCOOH$	10 μg/mL

*1 PFOS and PFOA are included in the list of regulated compounds under the Stockholm Convention on Persistent Organic Pollutants (POPs Convention) and have been studied more extensively than other PFAS.

*2 Hexafluoro-2-propanol (HFIP), used as an eluent in GPC analysis of polyamides and polyesters, are sometimes defined as a PFAS.

*3 Trichloroacetic acid (TCA), Dichloroacetic acid (DCA), and Monochloroacetic acid (MCA) are not PFAS, and they are referred to as haloacetic acids along with substances like bromoacetic acid. They are often important substances to monitor in the field of water quality analysis, thus they were included in this application study.

3. LC and MS settings

Analysis conditions and mass detector settings used throughout the study are summarized below. Details of eluents studied are described in "Optimization of Eluent"

LC conditions
Column   : Shodex HILICpak VT-50 2D
Eluent   : 50 mmol/L NH₄HCO₃ aq./CH₃CN
              50 mmol/L CH₃COONH₄ aq./CH₃CN
Flow rate   : 0.2 mL/min
Detector   : ESI-MS (negative, SIM), PDA
Column temp.: 40 ºC

MS settings
Instrument   : Waters ACQUTY UPLC H-Class/SQD2
ESI source temp.   : 150 °C
Capillary voltage : 0.75 kV
Desolvatation
   Temp.   : 400 °C
   Gas flow rate   : 800 L/hr
Cone voltage   : 50 V (PFOS), 50 V (PFBS), 45 V (TFMS),
                     35 V (PFOA), 20 V (PFBA), 20 V (PFPrA),
                     30 V (DFA), 30 V (TFA), 20 V (HFIP),
                     20 V (TCA), 20 V (DCA), 20 V (MCA)
m/z (-)   : 499 (PFOS), 299 (PFBS), 149 (TFMS), 413 (PFOA),
                     213 (PFBA), 163 (PFPrA), 95 (DFA), 113 (TFA),
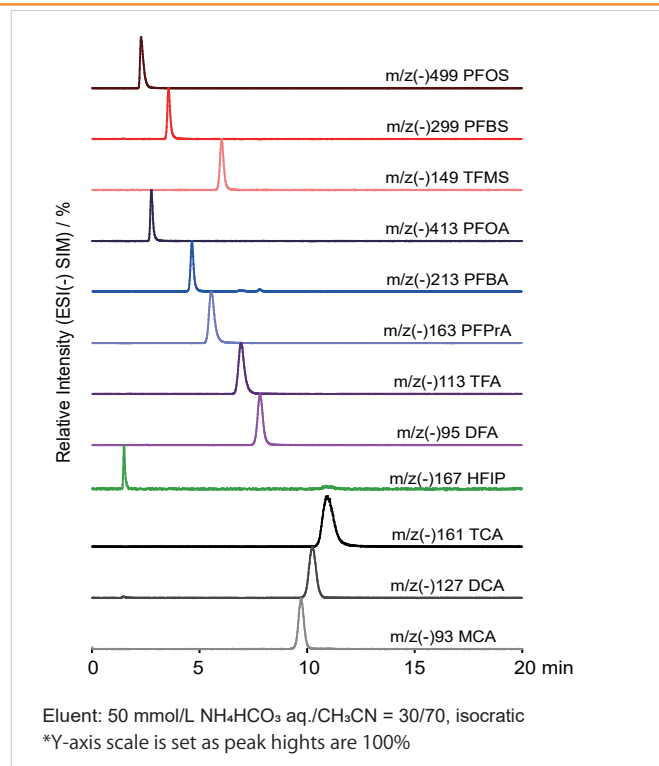   167 (HFIP), 161 (TCA), 127 (DCA), 93 (MCA)

Results

Optimization of Eluent

1. Acetonitrile Concentration

Using a mixed solvent of 50 mmol/L ammonium acetate aqueous solution and acetonitrile as an eluent, we examined the change in elution time of each analyte when the acetonitrile ratio was varied from 40 % to 80 % in 10 % increments. Figure 1 shows the chromatograms obtained with 70% acetonitrile. Figure 2 shows the relationships between acetonitrile ratio in the eluent and retention time (t_R).

The change in retention time exhibited complex behavior, such as the elution order of analytes being reversed depending on the acetonitrile ratio. The VT-50 2D column mainly works under hydrophilic interaction, but may also has ion-exchange and reverse-phase chromatography functions. The types of interactions and their strengths uniquely affected each analyte were very likely related to the combination of those separation modes.

2. Selection of Salt

Volatile acids or salts may be added to the eluent in LC/MS analysis. Ammonium acetate used in section 1 is volatile. We used another volatile salt, ammonium bicarbonate, in this study to examine its effects on sensitivity.

Aqueous solutions of ammonium acetate and ammonium bicarbonate were prepared in 50 mmol/L, then mixed with acetonitrile in a ratio of 30/70. Table 3 shows the comparison of peak S/N ratios. The S/N ratio percentage of ammonium acetate was calculated as considering the S/N ratio of ammonium bicarbonate as 100 %.
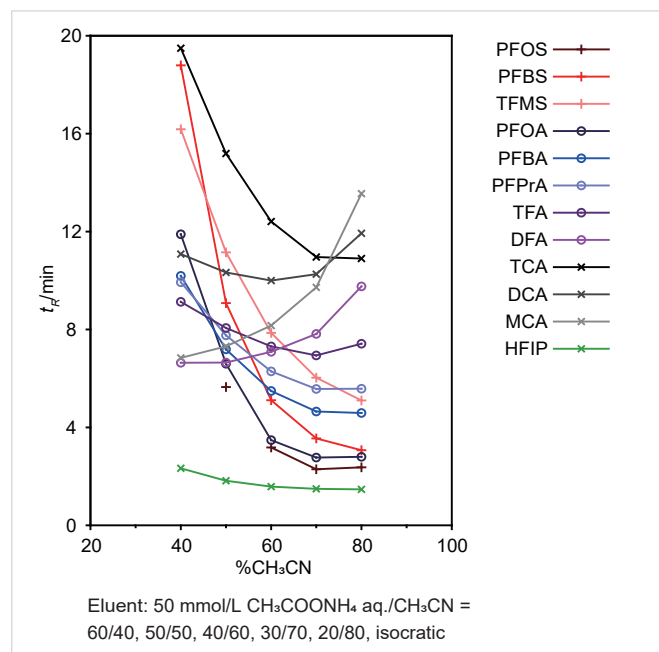

Eluent: 50 mmol/L NH₄HCO₃ aq./CH₃CN = 30/70, isocratic
*Y-axis scale is set as peak hights are 100%

Fig. 1 Chromatograms of PFAS analyzed in the study

Eluent: 50 mmol/L CH₃COONH₄ aq./CH₃CN = 60/40, 50/50, 40/60, 30/70, 20/80, isocratic

Fig. 2 Effects of acetonitrile ratio on retention time

Table 3. Effects of salt type on S/N

	NH₄HCO₃ aq.	CH₃COONH₄ aq.
PFOS	100 %	78 %
PFBS	100 %	46 %
TFMS	100 %	99 %
PFOA	100 %	130 %
PFBA	100 %	1565 %
PFPrA	100 %	603 %
TFA	100 %	541 %

Eluent: 50 mmol/L NH₄HCO₃ aq./CH₃CN = 30/70, isocratic
         50 mmol/L CH₃COONH₄ aq./CH₃CN = 30/70, isocratic

3. Concentration of Salt

Concentration of ammonium bicarbonate aqueous solution was varied while keeping the acetonitrile concentration in the eluent at 70 %. Figure 3 shows relationships between ammonium bicarbonate aqueous solution concentration in the eluent and retention time (t_R).
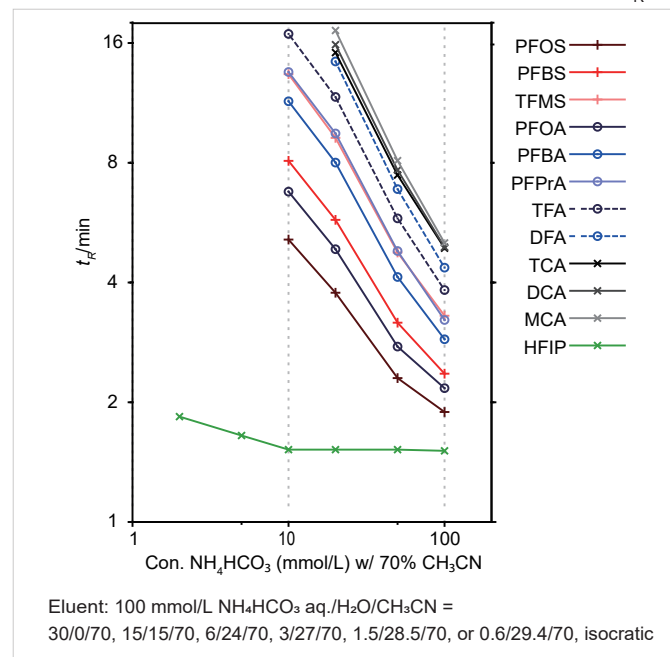

Eluent: 100 mmol/L NH₄HCO₃ aq./H₂O/CH₃CN = 30/0/70, 15/15/70, 6/24/70, 3/27/70, 1.5/28.5/70, or 0.6/29.4/70, isocratic

Fig. 3 Effects of salt concentration on retention time

A logarithm plot of the salt concentration against the retention time logarithm obtained a linear change. This suggests the presence of ion-exchange interactions between the column and the analytes.

4. pH

Next, the effects of eluent pH on retention time was examined. The pH of the eluent was adjusted by varying ratios of 50 mmol/L ammonium bicarbonate aqueous solution and 50 mmol/L ammonia water, while keeping the acetonitrile ratio at 70 %. The pH of 50-mmol/L ammonium bicarbonate aqueous solution before mixing with acetonitrile was 8.9, and the pH of the 50-mmol/L ammonia water was 9.7. Figure 4 shows the relationships between ammonia water ratio in the eluent and retention time (t_R).

When the pH of ammonium bicarbonate aqueous solution before mixing with acetonitrile was 8.9 to 9.7, there was no change in analytes' elution order. It was found that in general, higher pH resulted in stronger retention.
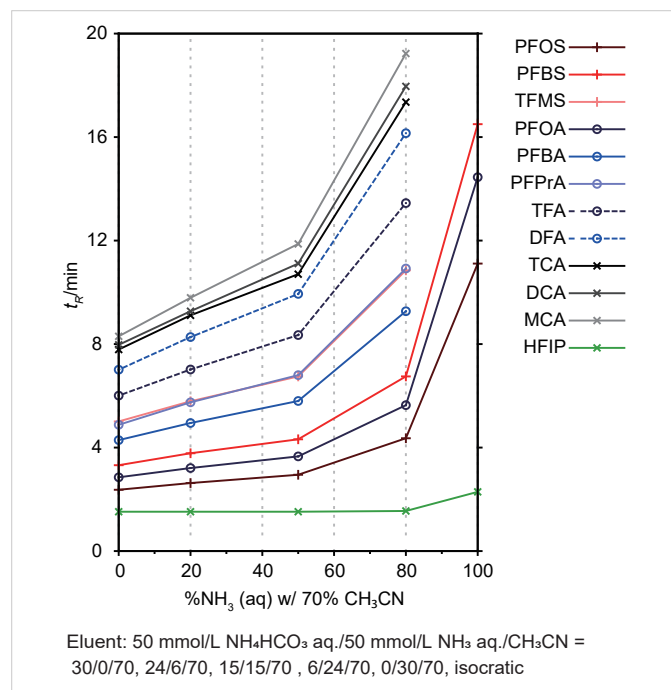

Eluent: 50 mmol/L NH₄HCO₃ aq./50 mmol/L NH₃ aq./CH₃CN = 30/0/70, 24/6/70, 15/15/70 , 6/24/70, 0/30/70, isocratic

Fig. 4 Effects of ammonia water ratio concentration on retention time

Conclusions

In this application, we achieved a simultaneous analysis of short-chain PFAS as well as long-chain PFAS such as PFOS and PFOA. From the perspective of sensitivity in mass detection, eluents with high acetonitrile ratios are advantageous. However, it was found that some analytes experienced stronger retention with higher acetonitrile ratios.

Further examination of salt concentration, salt type, and eluent pH, concluded that a 50-mmol/L ammonium acetate aqueous solution/acetonitrile = 30/70 was best suited for the simultaneous analysis of PFAS analyzed in this study.

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