FOOD CONTACT MATERIALS: THE SOURCE OF PFCs IN HUMAN DIET

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Introduction

Perfluorinated compounds (PFCs), a broad group of anthropogenic chemicals, are widely used in miscellaneous industrial and consumer applications, mainly thanks to their unique ability to repel both water and oil [1]. Within the last decade, PFCs have been identified as "emerging" food and environmental contaminants, due to their presence in various types of abiotic and biotic matrices, including human tissues and biotic fluids [2,3]. In order to enable a risk assessment associated with dietary exposure to PFCs, EFSA (the European Food Safety Authority) recommended that further data on their levels in food and in humans would be desirable. On this account, in March 2010, Commission Recommendation 2010/161/EU advised the Member States to monitor the presence of following PFCs: PFOS and PFOA and, if possible, their precursors such as perfluorooctane sulphonamide (PFOSA), N-ethyl perfluorooctane sulfonamidoethanol (NEtFOSE), 8:2 fluorotelomer alcohol (FTOH), compounds, similar to PFOS and PFOA, but with different chain length (C4–C15), and polyfluoroalkyl phosphate surfactants (PAPS) such as 8:2 diPAPS and 8:2 monoPAPS. The purpose was to estimate the relevance of these PFCs presence in foods [5]. Worth to notice thatmost of until now conducted studies have been focused on determination of perfluorocarboxylic acids (PFCAs) and perfluorosulfonic acids (PFSAs) of different chain length in drinking water and food of animal origin, which are supposed to be the main sources of PFCs in human diet. However, another important source of PFCs in food chain can be packaging materials (paper and board), which are treated by polyfluorinated surfactants (PFSs) to improve their properties. PFSs typically occurring in paper wraps are ionic PAPSs (which degraded via FTOHs to PFCAs), S-diPAPSs and SN-diPAPSs [6]. Since recently, non-ionic and polymeric PFSs represented by e.g. polyfluoro-alkoxylates or polyfluoro-acrylates, have been increasingly used [7]. Although some data on detection and migration of PFSs are available [7], the quantification of these compounds is still difficult, the spectrum of industrial PFSs-based blends is very broad and practically no analytical standards are commercially available.

In the presented study, DART (Direct Analysis in Real Time) ambient ion source coupled to mass spectrometer was used for screening of food packaging papers and boards used for the presence of fluorotelomers (mass spectra of PFSs typically contain a series of ions differing by Δ m/z 99.99). The positive samples were then extracted by ultrasonication with different organic solvents to isolate as much as possible insufficiently bound PFSs. For their identification, UHPLC-MS/MS and/or UHPLC-TOFMS were employed. Finally, migration tests on food contact papers obtained from fast food chain were carried out.

Materials and methods

Certified standards of 6:2, 8:2 and 10:2 monoPAPS, 6:2, 8:2 and 10:2 diPAPS and 6:2 triPAPS were purchased from Chiron (Norway). Methanol, tetrahydrofuran, dichloromethane, toluene and *n*-hexane were from Merck (Germany), acetonitrile, ethylacetate and cyclohexane were from Sigma-Aldrich, as well as ammonium hydroxide and ammonium acetate used as mobile phase additives. The paper and boards for food packaging were obtained at local stores and fast food chains in the Czech Republic.

DART-MS screening method

DART–MS system used in this study consisted of a DART ion source (DART-SVP) coupled to the Exactive™ benchtop mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). Vapur™ interface (IonSense, Saugus, MA, USA) was employed to hyphenate the ion source and the mass spectrometer and the distance between the exit of the DART gun and the ceramic transfer tube of the Vapur™ was set to 10 mm. Approximately 2 cm^2 piece was cut from the paper sample and taken for the analysis. To allow thermo-desorption of PFSs, the edge of the test paper

inner side was manually introduced to the ionization gas stream (while preventing the paper from blocking it) with the use of tweezers. The desorption time was approximately 5s.

UHPLC-MS/MS and UHPLC-TOFMS analysis

The PAPSs were analysed by a UHPLC-MS/MS system consisted of an Acquity Ultra-Performance LC system (Waters, USA) coupled to a Xevo TQ-S triple-quadrupole mass spectrometer. Acquity UPLC was equipped with a PFC column isolator to delay interferences coming from a LC system and all Teflon tubings were replaced by made those from PEEK.

Mono-, di- and triPAPSs were separated on an Acquity BEH C18 column (50×2.1 mm I.D., 1.7 µm, Waters, USA) and mobile phase consists of 18 MΩ Milli-Q water and methanol adjusted to pH ≈ 9.5 by ammonium hydroxide. The mono- and diPAPSs were analysed in ESI- and triPAPSs in ESI+. Since only a few PAPSs standards were available, the all theoretically possible MRM transitions were calculated and fragmentation voltages were extrapolated from known analytes.

Finally, extracts prepared from paper samples were analysed by a UHPLC-TOFMS system (LCT Premier XE, Waters, USA) to detect also other PFSs than PAPSs. Since the same UHPLC instruments were used, also the chromatographic conditions were the same as in the PAPSs analysis. The instrument was tuned on resolution 11 000 FWHM and all positive samples were analysed in both polarities. The list of PFSs was adopted from literature [7].

Migration tests

More than 250 cm^2 of fast food paper wrap was cut to 1 cm^2 squares. 10 cm^2 were extracted by 10 mL of methanol, dichloromethane or ethylacetate in ultrasonic bath to obtain highest possible migration of loosely bounded PFS. Simultaneously, the migration of PFSs into the water, 3% solution of acetic acid and olive oil was tested. Two different migration experiments were done (*i*) migration for 60 min in 60°C and (*ii*) migration at room temperature for 24, 48 and 72 hrs. The aliquot of water and 3% solution of acetic acid could be directly analysed by LC-MS/MS. Olive oil food simulant has to be purified before analysis. 1 g of olive oil was diluted in *n*-hexane and loaded on silica SPE cartridge. The cartridge was flushed by dichloromethane:*n*-hexan (1:1, *v/v*) and analytes were eluted by 3 mL of dichloromethane:methanol (1:1, *v/v*) and 10 mL of methanol. The extract was evaporated and reconstituted in 1 mL of methanol.

Results and discussion

DART-MS screening

The applicability of DART-MS technique for screening of various additives in food packaging materials has been documented by Ackerman [8]. In the current study, the same type of ambient mass spectrometry technique has been used for a rapid screening of PFSs in paper and board intended for packaging of food. The analysis was based on a search for a characteristic ion patterns with 99.99 Da differences corresponding to C_2F_4 increment. Although a number of in this context positive samples were identified among those collected at a market, only molecular ions related to PAPS were identified directly based on DART-MS spectra (see Figure 1). Various PAPSs were found microwave popcorn bags and popcorn cups from cinemas, boxes for pizza, muffin cups and paper wraps from fast food chains.

LC-MS/MS analysis of PAPSs

Samples of paper in which the presence of PFSs was indicated by DART-MS screening were extracted in ultrasonic bath at elevated temperatures with various organic solvents. To examine obtained extracts for PAPSs, UHPLC-MS/MS was employed. As mentioned in Experimental, all PAPSs provide ions in ESI-, however triPAPS are in-source fragmented to diPAPS [7], what does not allow identification of isomers of parent compounds. Based on generic experience with organophosphate triesters (e.g. organophosphate pesticides or organophosphate flame retardants) which are commonly analysed in ESI+ mode, triPAPS were also tested using this detection setting. The polarity change resulted in production of intensive $[M+H^+]$ molecular ions of triPAPs what provided significantly improved sensitivity (up to 50×). Although only one triPAPS standard was available during this study, it enables us to understand fragmentation mechanism of these compounds. IN ESI+,TriPAPS are (alike mono- and diPAPS in

ESI-) fragmented to phosphate via diPAPS and monoPAPS, thus it allowed us theoretical calculation of MRM transitions for all combinations of FTOHs with different chain length (FTOHs from 4:2 to 18:2 have been considered in our study). In selected "positive" samples were identified monoPAPS with masses from *m/z* 343 to *m/z* 943), diPAPS with molecular ions from *m/z* 689 to *m/z* 1489 and triPAPS with *m/z* from 1037 to 1737. Based on the MS/MS experiments, the identified FTOHs (neutral loss) bound in the PAPS have chain length varying from 4:2 to 16:2.

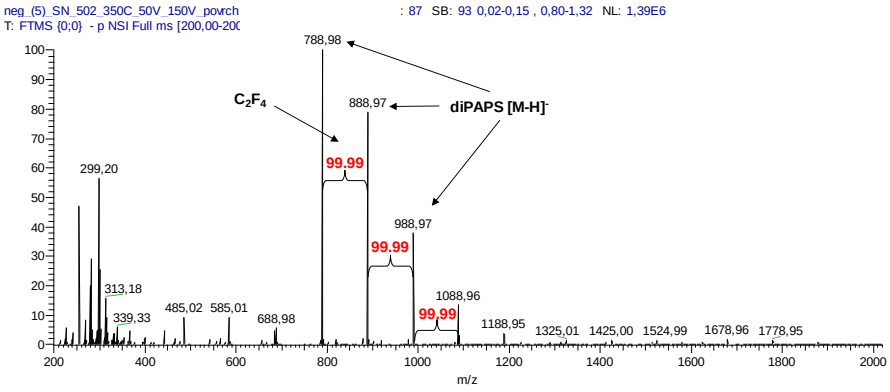

Figure 1: Example of DART-MS spectra of paper wrap from fast food chain.

The amount as well as the spectrum of extracted PAPSs was largely variable. MonoPAPSs and triPAPSs are considered as the side products of diPAPSs synthesis [7], however in some samples were diPAPSs (which should be the key components of some technical blends) detected in a significantly lower amount than the triPAPSs and monoPAPSs (see Figure 2).

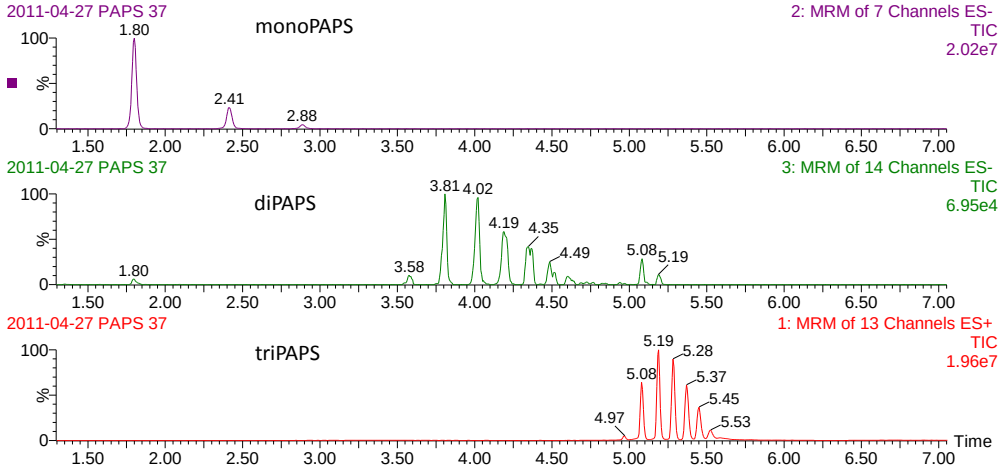

Figure 2: LC-MS/MS profile (TIC) of mono-, di- and triPAPSs extracted by methanol from muffin cup (in this sample intensities of mono and triPAPSs are cca 250× higher compared to diPAPSs).

LC-TOFMS analysis of other PFS

It should be noted, that PAPSs were not detected in all samples, which were identified by DART-MS analysis as possibly containing PFSs or fluoropolymers. In addition to UHPLC-MS/MS, sample extracts were also analysed by UHPLC-TOFMS,the potential PFSs were searched based on their exact masses. Only S-diPAPSs were

additionally detected in a few samples, surprisingly never in the same samples, in which PAPSs were identified. No other PFSs tested by Trier et al. [7] were identified, although characteristic telomere patterns were detected by DART-MS as well as UPLC-TOFMS. The identification of PFSs in these samples will be further investigated.

Food simulant migration tests

In a wide series experiments, migration potential of PAPSs into food matrices was documented. The positive samples were extracted by various solvents to get information on the amount of unbound PAPSs and and assess extractability in relation to their polarity. As expected, the most of polar monoPAPSs were extracted by methanol while less polar diPAPSs were more efficiently extracted with dichloromethane. Although the highest recovery of triPAPSs was obtained with *n*-hexane, the lower molecular mass triPAPSs (with a short chain FTOHs *m/z* 1037 and 1137) were absent in respective extracts. On this account, ethylacetate was selected as the most suitable solvent, extracting the entire spectrum of PAPSs isomers. Since analytical standards were not available, extracts prepared by sonication of respective sample in methanol, dichloromethane and/or ethylacetate (i.e. conditions representing the worst case scenario, when nearly all unbound additives migrate into the food) were used for comparison of (relative) migration rates.

Considering the high number of positive packaging materials we found in fast food chains, migration tests were designed to simulate typical conditions under which their contact with food occurs: temperatures up to 60°C, storage time 1–72 hrs. As food simulants, water, aqueous acetic acid (3%, *w/w*) and olive oil were used. In line with the earlier published data [6], the highest migration rate was into the olive oil. Interestingly, leaching for 1 hr at 60°C resulted in comparable amounts of diPAPSs that were found after 24 hrs at ambient temperature. No further increase of diPAPSs occurred at longer migration times (up to 72 hours). Migration of triPAPSs into olive oil was significantly lower in both experiments (1% of amount extracted by ethylacetate) and slightly increased with time of migration. While monoPAPSs were not detected in olive oil at all, the 6:2 monoPAPS was transferred into warm water (60°C) in the same extent as into the methanol, on the other hand, the migration intensity of 8:2 monoPAPS into water was only 20% of that content in methanolic extract, for 10:2 monoPAPS less than 1%. DiPAPSs migrated also into the water, but the amount was less than 0.1% of dichloromethane extract.

No migration of triPAPSs and diPAPSs was found into the acidic water. Acidification of water decreased migration of 6:2 and 8:2 monoPAPSs significantly, approx. 100 times.

In the follow-up experiments, attention will be paid to accurate quantification of migrating PFSs into a wide range of food matrices.

Acknowledgements

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