SEPARATION OF BRANCHED ISOMERS OF PERFLUOROOCTANESULFONATE

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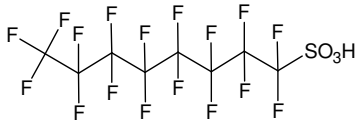
Introduction

Perfluorinated compounds (PFC) are found in a wide range of applications, because of their strong stability and their inert and non adhering surface properties. But it was observed, that perfluorocarboxylic acids (PFCA) and perfluoroalkylsulfonates (PFAS), in particular perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) are persistent, bioaccumulative and toxic in animal studies. In the EU the application of PFOS is prohibited by law but exemptions are granted (Dir. 2006/122/EC). In a survey[1] a concentration dependent carryover of PFC from soil to plant could be shown. If plants are used as feed for food producing animals, PFC can enter the food chain.

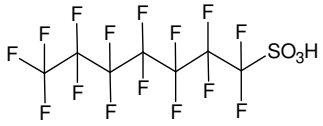
At the Federal Institute for Risk Assessment (BfR) in Berlin pilot feeding experiments with different animal species were arranged to investigate the carry-over from naturally contaminated feed into food producing animals. The experiments were focused on bioaccumulation in blood, meat and organs, and excretion via milk, urine and faeces.

Especially in the case of PFOS not only the linear PFOS but also a considerable amount of branched isomers exist. This may cause problems, because each laboratory has its own method to handle the branched isomers. The structure and the abbreviations of branched isomers are shown in Figure 1. This paper shows the separation of these compounds using HPLC-MS/MS.

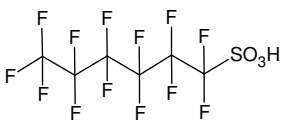
Compound	Abbreviation	CF_3-Group at Position	Parent Substance
Perfluorooctanesulfonate	n-PFOS		PFOS
Perfluoro-1-methylheptane sulfonate	PF1-MHpS	1	PFHpS
Perfluoro-2-methylheptane sulfonate	PF2-MHpS	2	PFHpS
Perfluoro-3-methylheptane sulfonate	PF3-MHpS	3	PFHpS
Perfluoro-4-methylheptane sulfonate	PF4-MHpS	4	PFHpS
Perfluoro-5-methylheptane sulfonate	PF5-MHpS	5	PFHpS
Perfluoro-6-methylheptane sulfonate	PF6-MHpS	6	PFHpS
Perfluoro-5.5-dimethylhexane sulfonate	PF5,5-DMHxS	5,5	PFHxS
Perfluoro-4.4-dimethylhexane sulfonate	PF4,4-DMHxS	4,4	PFHxS
Perfluoro-3.5-dimethylhexane sulfonate	PF3,5-DMHxS	3,5	PFHxS
Perfluoro-4.5-dimethylhexane sulfonate	PF4,5-DMHxS	4,5	PFHxS



Perfluorooctanesulfonate (n-PFOS)



Perfluoroheptanesulfonate (PFHpS)



Perfluorohexanesulfonate (PFHxS)

Figure 1: Structures and abbreviations of branched Isomers of perfluorooctanesulfonate (PFOS)

Materials and methods

Reagents:

Native and ^{13}C-labeled PFC were purchased from Wellington Laboratories, USA. Methanol absolute, acetonitrile, formic acid (99 %) and ammonium acetate (all ULC/MS) were purchased from Biosolve, The Netherlands. Sodium acetate anhydrous p.a. was obtained from Merck, Germany .Water was double distilled by using the distillation unit 2001/2 from GFL

Apparatus:

HPLC-MS/MS: Agilent 1290 SL LC/ Agilent 6460 Triple Quadrupole LC/MS

Sample preparation:

In plasma and urine, PFCA and PFAS were determined based on a method published by Kärrman et al. (2005)[2]. Internal standard solution and 4 ml of a mixture of formic acid 99% / water (1:1) were added to 1 ml of the sample. The solution was sonicated for 15 minutes. After sonication 3 ml water was added and after centrifugation the samples were purified and concentrated using SPE on an OasisWAX (60 mg/3ml) column. The conditioning-, washing- and eluting steps were described previously[3]. The SPE columns were each preconditioned with 2 ml 0.1% NH_4OH in methanol, methanol and water. After the transfer from the samples onto the weak anion exchanger, the SPE-columns were each washed with 2 ml 0.025 mol/l sodium acetate (pH 4) and methanol and subsequently they were put under vacuum suction until dryness. Elution was performed with 2 ml 0.1% NH_4OH in methanol. The samples were evaporated in a gentle stream of nitrogen after addition of 30 µl glycerine as keeper. After reconstitution with a mixture of methanol (40 %) and water (60 %) the samples were measured using HPLC-MS/MS.

HPLC-MS/MS Analysis:

A small column (Eclipse Plus, C18, 3.5 µm, 4.6 x 30 mm Agilent Technologies) was applied as a pre column between purge valve and autosampler to separate background PFCA and PFAS. Also an in-line filter (Replacement frits 4.6mm, 0.2 µm Agilent Technologies) between autosampler and column was used to filter remaining particles from the samples. An injector program was used to minimize the carryover from heavy contaminated samples as far as possible.

HPLC-Parameters for linear PFC:

The separation was performed on an Agilent 1200 SL HPLC-System. A Gemini column (3 µm, C18, 110 A, 150 x 2 mm) from Phenomenex was the appropriate column to separate the individual analytes. The column temperature was hold at 50°C. A mixture of 2 mM ammoniumacetate (95 %) and acetonitrile (5 %) (v/v) was used as solvent A and a mixture of methanol (40 %) and acetonitrile (60 %) (v/v) was used as solvent B. The flow rate was 0.3 ml/min. Gradient: 10 % B (2 min. hold), 10 % B to 85 % B (7 min.); 85 % B to 98 % B; (3 min.) 98 % B (hold 7 min.), equilibration 10 min. 5µl of the extract were injected into the HPLC-MS/MS system. The total run time was 30 minutes.

Specific HPLC-Parameters for branched isomers:

This separation was also performed on an Agilent 1200 SL HPLC-System. A PRONTOSIL 200-3-C30 3.0 column (150 x 3 mm) from Bischoff Chromatography was the appropriate column to separate the individual analytes. The column temperature was hold at 50°C. 8 mM ammoniumacetate (95 %) adjusted to pH 9 was used as solvent A and a mixture of methanol (80 %) and acetonitrile (20 %) (v/v) was used as solvent B. The flow rate was 0.4 ml/min Gradient: 40 % B (15 min. hold), 40 % B to 60 % B (30 min.); 5 min equilibration. 3µl of the extract was injected into the HPLC-MS/MS system. The total run time was 50 minutes.

Source parameters:

MS/MS-detection was performed with an Agilent 6460 triple quadrupole mass spectrometer equipped with an electrospray interface (ESI) operating in the negative ion mode.

Gas temperature: 300 °C; gas flow: 4 l/min; sheath gas temperature: 350 °C; sheath gas flow: 10 l/min; nebulizer: 43 psi; capillary 3000 V (negative); nozzle voltage: 0V. MRM settings for linear PFC are published elsewhere[4], MRM settings for the branched Isomers are stated below (Table 1).

Results and discussion:

Not only in the case of PFOS but also in the case of other PFC like PFHxS and PFHpS a considerable number of branched isomers exist. The focus here is on the branched isomers of PFOS, because only in the case of PFOS standards of branched isomers are commercially available. The chromatograms of a plasma and an urine sample,

both from the feeding experiments with cows containing 0.5 µg/l linear PFOS, show that the branched isomers contribute considerably to the total PFOS amount (Figure 2 and 3).

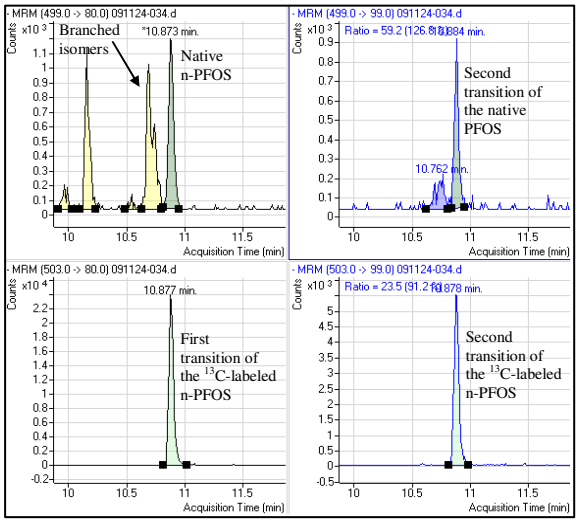


Figure 2: First and second transition of native and 13C-labelled linear PFOS in a cow plasma sample containing 0.5 µg/l linear PFOS. The HPLC-method for linear PFC was used.

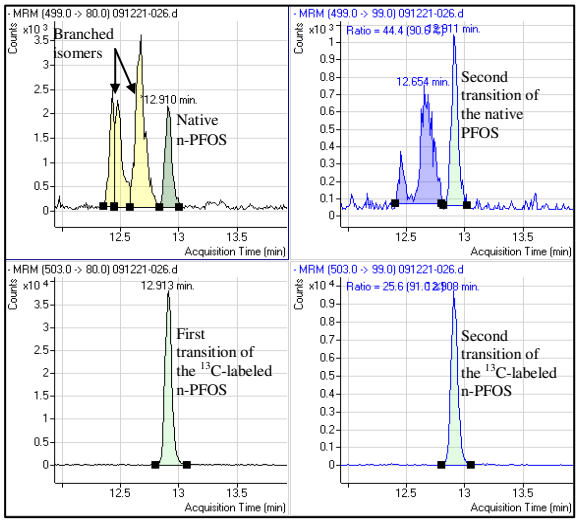


Figure 3: First and second transition of native and 13C-labelled linear PFOS in a cow urine sample containing 0.5 µg/l linear PFOS. The HPLC-method for linear PFC was used.

For the interpretation of the feeding experiments first of all only the concentrations of the linear PFOS were of interest. The question was, if the peak with the same retention time as the linear ^{13}C-labeled PFOS is only the linear PFOS, or contains other isomers with the same retention times and the same transitions. Therefore the transitions of the branched isomers were determined. The two most intensive transitions are shown in Table 1.

Abbreviation	Precurser	Frag (V)	Product-Ion 1	CE 1 (V)	Product-Ion 2	CE 2 (V)
n-PFOS	499	200	80	65	99	45
PF1-MHpS	499	160	419	25	99	35
PF2-MHpS	499	200	80	65	99	45
PF3-MHpS	499	185	80	70	130	50
PF4-MHpS	499	200	80	70	230	40
PF5-MHpS	499	200	80	70	130	50
PF6-MHpS	499	200	80	70	169	45
PF5,5-DMHxS	499	200	80	75	130	50
PF4,4-DMHxS	499	180	269	35	80	75

Table 1: The two most intensive transitions of the linear PFOS and the branched isomers of PFOS

With the exception of PF1-MHpS all branched isomers show the transition 499 to 80. Therefore it is not necessary to separate n-PFOS and PF1-MHpS chromatographically from each other. All the other branched isomers have to be separated chromatographically from the linear PFOS. Using the standards of the branched isomers it could be ascertained that all branched isomers are chromatographically separated from the linear PFOS apart from PF1-PFHpS using the HPLC parameters for linear PFC specified above.

The next step is to investigate whether the branched isomers show differences in ingestion and excretion in comparison to the linear PFOS. For this purpose the chromatographic separation of the branched isomers with the exception of PF1-MHpS is necessary. Good separations could be achieved by using a C30 phase and a pH value of 8 or higher. The results are shown in Figure 4.

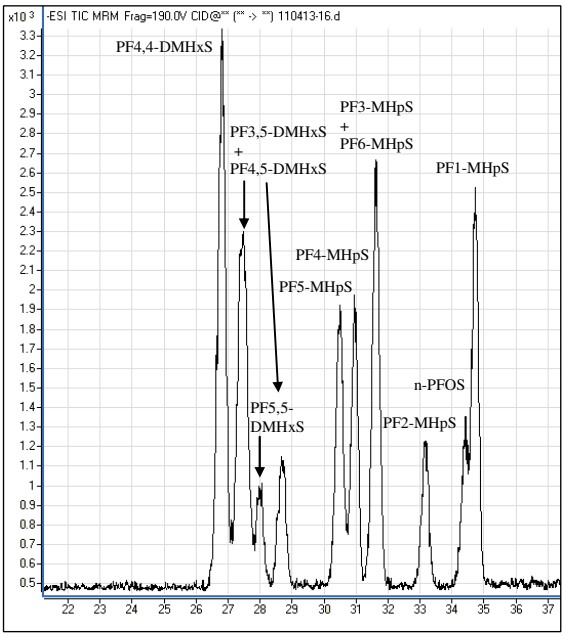


Figure 4: Total ion chromatogram of the separation of the branched isomers of PFOS using a C30-phase and the HPLC-parameters for the branched isomers.

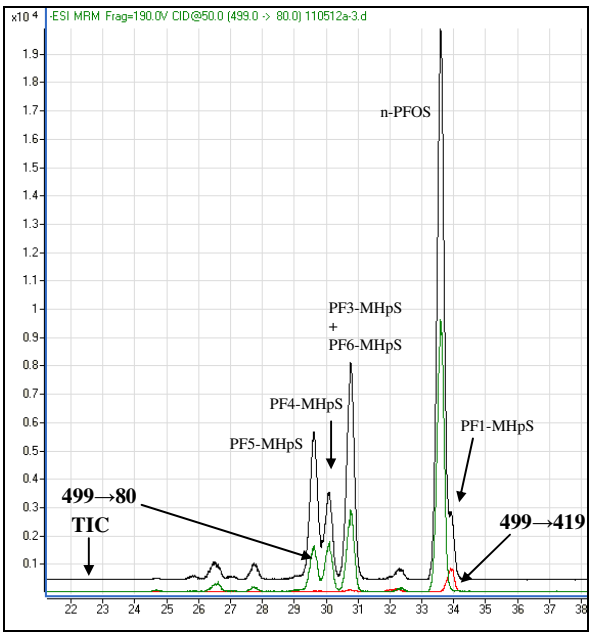


Figure 5: Total ion chromatogram and the chromatograms of the transitions 499 → 80 and 499 → 419 of a pig plasma sample (branched isomers HPLC parameters)

Until now perfluoro-3-methylheptane sulfonate and perfluoro-6-methylheptane sulfonate (Figure 4) can not be separated. All other isomers can be separated partially. In Figure 5 a chromatogram of n-PFOS and branched isomers in a plasma sample from the feeding experiments with pigs is shown. The sample contains n-PFOS basically, but also PF4-, PF5- and PF3- respectively PF6-MHpS could be detected in considerable concentrations. By using the special transition from P1-MHpS this substance could also be identified.

The branched isomers lead to problems in inter laboratory comparisons. One possibility is to integrate all peaks showing the same transitions as PFOS. The peril is that matrix components which show the transition 499 to 80 too, were also integrated. Another problem is that transitions and the intensity of transitions of the branched isomers depend on the type of mass spectrometer and the used parameters. The other possibility is to integrate only the peak with the same retention time as the linear standard, but in this case it is essential to check whether the linear PFOS is separated chromatographically from the other branched isomers. But if all laboratories would check if they separate the linear PFOS, the results of the different laboratories would be more comparable.

References:

1. Stahl T, Heyn J, Thiele H, Hüther J, Failing K, Georgii S, Brunn H. (2009); *Arch. Environ. Contam. Toxicol.* 57: 289-298
2. Kärrman A, van Bavel B, Järnberg U, Hardell L, Lindström G. (2005); *Anal. Chem.* 77: 864-870
3. Taniyasu S, Kannan K, So MK, Gulkowska A, Sinclair E, Okazawa T, Yamashita N. (2005); *Journal of Chromatography A* 1093: 89-97
4. Bernsmann T, Fürst P. (2008); *Organohalogen Compounds* 70: 718-721