SEPARATION OF BRANCHED PFOS ISOMERS BY UPLC WITH MS/MS DETECTION

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OVERVIEW

UPLC is shown to achieve rapid separation of perfluoroalkane sulphonate homologs and isomers. Tandem quadrupole MS/MS detection provides rich spectral information to facilitate compositional analysis and structural elucidation of perflourinated alkane sulphonates. Sensitive quantitation of PFOS isomers is demonstrated using the mass spectrometer in the highly selective MRM mode.

INTRODUCTION

Perfluorooctane sulfonate (PFOS) is a fully fluorinated anion that can be formed by degradation from a large group of related substances, close to 100 in total, referred to as PFOS-related substances. These substances are used in a wide variety of applications including the paper and packaging industries, the textiles/upholstery industries and as a surfactant. Extensive biological monitoring of PFOS and other related perfluorinated compounds in recent years has revealed PFOS as a global pollutant[1] and perfluorinated compounds have even been found in remote areas of the Artic.[2]

PFOS has been detected at parts per billion levels in human serum and the livers of fish, birds, and marine mammals[2,3,4]. Toxicity tests in rodents have raised concerns about potential developmental, reproductive, and systemic effects of PFOS. Evidence of the toxic effects have been reported. It is for this reason that sensitive detection of all perfluorinated compounds including PFOS and its branched isomers is of great importance.

A method based on LC/MS/MS analysis using negative ion electrospray is described. Rapid separation of PFOS isomer groups was performed by ACQUITY UPLC™ with tandem quadrupole mass spectrometry detection. MS/MS detection provided useful structural information for analysis of isomer composition and proved suitable for sensitive quantitative analysis.

METHODS

LC Conditions—Compositional Analysis

LC System: Waters ACQUITY Ultra Performance LC™

LC Column: Waters ACQUITY UPLC™ BEH Shield RP18 2.1 x 100mm, 1.7µm.

Column temperature: 50°C

Mobile phase: A: 10mM aqueous ammonium acetate
B: 10mM ammonium acetate in 80/20 v/v methanol/acetontirile

Time (min)	Flow Rate µL min^{-1}	% A	% B	Curve
Initial	600	53	47	Initial
5.0	600	45	55	6
6.0	600	45	55	6
6.1	600	53	47	1

LC Conditions—Total PFOS Quanitative Analysis

LC Column: Waters ACQUITY UPLC™ BEH C8 2.1 x 50mm, 1.7µm.

Column temperature: 50°C

Time (min)	Flow Rate µL min-1	% A	% B	Curve
Initial	600	53	47	Initial
1.0	600	10	90	6
1.2	600	10	90	6
1.3	600	53	47	1

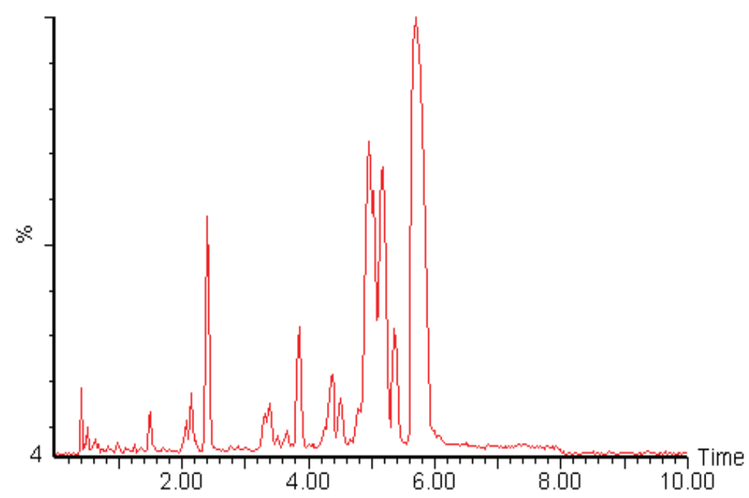
MS CONDITIONS

MS System: Quattro Premier™ XE
Ion Mode: Negative ion ESI
Cone Voltage: 20-50V
Collision Energy: 25-40eV
Full scan data: m/z 100-800
Product ion scan data: m/z 65-510 from precursor m/z 499
MRM data: See table below

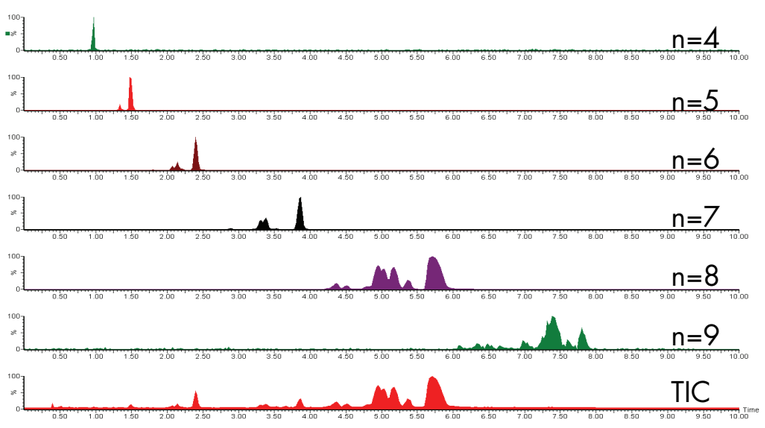
Channel	Precursor Ion	Product Ion	Dwell (s)	Cone (V)	Collision Energy (eV)
1	499	80	0.10	50	50
2	499	99	0.10	50	40
3	499	130	0.10	50	40
4	499	169	0.10	50	40

RESULTS AND DISCUSSION - COMPOSITIONAL ANALYSIS

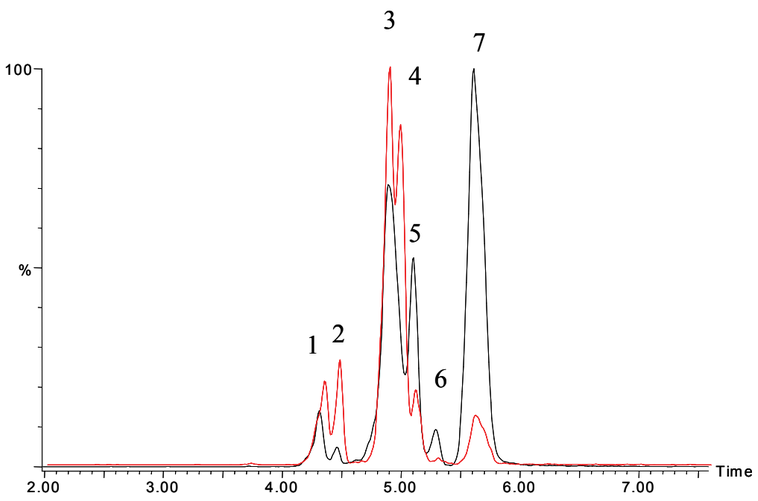
Analytical methods which provide good chromatographic separations of perfluorinated alkane sulphonates, when coupled with mass spectroscopic detection, allow for the characterisation and/or quantitation of complex mixtures. The chromatographic separation of a Technical Grade sample of PFOS in under 7 minutes using Waters ACQUITY UPLC™ technology is shown below.



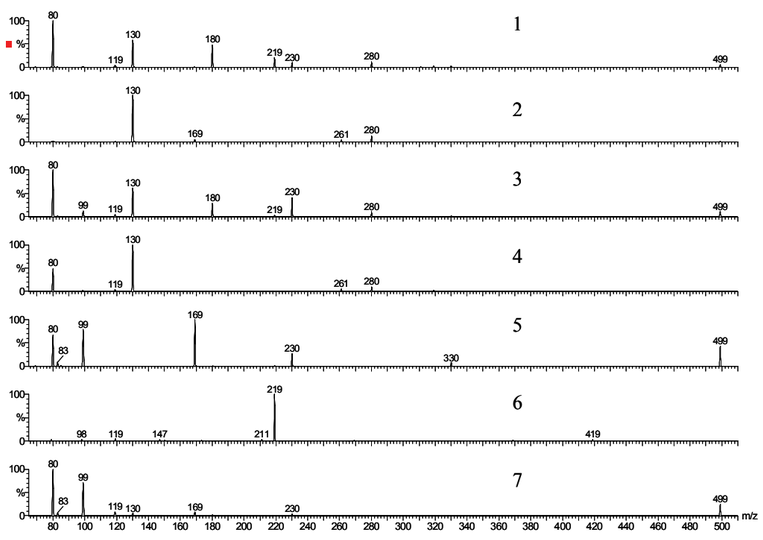
MS data were collected in full scan mode. Extracted ion chromatograms (XICs) corresponding to the homologous series $[C_nF_{n+1}SO_3]^-$ from n=4 to n=9 are shown below. This shows the Technical Grade product to contain material of different chain lengths. The number of isomers for each homolog is seen to increase with n as expected.



Further experiments focussed upon the group of isomers associated with PFOS (n=8 homolog from above chromatogram). A product ion scan experiment monitoring product ions of m/z 499 was conducted. Below are the XICs for product ions m/z 80 and m/z 130 (red trace).



The chromatograms show different relative intensity profiles depending upon which product ions are monitored. This indicates that the relative intensities of product ions in the spectra of PFOS isomers are different. These differences are the result of structural differences between isomers. Mass spectrometry can therefore provide useful information for structural elucidation. To illustrate this further the spectra of several isomer groups labelled in the chromatograms are shown below.



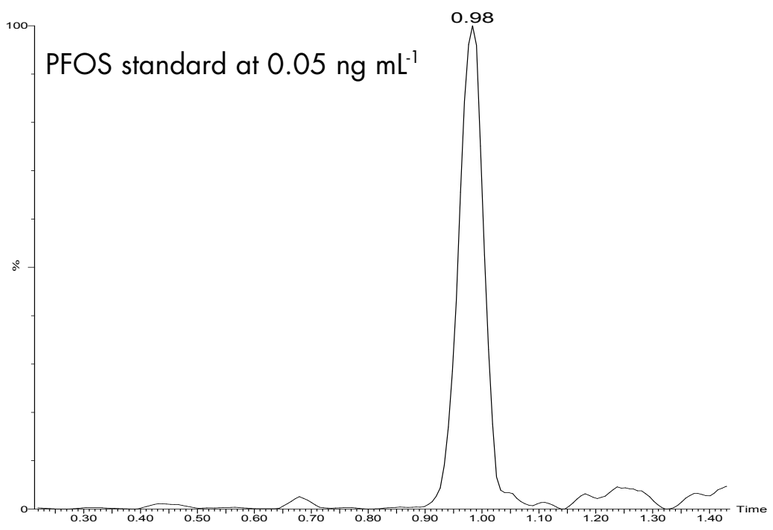
Further investigations revealed a minor eighth peak with common fragments at Rt 3.71 min and there are clearly further unresolved components within the labelled isomer groups. Spectral information was obtained over the range *m/z* 65-510 with the use of tandem quadrupole MS/MS enabling structurally significant low mass fragments to be observed which would not be seen on a conventional ion trap mass spectrometer due to low mass cut-off.

The peaks observed in the product ion spectra from the precursor *m/z* 499 generally fit the series $[C_nF_{2n+1}]^-$ for peaks from *m/z* 119 to *m/z* 419; **or** $[C_nF2_nSO_3]^-$ which is characterised by peaks from *m/z* 80 to *m/z* 330. Previous work[5] has postulated that the relative abundance of such series may be an indicator of degree of branching in the isomers, which is an important structural characteristic.

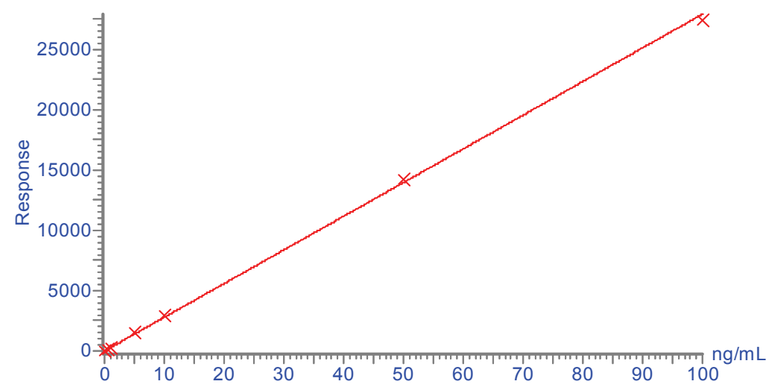
Further optimisation of chromatographic separation and MS/MS fragmentation is proposed in order to enable better characterisation of the complex PFOS mixtures.

RESULTS AND DISCUSSION - QUANTITATION

In addition to determining the structural composition of PFOS it also important to offer sensitive quantitative methods for the analysis of the material in a variety of environmental matrices. To improve quantitation a BEH C8 column chemistry was used to produce a rapid separation of PFOS as a single chromatographic peak. The mass spectrometer was operated in MRM using the conditions described in the method section. MRM use offers the ultimate combination of selectivity and sensitivity as demonstrated by the chromatogram below. This shows the trace for the MRM tranisition m/z 499>80. The calibration curve for PFOS over the range 0.01-100 ng mL^{-1} is also shown.



Compound name: PFOS ii
Correlation coefficient: r = 0.999646, r^2 = 0.999292
Calibration curve: 279.193 * x + 0.871978
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



CONCLUSIONS

The combination of UPLC with tandem quadrupole mass spectrometry has considerable potential for the determination of PFOS composition and quantitation.

Rapid separation of perflourinated alkane sulphonate homologs can be achieved in under 7 minutes.

Tandem quadrupole mass spectral data provides useful information for determining the structural composition of PFOS isomers.

The use of MRM enables highly selective detection of PFOS at low levels.

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