Chromatographic separation of branched perfluorooctanesulphonate isomers by UPLC with tandem quadrupole MS/MS detection

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Introduction

Perfluorooctanesulfonate (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] Perfluorinated compounds have been found in remote areas of the Arctic.[2] PFOS has been detected at parts per billion levels in human serum and the livers of fish, birds, and other 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. The assay was linear over the range 0.1-500ng mL^{-1}. The use of MS/MS detection provided useful structural information for analysis of isomer composition.

Materials and Methods

Standards and Reagents

Standards of technical perfluorooctanesulfonate potassium salt in methanol were provided by Wellington laboratories (Guelph, Ontario, Canada). Acetonitrile, Methanol (Optima grade) and Water (HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Ammonium Acetate was purchased from Sigma Aldrich (St. Louis, MO, USA).

Instrumentation

Chromatographic separations were preformed using an Acquity Ultra Performance Liquid Chromatographic (UPLC) system™ (Waters Corporation, Milford, MA, USA). The instrument is capable of delivering mobile phase and withstanding system pressures up to 15000 psi. Gradient elution of the PFOS isomers was performed using an Acquity UPLC™ column (BEH Shield RP 18, 2.1 x 100mm, 1.7µm) (Waters Corporation, Milford, MA, USA) at a flowrate of 600µL min^{-1}. The column temperature was maintained at 50 ^{0}C. The linear gradient was operated from 47 to 55% eluent B over 5 minutes, then held at 55% eluent B for 1 minute. Eluent A was 10 mM aqueous ammonium acetate, and eluent B was 10 mM ammonium acetate in methanol:acetonitrile 80:20 (v/v).

All of the mass spectra were acquired using a Waters Micromass Quattro Premier XE Tandem Quadrupole Mass Spectrometer with a Z-spray interface (Waters Corporation, Manchester, UK) operated under negative ion electrospray mode. Cone voltage and collision energies were optimised by infusion of a standard of concentration 1µg mL^{-1} using the built in syringe pump at a flow rate of 10µL min^{-1}. The capillary voltage was 3.5 kV. The source block and desolvation heaters were 130 and 400 ^{0}C respectively. The cone gas and desolvation gas flow rates were 50 and 750 L h^{-1}. Argon was used as the collision gas 0.4 mL min^{-1}. Optimised operating parameters are listed in Table 1.

Channel	Precursor	Product	Dwell (s)	Cone (V)	Collision (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

Table 1.

Optimised MS/MS conditions

Quantitation was achieved using multiple reaction monitoring (MRM) mode for several Precursor to Product transitions. Dwell times were 0.10 s. Each ion monitored used the optimal conditions listed in Table 1. Full scan MS/MS was employed to examine different fragmentation ratios of the PFOS isomers *(Figure 2).*

Results and Discussion

Analytical methods which can provide good chromatographic separations of PFOS coupled with mass spectrometric detection allow for the characterisation and/or quantitation of multiple isomers. Chromatographic separation using Waters Acquity UPLC™ Technology allowed several PFOS isomer groups to be distinguished in under 6.0 minutes *(Figure 1).*

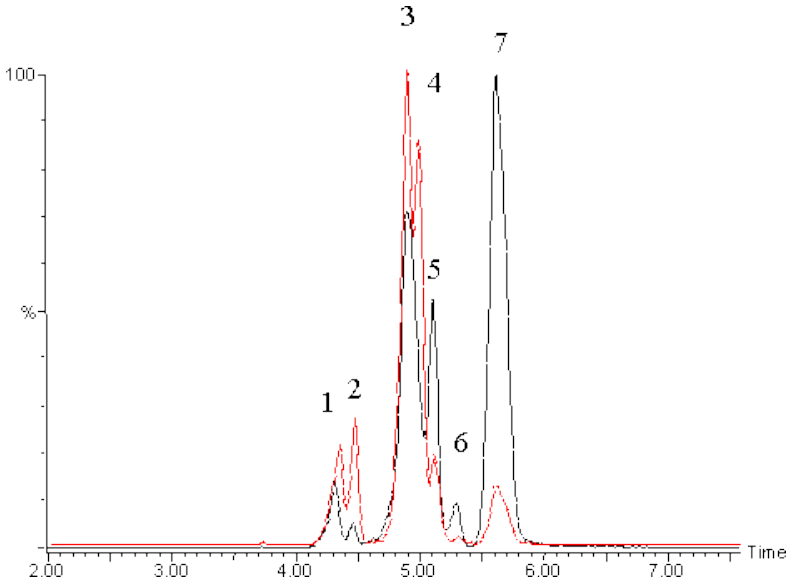

Figure 1

Chromatographic separation of PFOS isomer groups using full scan MS/MS monitoring products ions of m/z 499. Extracted ion chromatograms of m/z 80 and 130 (red trace) are shown.

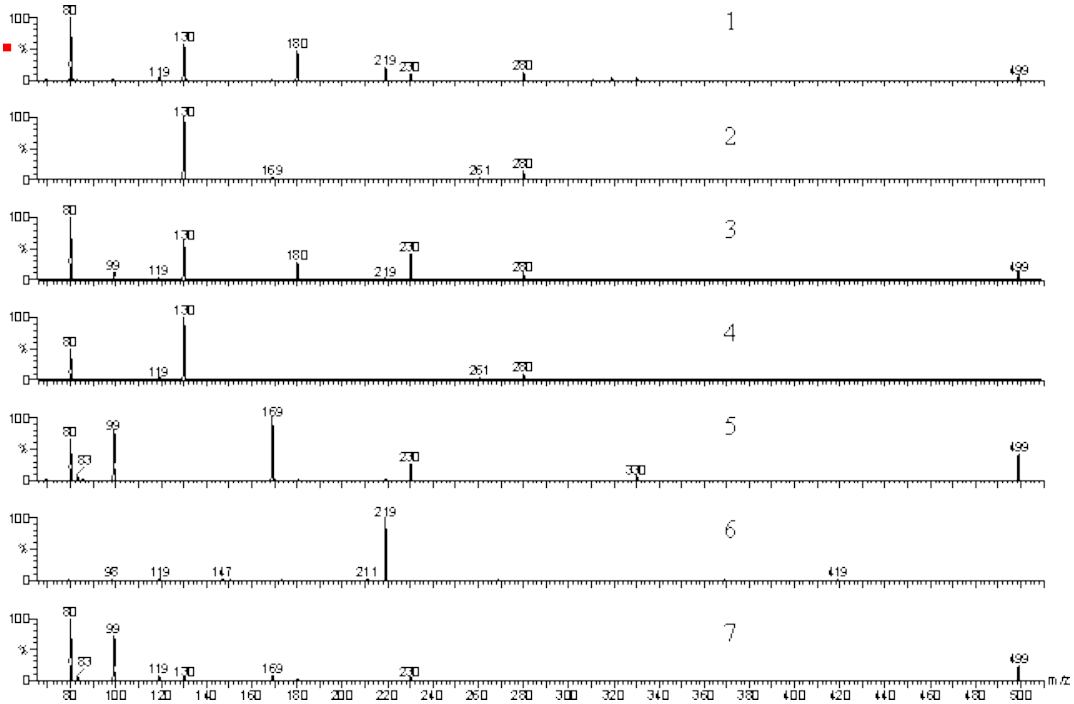

Figure 2

MS/MS spectra corresponding to Chromatographic peaks labelled (1) –(7) showing different fragmentation ratios of PFOS isomers.

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.

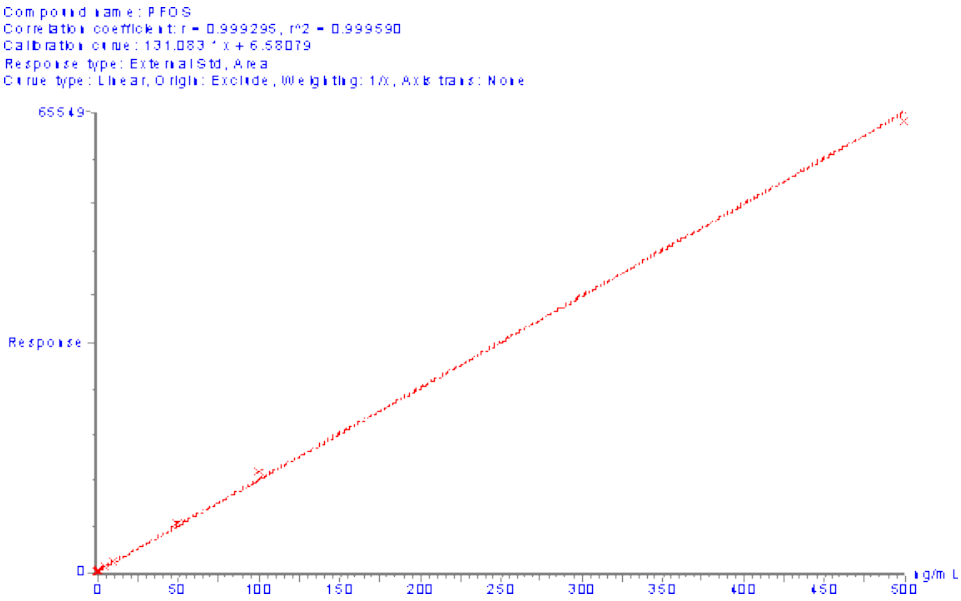

Figure 3

Calibration curve of standard solutions of technical PFOS in the range 0.1-500 ng mL^{-1}.

Quantitative determination of PFOS is of major interest and the Figure 3 demonstrates the excellent linearity obtained using this method over the range 0.1-500 ng mL^{-1}.

Acknowledgements

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