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Determination of the best stationary phase for PFAS with LC-MS/MS

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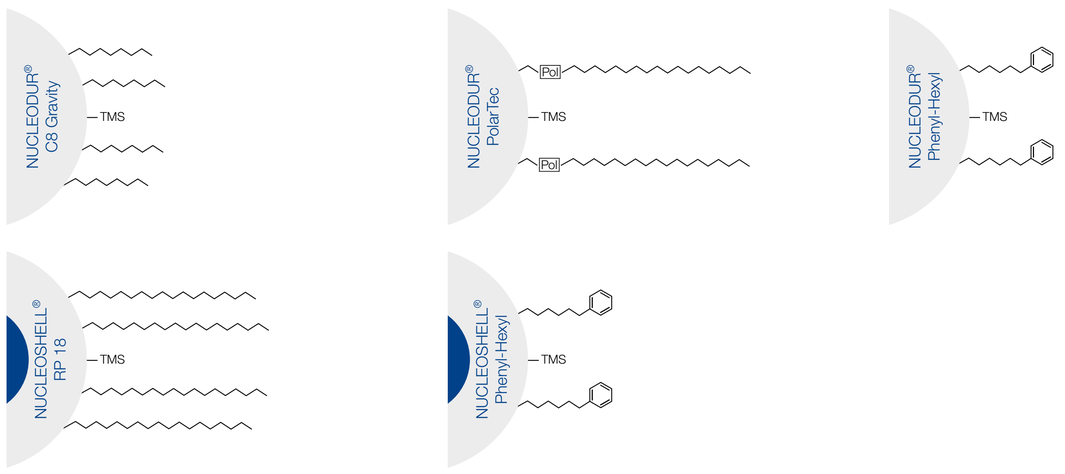
Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of persistent organic pollutants comprising up to 4700 different chemicals[1]. Due to their unique properties, they have been widely used as coatings and fire-fighting foams, resulting in high concentration levels in the environment. The consideration and analysis of these substances is becoming increasingly important due to their biomagnification and bioaccumulation, as well as new toxicological findings [2]. These include adverse effects on cholesterol levels, the immune system, especially in children, and possible neuro-, geno- and reproductive toxicity [3]. Due to their prevalence, PFAS are often analysed in environmental samples, food and drinking water as well as in biological samples, making it a major challenge to find an applicable method to analyse these different sample conditions over a wide range of concentrations [4].

The recognised analysis of PFAS involves LC-MS/MS and uses a reversed phase C_{18} mechanism. In this study, 29 analytes from the six major groups of PFAS, of perfluoroalkyl carboxylic acids, perfluoroalkyl sulfonic acids, fluorotelomer sulfonic acids, perfluoroether carboxylic acids, chlorinated perfluoroalkyl ether sulfonic acids and perfluorooctane sulfonamides, were investigated. To determine the most suitable stationary phase, a variety of 9 NUCLEODUR® and 6 NUCLEOSHELL® phases were tested and narrowed down to those with the least column bleed and the best seperation perfomance. These were further compared and are shown in figure 1.

Figure 1:



Five most suited columns and their stationary phases

HPLC Conditions

Eluent A	5 mM ammonium acetate in water
Eluent B	5 mM ammonium acetate in methanol
Gradient	hold 40 % B for 1 min, in 8 min from 40 % B to 95 % B, hold 95 % B for 3 min, in 0.1 min to 40 % B, hold 40 % B for 2.9 min
Flow rate	0.3 mL/min
Temperature	40 °C
Injection volume	2 µL
Interface	ESI
Polarity	negative

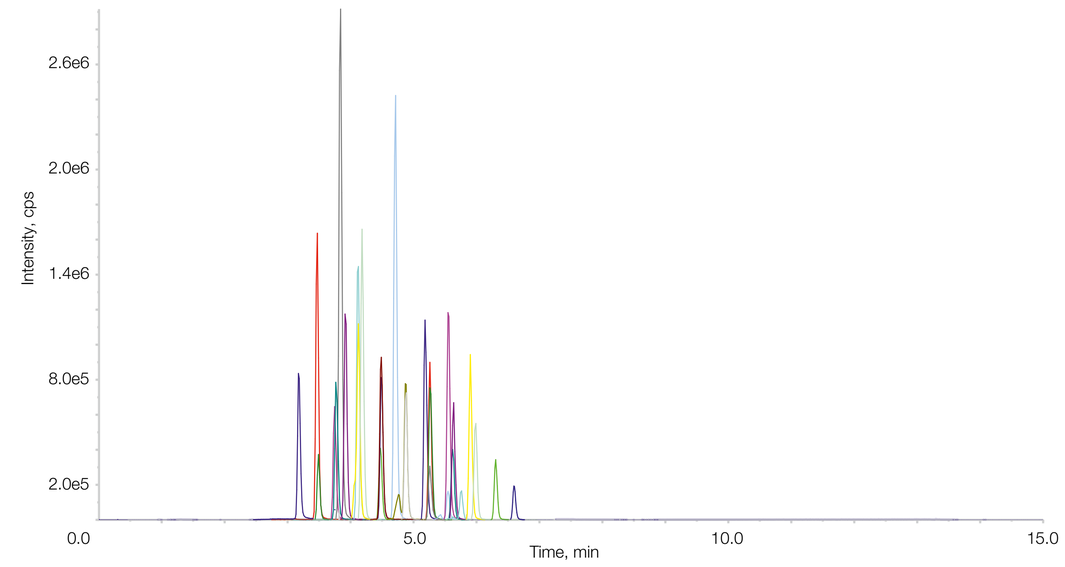
Results

		Selectivity	Area to Height	Peak Symmetry	Signal to Noise	Isomer Separation
NUCLEODUR®	C_8 Gravity	++	+	+++	+	+
NUCLEODUR®	PolarTec	+++	o	++	o	+
NUCLEODUR®	Phenyl-Hexyl	++	+	+	+	+++
NUCLEOSHELL®	RP18	o	+++	o	++	+++
NUCLEOSHELL®	Phenyl-Hexyl	++	++	++	+++	+++

Table 1: Results of the comparison of five HPLC columns

Large Volume Injection

Figure 2:



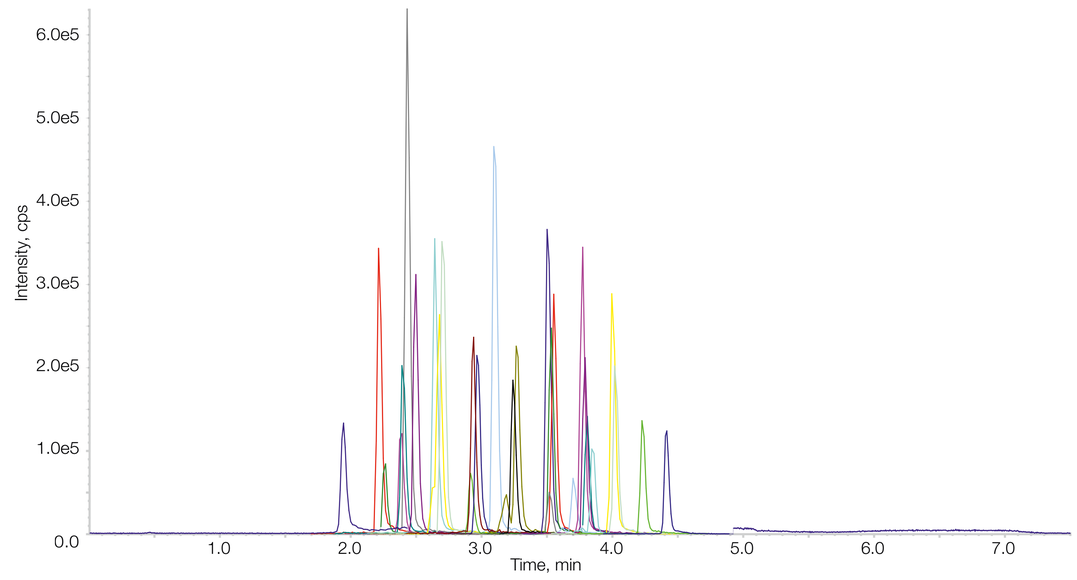
Chromatogram of a standard solution (β = 0.125 ng/mL)

HPLC Conditions for Large Volume Injection

Column	EC 100/2 NUCLEODUR® Phenyl-Hexyl, 3 µm (REF 760576.20)
Eluent A	5 mM ammonium acetate in water
Eluent B	5 mM ammonium acetate in methanol
Gradient	hold 5 % B for 0.5 min, in 1 min from 5 % B to 60 % B, in 5.5 min from 60 % B to 95 % B, hold 95 % B for 5 min, in 0.1 min to 5 % B, hold 5 % B for 2.9 min
Flow rate	0.3 mL/min
Temperature	40 °C
Injection volume	80 µL
Sample Conditions	Ratio of 50:50 Water/Methanol and 1 % glacial acetic acid

Shorter Analysis Time and UHPLC

Figure 3:

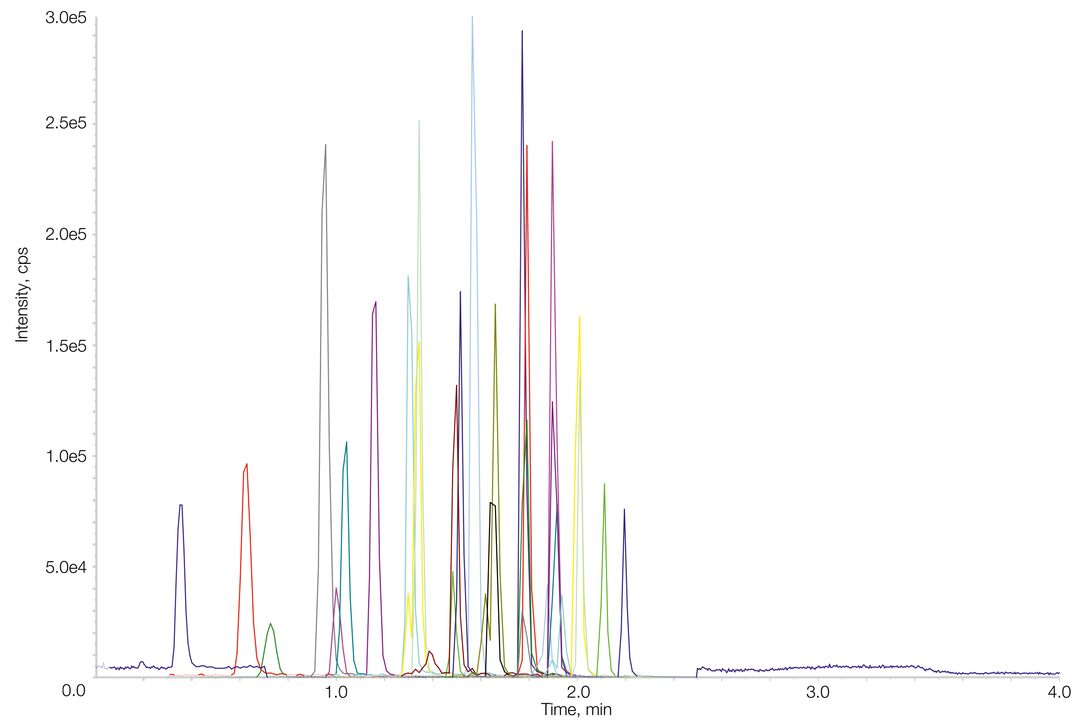


Chromatogram of a method in 7.5 minutes (β = 1 ng/mL)

Conditions

Column	EC 50/2 NUCLEOSHELL®Phenyl-Hexyl, 2.7 µm (REF 763732.20)
Gradient	hold 5 % B for 0.5 min, in 0.5 min from 5 % B to 60 % B, hold 60 % B for 0.5 min, in 3 min from 60 % B to 95 % B, hold 95 % B for 5 min, in 0.05 min to 5 % B, hold 5 % B for 1.45 min

Figure 4:



Chromatogram of a UHPLC method in 4 minutes (β = 1 ng/mL)

Conditions

Column	EC 50/2 NUCLEODUR® Phenyl-Hexyl, 1.8 µm (REF 760566.20)
Gradient	hold 40 % B for 0.25 min, in 2 min from 40 % B to 95 % B, hold 95 % B for 1.75 min, in 0.1 min to 5 % B, hold 5 % B for 2.9 min
Flow rate	0.9 mL/min

Conclusion

This study presents the most suitable separation columns with appropriate gradients for the analysis of PFAS in terms of performance, large volume injection and reduced analysis times. After comparing 15 different stationary phases, the NUCLEOSHELL® Phenyl-Hexyl gave the best overall results with a low signal-to-noise ratio and good peak shapes.

In the field of large volume injection, high interaction of the analytes with the stationary phase is required. For this reason the PolarTec showed the best conditions with a different selectivity and therefore much higher retention times. To ensure a good peak shape, it is necessary that the sample is present in a certain water/methanol ratio and is acidified. However, acidification of the sample led to further interactions of the PolarTec stationary phase with the analytes, which meant that reproducibility could not be guaranteed. In tests with other HPLC columns, the best results were subsequently obtained with NUCLEODUR® Phenyl-Hexyl. With this method, good reproducibility was achieved and standards as low as 1 ng/L could be analysed.

Another aspect that is becoming increasingly important is the cost effectiveness of the method, so analysis time and solvent consumption are important factors. As the NUCLEOSHELL® Phenyl-Hexyl proved to be a good phase, a shorter column was used to create a method with an analysis time of 7.5 minutes instead of 15 minutes. In addition, NUCLEODUR® Phenyl-Hexyl with a particle diameter of 1.8 µm confirmed to be a very good column for UHPLC, allowing the analysis time to be further reduced to 4 minutes by using a higher flow rate.

References

[1] Umweltbundesamt, PFAS Gekommen, um zu bleiben, Schwerpunkt, 2020

[2] EFSA CONTAM Panel et al., Risk to human health related to the presence of perfluoroalkyl substances in food, EFSA Journal, 2020

[3] Bundesinstitut für Risikobewertung, PFAS in Lebensmitteln: BfR bestätigt kritische Exposition gegenüber Industriechemikalien, Stellungnahme Nr. 020/2021, 2021

[4] A. Androulakakis et al., Current progress in the environmental analysis of poly- and perfluoroalkyl substances (PFAS), Environmental Science Advances, 2022