

Marian Twohig¹, Nicholas Ellor¹, Keith Worrall², Tim Jenkins², Gordon Kearney²

¹Waters Corporation, 100 Cummings Center, Beverly, MA 01915, USA;

²Waters Corporation, Atlas Park, Simonsway, Manchester M22 5PP, UK

SUMMARY

Waters® ACQUITY Ultra Performance LC™ (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 perfluorinated alkane sulphonates. Sensitive quantitation of perfluorinated octyl sulphonate (PFOS) isomers is demonstrated using the mass spectrometer in the highly selective multiple reaction monitoring (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 pollutant¹ and perfluorinated compounds have even 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 marine mammals.^{2,3,4} Toxicity tests in rodents have raised concerns about potential developmental, reproductive and systemic effects of PFOS. It is for this reason that sensitive detection of all perfluorinated compounds including PFOS and its branched isomers is of great importance.

EXPERIMENTAL DESCRIPTION

An LC/MS/MS method of analysis using negative ion electrospray is described. Separations were performed using a Waters ACQUITY Ultra Performance LC system. Sample components were separated using a Waters ACQUITY UPLC column and gradient elution. All mass spectra were acquired using a Waters Quattro Premier™ XE tandem quadrupole mass spectrometer with ZSpray™ interface. When operated in negative ion electrospray mode, the mass spectra acquired provided useful structural information for analysis of isomer composition and proved suitable for sensitive quantitative analysis.

In the first instance, UPLC/MS/MS was shown to provide fast and efficient separation of perfluorinated alkyl sulphonates in the homologous series containing C₄ to C₉. Rapid separation of the PFOS isomer group was achieved and structural information for isomers was obtained from analysis of product ion spectra. Subsequent analysis targeted the PFOS isomer group demonstrating the quantitative potential of the UPLC/MS/MS approach.



Waters ACQUITY UPLC and Quattro Premier XE Mass Spectrometer.

EXPERIMENTAL CONDITIONS**LC conditions - Compositional Analysis**

System: ACQUITY Ultra Performance LC
 Column: ACQUITY UPLC BEH Shield RP18
 2.1 x 100 mm, 1.7 μ m.
 Column temp: 50 °C
 Mobile phase: A: 10 mM aqueous ammonium acetate
 B: 10 mM ammonium acetate in 80/20 v/v methanol/acetone
 Flow rate: 600 mL min⁻¹
 Gradient: Time (mins) %B
 0.0 47
 5.0 55
 6.0 55
 6.1 47

LC conditions – Total PFOS Quantitative Analysis

As for compositional analysis except:

Column: Waters ACQUITY UPLC BEH C8
 2.1 x 50 mm, 1.7 μ m.
 Gradient: Time (mins) %B
 0.0 47
 1.0 90
 1.2 90
 1.3 47

MS Conditions

MS System: Quattro Premier XE
 Ion Mode: Negative ion ESI
 Cone Voltage: 20 - 50 V
 Collision Energy: 25 - 40 eV
 Full scan data: m/z 100 – 800
 Product ion scan: m/z 65 - 510 (precursor m/z 499)
 MRM data: See Table 1 below

Software

Data was acquired and processed with Waters MassLynx™ Software.

Channel	Precursor ions m/z	Product ions m/z	Dwell Time (s)	Cone (V)	Collision Energy (eV)
1	499	80	0.1	50	50
2	499	99	0.1	50	40
3	499	130	0.1	50	40
4	499	169	0.1	50	40

Table 1. MRM conditions for monitoring of PFOS.

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 characterization and/or quantitation of complex mixtures.

The chromatographic separation of a Technical Grade sample of PFOS in under seven minutes using Waters

ACQUITY UPLC Technology is shown in Figure 1 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 in Figure 2 below. This shows that the Technical Grade product contains material of different chain lengths. The number of isomers for each homolog is seen to increase with n as expected.

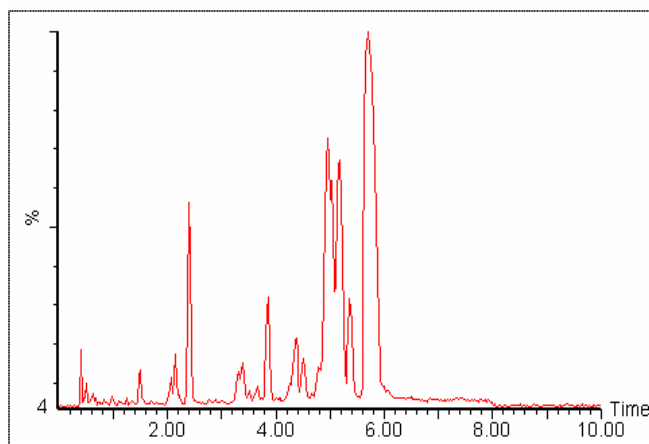


Figure 1. Total Ion Chromatogram of Technical Grade PFOS Sample.

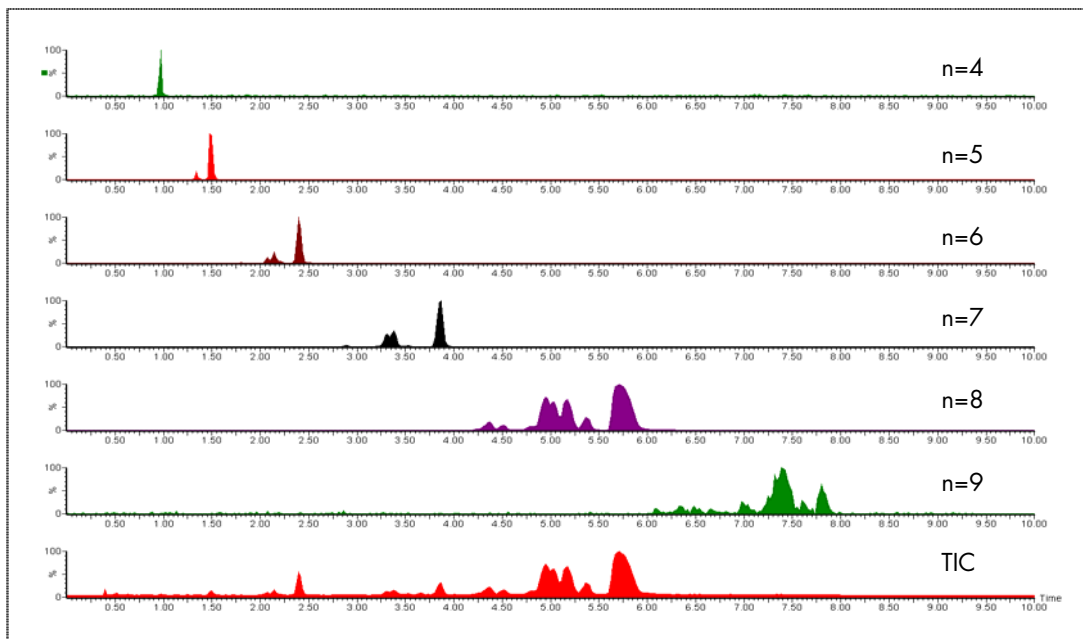


Figure 2. Extracted ion chromatograms from sample of Technical Grade PFOS.

Further experiments focused upon the group of isomers associated with PFOS ($n=8$ homolog from Figure 2). A product ion scan experiment monitoring product ions of m/z 499 was conducted. Below, in Figure 3, 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 variations 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 in Figure 5 overleaf. Further investigations revealed a minor eighth peak with common fragments at R_t 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** $[C_nF_{2n}SO_3]^-$, which is characterized by peaks from m/z 80 to m/z 330.

Previous work⁵ 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. In particular, the $[C_nF_{2n+1}]^-$ ions are indicative of cleavage in the α -position of a CF_3 branched chain and the $[C_nF_{2n}SO_3]^-$ series of the linear alkyl sulphonate portion of the molecule (Figure 4).

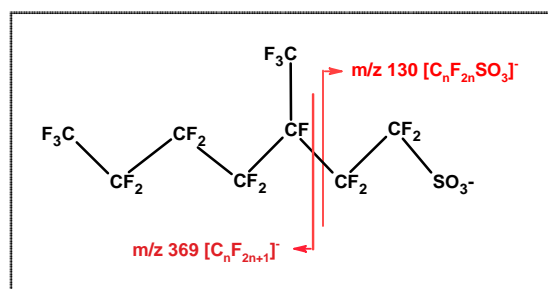


Figure 4. Ions resulting from alpha-Cleavage of branched PFOS.

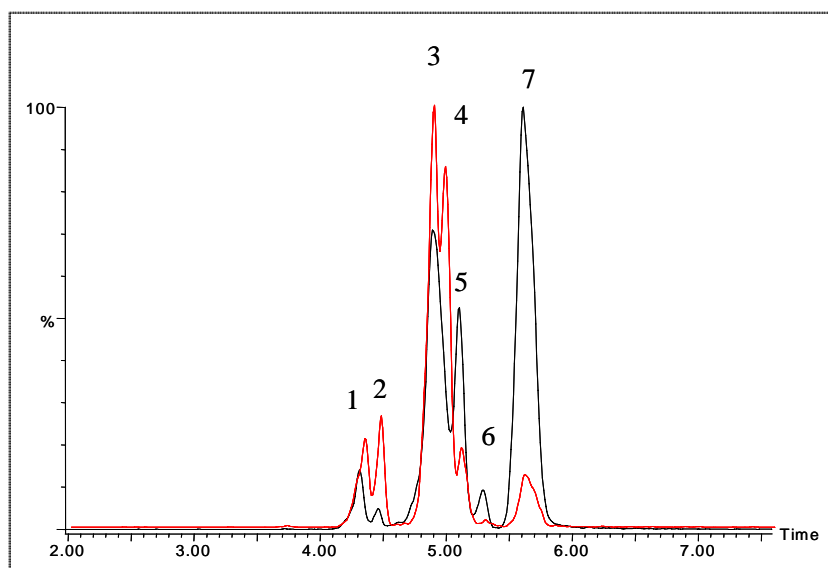


Figure 3. Extracted ion chromatograms for m/z 80 and m/z 130 (red trace).

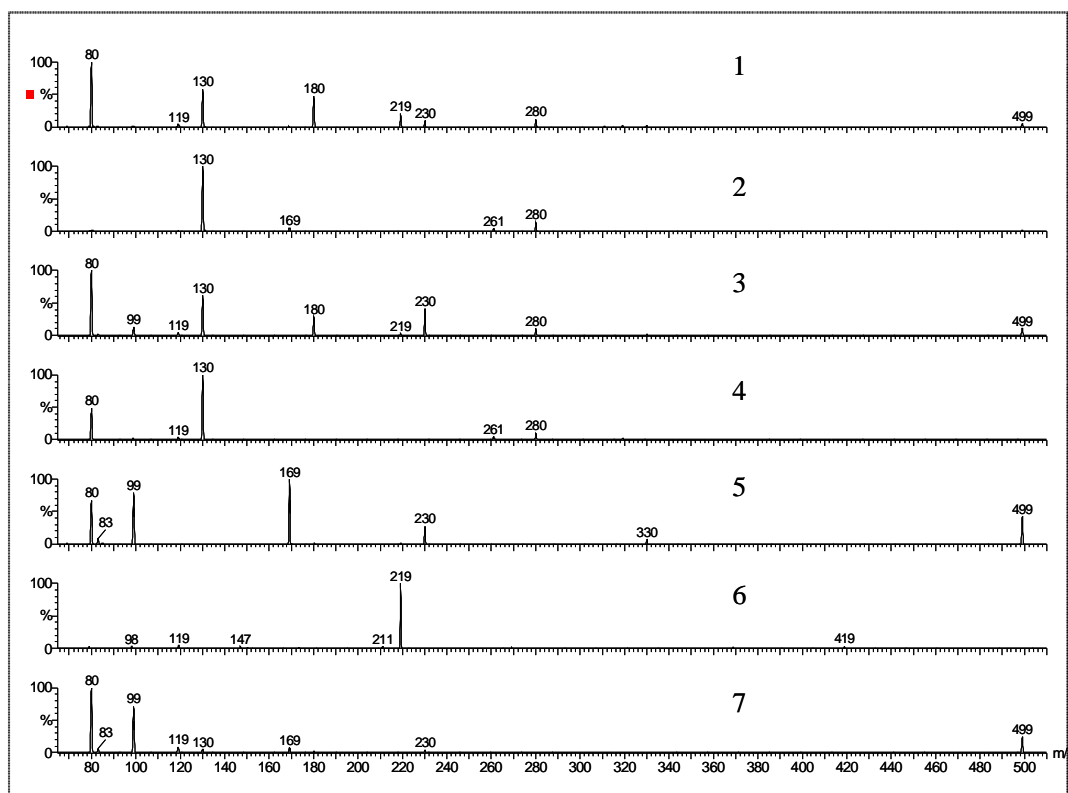


Figure 5. Product ion spectra corresponding to peaks in the UPLC separation of PFOS isomers shown in Figure 3.

The mixture analyzed is a complex one and the spectra recorded show the characteristic fragments associated with PFOS. Further work with branched model compounds would facilitate the assignment of exact structures. Nevertheless, it is clear that MS/MS can produce useful spectral information containing structurally significant information.

Further optimization of chromatographic separation and MS/MS fragmentation is proposed in order to enable better characterization of complex PFOS mixtures. Investigation of simpler systems containing specific isomers will enable better characterisation of the perfluorinated alkyl compounds.

The obvious differences in response factor also have an implication for accurate quantitation of PFOS mixtures.

Quantitative Analysis

In addition to determining the structural composition of PFOS it is 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 mode using the conditions described in the method section. MRM use offers the ultimate combination of selectivity and sensitivity as demonstrated by the chromatogram shown in Figure 6. This shows the trace for the MRM transition m/z $499 > 80$. The calibration curve for PFOS over the range 0.01 - 100 ng/mL is also shown (Figure 7).

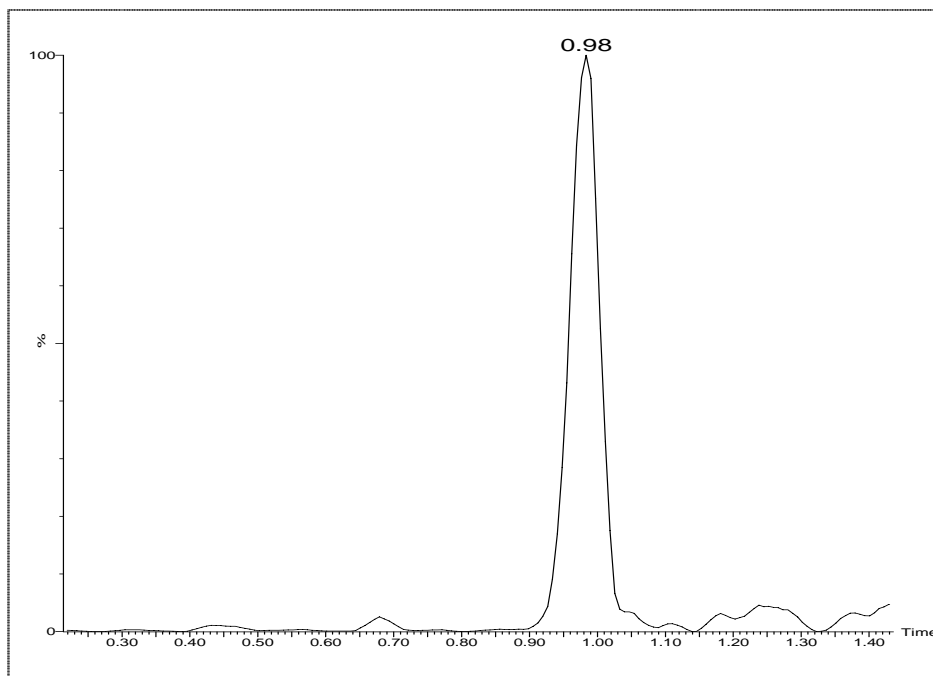


Figure 6. PFOS standard at 0.05 ng/mL.

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

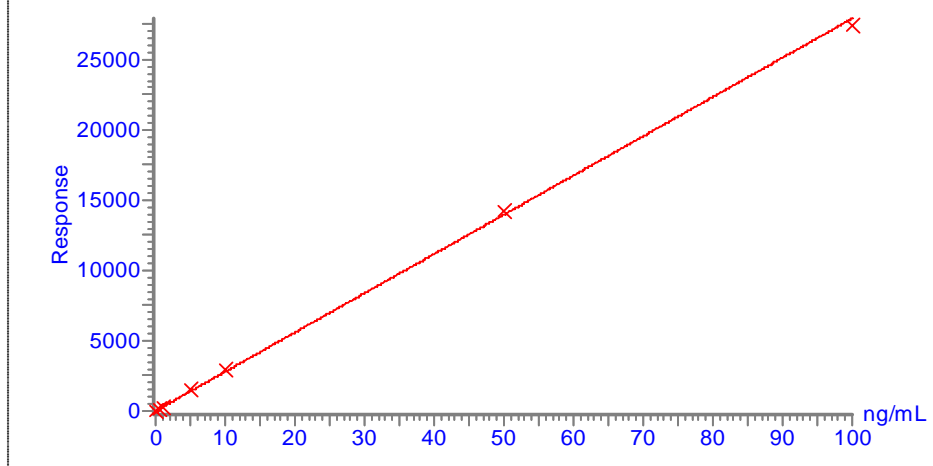


Figure 7. PFOS calibration curve.

CONCLUSIONS

The combination of UPLC with tandem quadrupole mass spectrometry has considerable potential for the compositional analysis and quantitative determination of perfluorinated alkyl compounds.

Rapid separation of perfluorinated alkane sulphonate homologs can be achieved in under seven 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.

REFERENCES

1. Giesy J.P., Kannan K., *Environ. Sci. Technol.* 35 (2001) 1339.
2. Bossi R, Riget FF, Dietz, Sonne C, Fauser P, Dam M, Vorkamp K. *Environ Pollut.* 2005 Jul; 136 (2):323-9.
3. Olsen G.W., Church, T.R., Miller J.P., Burris J.M., Hansen K.J., Lundberg J.K., Armitage J.B., Herron R.M., Medhdizadehkashi Z., Nobiletti J.B., O'Neill M.E., Mandel J.H., Zobel L.H., *Environmental Health Perspectives.* Vol 111, No.16, Dec 2003.
4. Koichi I, Okado F, Ito R, Kawaguchi M, Okanouchi N, Nakazawa H., *Journal of Chromatography B*, 810 (2004) 49-56.
5. Langlois I, Oehme M; *Organohalogen Compounds*, Volume 66 (2004) 3973-3978.

ACKNOWLEDGEMENT

The authors would like to thank Wellington Laboratories (Guelph, Ontario, Canada) for the provision of perfluorinated standards.

WATERS CORPORATION
34 Maple St.
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com

Waters

For Complete  Confidence

Waters, ACQUITY Ultra Performance LC, ACQUITY UPLC, UPLC, Quattro Premier, ZSpray, and Masslynx are trademarks of Waters Corporation.
All other trademarks are the property of their respective owners.
©2006 Waters Corporation Produced in the U.S.A. May 2006 720001694EN SE-PDF



001