Identification of the isomer composition in technical perfluorooctane sulfonate solution by LC-ESI(-)-IT-MS/MS

Ingrid Langlois[1], Michael Oehme[1]

[1]University Basel

Introduction

Perfluorinated compounds (PFC) have been detected in the environment worldwide. Among them, perfluorooctanesulfonate (PFOS, $C_8F_{17}SO_3^-$) is the most dominant contaminant[1]. Its presence in biota can be explained by the application of PFOS for more than 50 years as well as its formation by biodegradation from perfluorooctanesulfonamide[2]. PFOS is very persistent and has shown different toxicological effects such as peroxisome proliferation and inhibits gap junction intercellular communication[3].
Reversed-phase-HPLC combined with triple quadrupole (TQ) mass spectrometry (MS) is the method of choice for the quantification of PFOS[1,2,4]. PFOS contains several isomers, which are detectable in biota[4]. These are usually not completely separated and reported as an additional signal "shoulder"[4]. Hundreds of structural PFOS isomers ($C_8F_{17}SO_3^-$) are theoretically possible. Currently, nearly no information is available about the structure and the abundance of the isomer patterns in biota and in technical product. PFOS is produced mainly by an electrochemical process[5]. It forms a main PFOS isomer with a linear chain (70 %)[5] and many branched isomers.
The aim of this work was to characterize the isomer composition of commercial PFOS solutions and to separate as many isomers as possible. Moreover, the fragmentation behavior of PFOS isomers was investigated using ion-trap (IT) mass spectrometry (MS) with electrospray ionization in negative mode (ESI(-)) for structure elucidation.

Methods and materials

Chemicals and HPLC separation: Technical PFOS (98 %) was obtained as potassium salt from ABCR (Karlsruhe, Germany) and was dissolved in methanol. Methanol (≥ 99.8 %) was delivered by SDS (Peypin, France). Water was purified by an Elgastat Maxima HPLC water purification unit (Elga Ltd., Bucks, England). Ammonium acetate (98.0 %) was purchased from Merck (Darmstadt, Germany). A fluophase PFP (Thermo Electron, 150 mm column length, 2.1 mm i.d., 5 µm particles, 100 Å pore size) and a X-Terra C18 phase (Waters, 100 mm length, 3.00 mm i.d., 3.5 µm particles, 125 Å pore size) were employed for the separation of PFOS isomers. A Rheos 2000 low-pressure binary gradient pump (Flux instruments, Basel, Switzerland) was used with a flow rate of 200 µl/min. All solvents were degassed with helium. The following gradient of H_2O with 4mM ammonium acetate and methanol was applied: 30 % methanol for 1 min, to 65 % within 12 min,

kept for 6 min, to 85% within 6 min, isocratic for 6 min. Then, the column was rinsed with 100 % methanol for 1 min and returned to the starting conditions within 1 min.

Mass spectrometry: An ion-trap mass spectrometer (LCQ, Thermo Finnigan, San Jose, CA, USA) was employed using electrospray ionization in the negative ion. The ion-trap parameters were optimized for maximal transmission of $[M-K]^-$ resulting in the following instrument parameters: nitrogen sheat gas flow 60 arbitrary units, heated capillary temperature 200 °C, spray voltage 4.5 kV, capillary voltage –22 V, tube lens offset 10 V. MS^2 spectra of $[M-K]^-$ were recorded with a collision energy (CE) of 40 % and an excitation time of 20 ms. Helium was used as collision gas.

Results and discussion

Mass spectra: The full scan ESI(-)-spectra of PFOS isomers were simple containing only the molecular ion $[M-K]^-$ *m/z* 499. The instrument related low mass cut-off for the MS^n spectra was *m/z* 135. Therefore, the main product ions observed by triple quadrupoles at *m/z* 80 $[SO_3]^-$ and *m/z* 99 $[FSO_3]^{-2}$ were not visible. Within the reduced mass range, fragmentation of the molecular ion led to the formation of two series of fragments. The less abundant series $[C_nF_{2n+1}]^-$ ranged from *m/z* 169 to *m/z* 419 and the major one $[C_nF_{2n}SO_3]^-$ from *m/z* 180 to *m/z* 430 with consecutive losses of 50 u (CF_2). Figure 1 shows the total MS^2 spectrum of a PFOS isomer mixture.

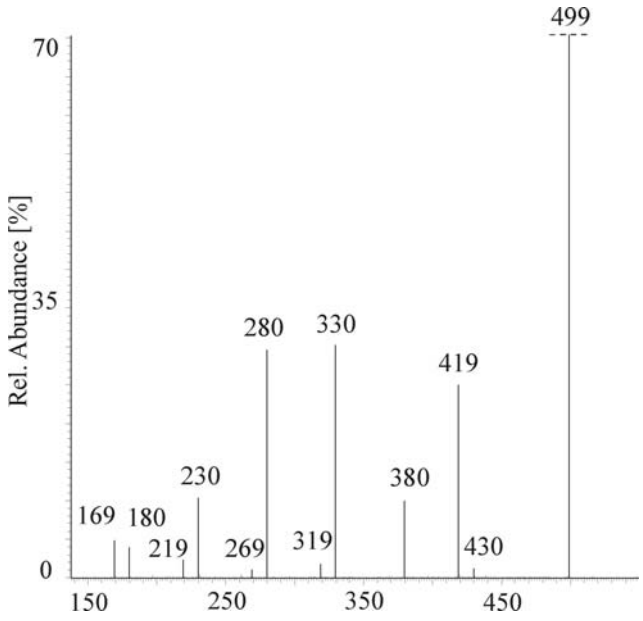

Figure 1: Total ESI(-)-MS^2 spectra of the $[M-K]^-$ ions (*m/z* 499) of a technical PFOS mixture.

All isomers groups provided these fragments. However, depending on the degree of branching some fragments of both series were present or absent.

Isomer separation and differentiation: PFOS isomers were separated with a PFP and a C18 column. The base peak ESI(-)-MS^2 chromatograms are compared in Figure 2. Series of (partly not resolved) signals of different isomer groups were present at both separations. The PFP phase allowed a separation into at least eight isomer groups compared to the three main signals obtained with the C18 column. The PFP phase showed more structure specific interactions with isomers. This resulted in an improved separation[5]. Some minor isomer groups eluted first followed by some medium abundant groups and the most abundant signal was at the end. The MS^2 spectra showed distinct differences in the fragmentation behavior of the different isomer groups. The mass chromatograms of the most abundant product ions are shown in Figure 2. They were *m/z* 380, 330, 280 and 230 from the main series and only *m/z* 419 from the minor series. As can be seen from Figure 2, the difference in the abundance of these selected ions allowed to recognize even more isomer groups.
Under reversed phase HPLC conditions non-branched chains eluted last due to the more lipophilic character of such structures. Therefore, it was assumed that the last eluting group of both separations contained at least linear PFOS.

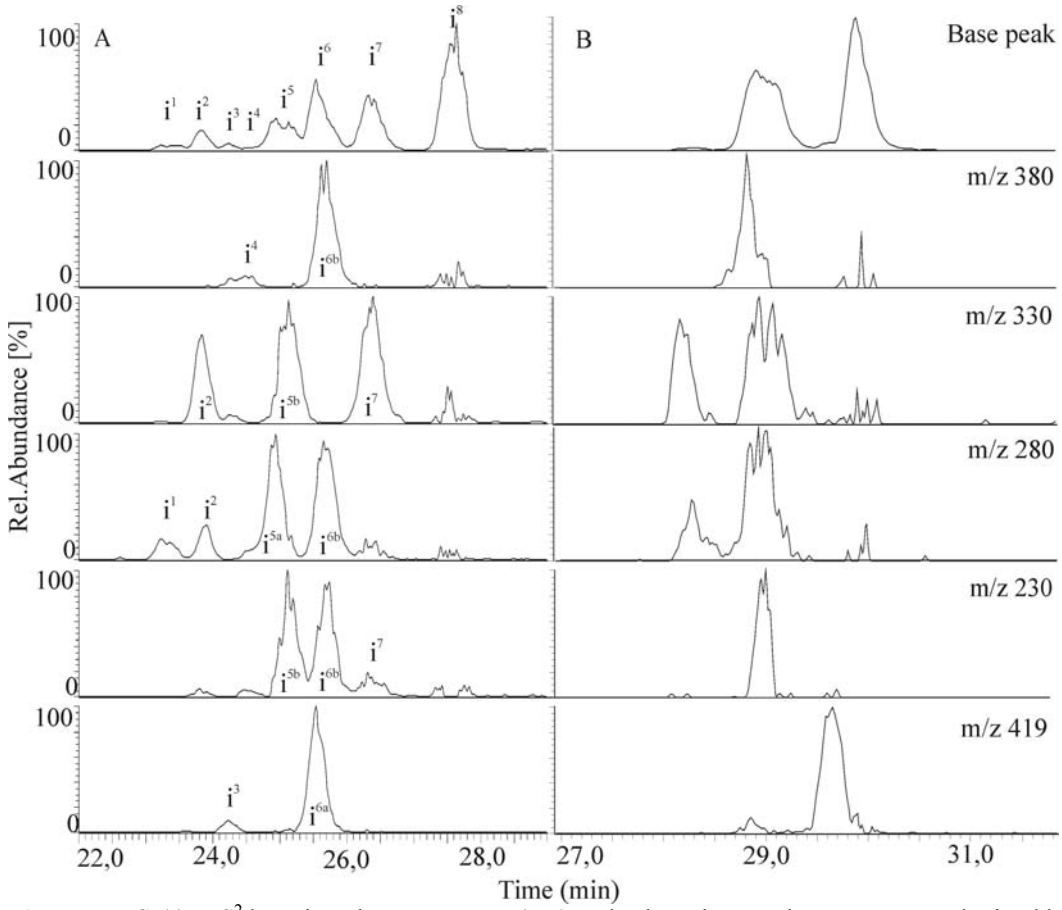

Figure 2: ESI(-)-MS^2 base ion chromatograms (top) and selected mass chromatograms obtained by separation of a PFOS standard on a PFP (A) and on a C18 (B) column. The $[M-K]^-$ molecular ion *m/z* 499 was fragmented. Different isomer groups are assigned with an "i" and a number.

The main ion *m/z* 419 of the minor series was only formed by one isomer group (i^{6a}). It allowed to differentiate the separated isomer group i^6 into two co-eluting groups (i^{6a} and i^{6b}) (Figure 2A). Moreover, differences in retention times of the mass chromatograms signals *m/z* 280 and 330 and the similar ones between *m/z* 330 and 230 allowed to split the isomer group i^5 into two sub groups (i^{5a} and i^{5b}) and to confirm the presence of the sub groups i^{6a} and i^{6b}. In total, ten groups of isomers could be differentiated due to their different fragmentation patterns. Figure 3 shows the MS^2 spectra of nine of these isomer groups. The groups i^{6a} and i^{6b} overlapped too much and did not allow to register single spectra. The relative abundance of the product ions in the MS^2 spectra of each group is summarized in Table 1 and should allow to identify their presence in other technical PFOS solution as well as in environmental samples.

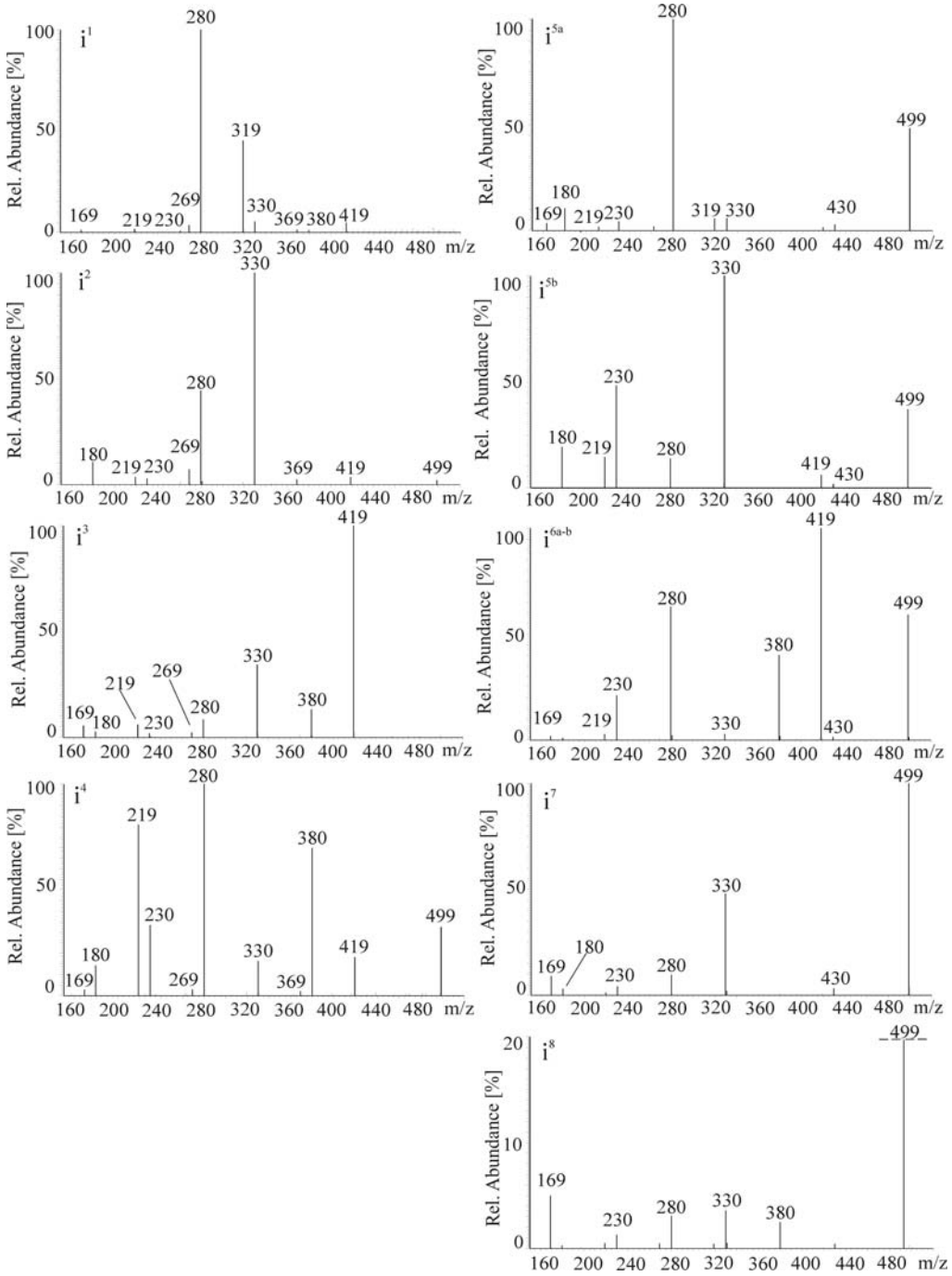

Figure 3: ESI(-)-IT-MS^2 spectra of [M-K]$^-$ ions of the isomer groups, i^1 to i^8 (40 % CE).

Table 1: Relative abundance (in %), of product ions of the different isomer groups (≥ 1.5 %) obtained by ESI(-)-IT-MS^2 of the $[M-K]^-$ molecular ion *m/z* 499 (40 % CE).

	i^1	i^2	i^3	i^4	i^{5a}	i^{5b}	i^{6a-b}	i^7	i^8
169	1.5	-	5.6	1.7	2.9	-	1.8	8.7	5.0
180	-	10.5	2.2	9.5	6.9	19.1	-	2.9	-
219	3.7	3.5	6.5	74.6	1.8	14.5	2.6	-	-
230	1.9	2.9	2.4	25.5	3.6	48.2	19.2	4.0	1.5
269	18.2	7.2	2.3	-	-	-	-	-	-
280	100	43.7	7.3	100	100	13.6	57.2	9.5	3.0
319	58.6	-	-	-	4.6	-	-	-	-
330	11.3	100	28.6	4.3	5.5	100	1.6	47.9	3.6
369	-	2.4	-	1.6	-	-	-	-	-
380	1.5	-	14.2	45.2	-	-	36.3	-	2.6
419	7.9	3.4	100	12.5	-	5.9	100	-	-
430	-	-	-	-	2.5	1.6	1.5	3.4	-
499	-	1.9	-	32.1	52.9	37.0	53.5	100	100

An estimation of the overall composition of technical PFOS is currently not possible due to different fragmentation pathways and, consequently, varying response factors. So far, only speculative assumptions can be made about the basic structures of the observed isomer groups. Early eluting groups may be more branched indicated by the pronounced fragmentation of groups i^1 to i^3 not forming the fragment >419 u (Table 1) at a relative abundance > 2 %. This is in agreement with the presence of the $[M-K]^-$ molecular ion *m/z* 499 and the fragment *m/z* 430 in groups i^5 to i^7 indicating less branching. The probably non-branched PFOS i^8 showed very little fragmentation. Currently, information about number and position of branching points is gained by gas chromatography combined with conventional electron and chemical ionization MS and derivatisation.

References

1. Martin J.W.; Smithwick M.M.; Braune B.M.; Hoekstra P.F.; Muir D.C.G.; Mabury S.A. (2004) Environ. Sci. Technol. 38, 373.
2 Tomy G.T.; Tittlemier S.A.; Palace V.P.; Budakowski W.R.; Braekevelt E.; Brinkworth L.; Friesen K. (2003) Environ. Sci. Technol. 38, 758.
3 Grasty R.C.; Grey B.E.; Lau C.S.; Rogers J.M. (2003) Birth Defects Research (B). 68, 465.
4 Hansen K.J.; Clemen L.A.; Ellefson M.E.; Johnson H.O. (2001) Environ. Sci. Technol. 35, 766.
5 3M report (2000) Federal register, Docket No. OPPT-2002-0043-0005, St Paul, MN, 51 pages.
6 Przybyciel M. (2003) Recent Developments in LC Column Technology. Special issue of LC-GC Europe (June 2), 2.