Polymeric type polyfluorinated compounds in technical standards and from food contact materials

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INTRODUCTION

Polyfluorinated compounds (PFCs) are increasingly under investigation as persistent organic pollutants to both humans and the environment. Whereas the sources and levels of PFCs in the environment have been investigated in some detail over the past decade, fewer studies have focussed on PFCs migration to food due to food contact materials. Furthermore, most analytical techniques and hence studies have focussed on the relatively small PFCs, including the perfluorinated alkyl acids (PFAA's), perfluorinated alkyl sulphonates (PFAS's) and fluorotelomer alchols (FTOH's)[1], while only a few methods have been developed for the complex mixtures of the technical standards used by industry [2, 3, 4, 5, 6]. A likely reason is that analytical standards are not commercially available and must be synthesized[7], and only a few technical standards are sold for research purposes. Other technical standards must be obtained by donations or bulk buys (> 50 kg), but often the chemical producing companies are reluctant, and even refuse to sell standards for analytical research purposes. The aim of this work is therefore to a obtain accurate mass data and typical fragmentation patterns for available PFC technical standards, some of which are used on food contact materials as grease-and-water repellent coatings (or precursors hereof). This work will aid in the identification and quantification of other so far unknown PFCs from food contact materials, in food and in the environment.

MATERIALS AND METHODS

Sample preparation. Technical standards were obtained from Sigma Aldrich (Zonyl FSN, Zonyl BA-L, Zonyl TM, Zonyl FSE, Zonyl UR, Zonyl FSO, trademark of Dupont) and donated by scientific colleagues (Zonyl NF). Analytical standards of the perfluorinated acids C_5, C_8, C_9, C_{10}, C_{12} , the fluorotelomers 6:2, 8:2, 10:2, the sulphonates L- and T-PFOS and the sulphonamide PFOSA were obtained from Sigma Aldrich and from Wellington. Stock solutions of 1-5 mg/mL were prepared in methanol, and diluted in MeOH/water to working solutions of 0.025-5 µg/mL. For these qualitative screening purposes, food contact materials were exposed to extraction rather than migration conditions, but with a solvent relevant for further migration studies: Food was removed from the containers of paper or polystyrene pre-cleaned in MilliQ water, and samples were collected from sections of the food contact material, which had not been in contact with food. 40 mL of 60 °C, 95% ethanol was poured onto cut pieces of materials, placed in a 60 mL polypropylene centrifuge screw-cap tube, ultrasonicated for 2 hours at 60 °C. The tubes were then centrifuged and the supernatant filtered through a 0.2 µm polypropylene filter and into HPLC glass vials.

HPLC. A Waters UPLC Aquity system (Waters Corp., Milford, MA, USA) was used, employing a C_{18} BEH column (Waters, 2.1 x 100 mm × 1.7 µm particle size, pH tolerance 2-12). The mobile phases (A: MilliQ water with 5% methanol added and B: methanol (LC-MS grade)) were initially tested with 2 mM ammonia formate (pH 6), but were in the final method adjusted with ammonia (24%) to pH 9.7. The gradient was: The initial composition (95% A/5% B) was changed linearly from 0 to 19 min (2% A/98% B), held constant to 21 min, returned linearly to the initial composition at 23 min, and held constant until 25 min. Flow: 0.3 mL/min.

Quadrupole-Time-of-flight (Q-TOF) mass spectrometry. A Micromass Q-TOF Ultima mass spectrometer (Micromass, Manchester, UK) was operated in negative ESI mode. Temperatures: source 120 °C, desolvation 300 °C. Voltages: capillary 3 kV, cone voltage 90 V. N_2 as desolvation gas (700 L/hr) and cone gas (50-100L/hr). Argon was used as collision gas with a partial pressure of 2.59 mbar. The TOF mass analyzer was operated in the V-mode, and was initially calibrated with solidum formate (5^{th} order polynomial, multi-point, N_p=0). Thereafter a single-point lock mass correction was used to adjust for mass drift during the run. The de-protonated dimer of Leucine Enkephalin ($C_{56}H_{73}N_{10}O_{14}$, 1109.5308 Th) was used as a single lock mass calibrant, and was introduced post-column via a tee by a syringe pump at a flow rate of 2 µL/min of 1 ng/µL (however 20 µL/min of 0.1 ng/µL is more reproducible) of Leucine-Enkephalin. Masses from 75 (100) - 1500 Th were recorded in the continuum mode at an acquisition rate of 1 spectrum/sec. MassLynx v. 4.1 was used for dataprocessing, while the exact theoretical mass was calculated with Molecular Weight Calculator v. 6.45, 2007 (freeware available at www.alchemistmatt.com).

RESULTS AND DISCUSSION

Initially the analysis was performed at neutral pH, which gave good responses for acids, sulphonates, sulphonamides and phosphonates. However, the fluorotelomer alcohols (6:2 and 8:2 FTOH and Zonyl BA-L) and the fluoroalkoxylates (e.g. Zonyl FSN, FSO) had poor responses. The pH was therefore raised to pH 9.7, which is closer to the pK_a of alcohols, and this gave a good response for all the PFCs tested, without hampering the separation.

Numerous homologue series were seen for PFCs separated by Δm=100 (C_2F_4) or Δm=50 (CF_2), and in this way both smaller PFAAs and larger bits of polymer chains (oligomers) could be found by extracting the $m/z \pm n \cdot 50$ Da, n

being an integer, (*Figure 1,4,5,6)*. Initially a span of 0.2 Da was set around the expected mass, and then once the peak was found and the exact *m/z* was seen, then the span was narrowed down to 0.05 - 0.10 Da around this exact *m/z* in order to reduce the noise, and hence to increase the S/N. This procedure was necessary, as TOF instruments with Time-to-digital-converters (TDC), in contrast to Analog-to-digital-converters (ADC), easily goes into dead-time at spectrum counts > 200, with the consequence that in-correct lower *m/z* are recorded [8] (*Figure 2)*.

Typically the de-protonated ions $(M-H)^-$ were seen, but also adduct ions such as $(M-2H^-+Na^+)^-$ and at high concentrations (ca. 10^4 counts) the de-protonated dimer $(2M-H)^-$ could be present. PFCs containing Hydrogen atoms could also show one or several losses of Δm=20 (HF), see *Figure 1*. On MS-MS instruments such as triple quadrupole instruments, this can be exploited in neutral loss scans, which however can be hampered by low sensitivity.

Figure 3 shows the MS-MS spectrum of a migrate of one of several Danish microwave popcorn bags, that had both the homologue series spaced Δm=100 apart of 789-1389 (polyfluorotelomer-phosphonate) and the 921-1321 (polyfluoro-thioether-phosphonate) series previously reported by Begley et al.[4,5] (*Figure 4)*. The MS-MS fragmentation of 889 shows, that the chains likely are 6:2 (443 Th) and 8:2 (543 Th) fluorotelomers. The 789-1489 series was also found in burger wrap paper, where the 921-1421 series was not found. This 789-1489 is also present in Zonyl NF, as shown in *Figure 4*. The PFC alkyl ethoxylate types (Zonyl FSN, FSO) with the molecular formula $F(CH_2CH_2O)_x(CF_2)_yC_2H_4OH$ (given by Aldrich), show typical homologue series with Δm=44, corresponding to x (CH_2CH_2O) units. For each x, there is a series corresponding to y (CF_2), as illustrated in *Figure 5*. Zonyl FSN is one of the Zonyl products for which a patent has been taken to enhance the coffee retention of thermoplastic foam cups, such as expanded polystyrene (EPS)[9]. The polystyrene cup tested showed some of the same m/z value as Zonyl FSN: 791.32 and 891.32 Th, which fits the theoretical structure formula $F(CH_2CH_2O)_x(CF_2)_yC_2H_4O^-$ for x=4 and 6 and y=12 and another series 877, 977 (*Figure 6)*. The tested polymeric technical standards are just a few of the many commercial PFCs in use, which are not routinely measured for today.

The finding of polymeric PFCs in food contact materials is believed to be the first of its kind in Europe. Contamination of food with such PFCs via single-use food contact materials could be a significant source of repeatable exposure to humans, and it is possible that this can explain some of the difference between the total fluoride bound in organic compounds in human blood, compared with the fluoride content in the identified organofluorine compounds. Polymeric PFCs also constitute an extra source of PFC contamination in the environment, as they, more or less, are amenable to bio-degradation[7]. Development of accurate and quantitative analytical methods are urgently needed, but are challenged by the demands for high sensitivity, and selectivity - due to the presence of numerous compounds - and in particular by the lack of analytical chemical standards. Future work will continue on the structure elucidation of commercial PFCs, and the influence of their surfactant behaviour on quantitative analysis. This will be used to identify PFCs in migrates from FCMs and in food, and in developing quantitative PFC methods for food safety enforcement.

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Figure 1 Typical fragmentation pattern of PFC standards: Δm=20 (HF), 50 (CF_2) and 100 (C_2F_4).

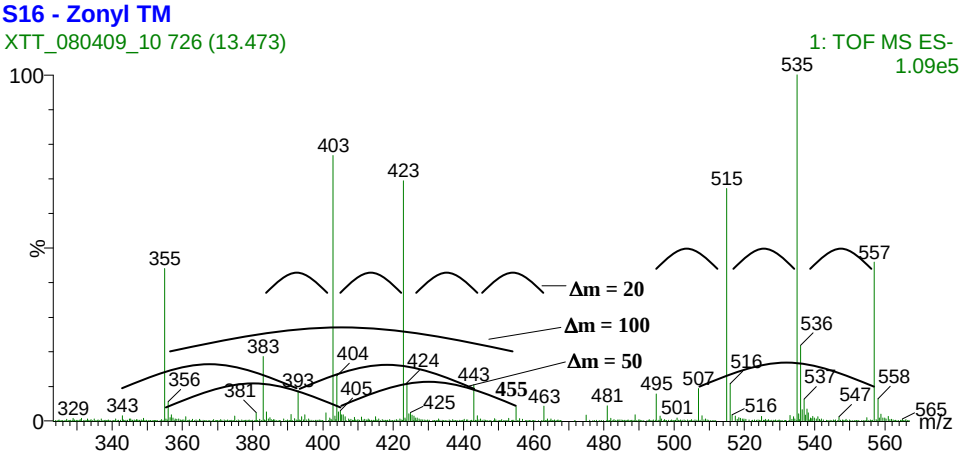


Figure 2. Incorrect *m/z* values at the apex of a **FSN** peak, due to overload of the TOF TDC detector (counts > 200).

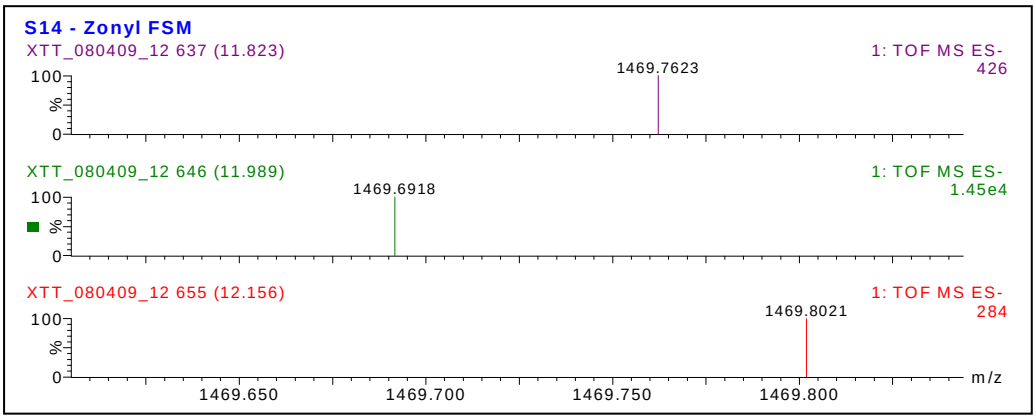


Figure 3. MS-MS of *m/z* 889 (span 5 Da) for a Microwave popcorn bag at Collision energies 10V, 20V and 30V. Two fragmentation major products 443 (containing 6:2 chain) and 543 (containing 8:2 chain) are seen.

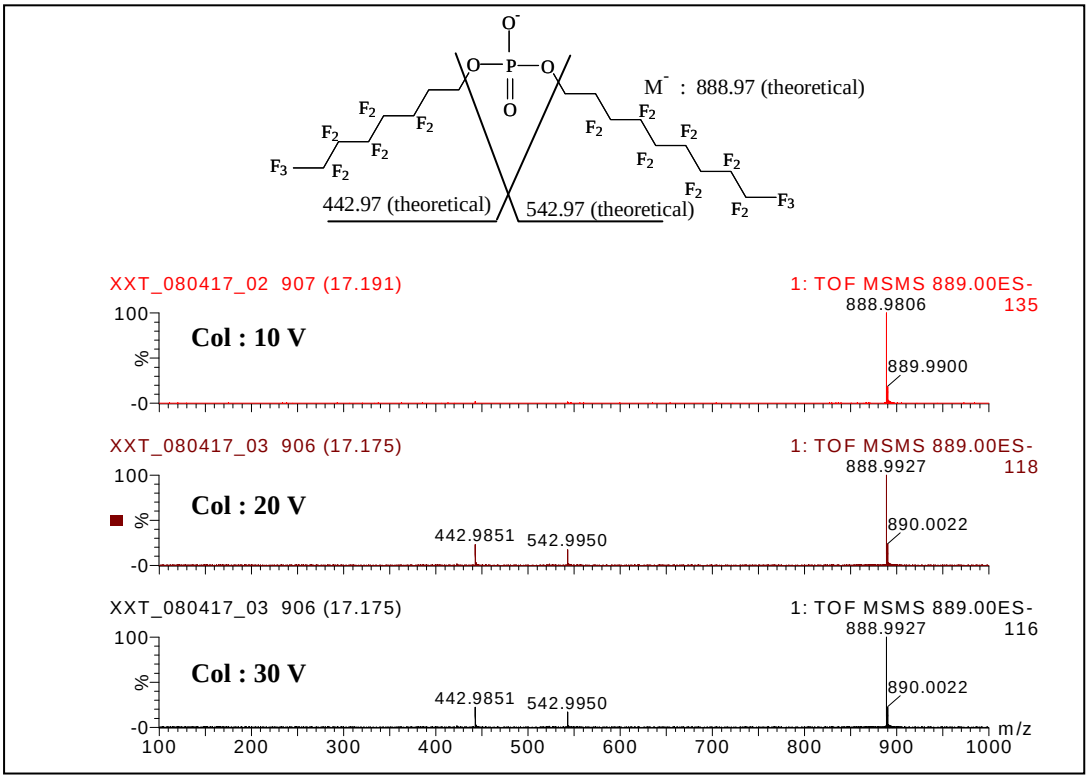


Figure 4. Homologue series Δm=100 apart in an extract from a Microwave popcorn bag, and from Zonyl NF. The 921 series belongs to a polyfluoro-thioether-phosphonate as described by Begley et al.[5]

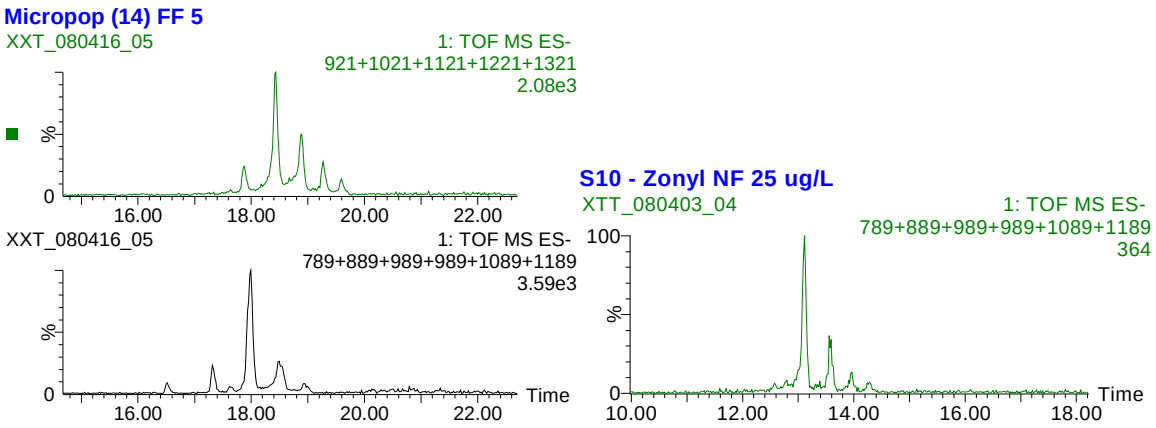
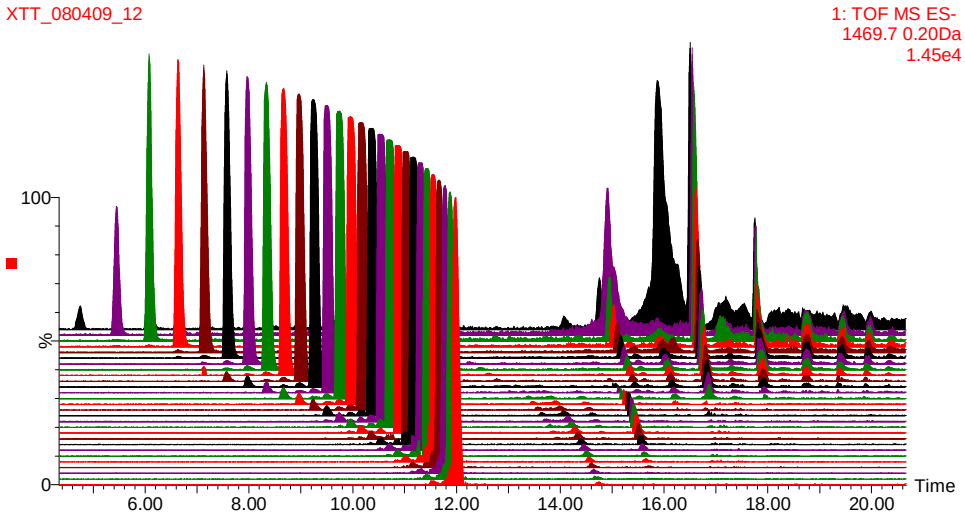
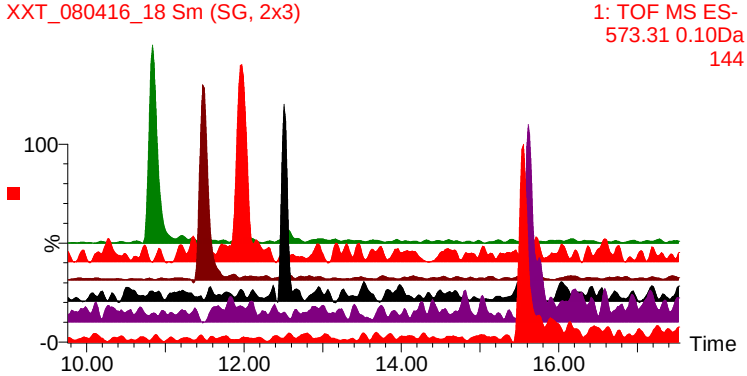


Figure 5. One of the many homologue series for Zonyl FSN, with Δm = 44 (CH_2CH_2O). Note, that each mass has several peaks, indicating, that these fragment ions likely also are part of different molecules.



m/z of peaks (Th)	
281.15	941.39
325.17	985.41
369.17	1029.43
413.20	1073.44
457.26	1117.46
501.23	1161.50
545.25	1205.52
589.26	1249.54
633.27	1293.58
721.13	1337.63
765.32	1381.63
809.34	1425.67
853.35	1469.70
897.37	

Figure 6. Homologue series for Polystyrene cup, containing some of the same *m/z* (791.32, 891.32 Th) as Zonyl FSN.



m/z of peaks (Th)
791.32
573.31
891.37
673.31