6th MS-FoodDay
Camerino, September 25-27, 2019

PERFLUOROALKYLATED POLLUTANTS IN LIVER OF FARM ANIMALS BY LC-Q-ORBITRAP: METHOD DEVELOPMENT AND VALIDATION

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Keywords: *Perfluoroalkyl substances (PFASs), animal liver, LC-Q-Orbitrap*

Introduction

Perfluoroalkylated substances (PFASs) have been produced since the 1950s and they represent a wide group of highly stable synthetic compounds used in various industrial applications. They are found, for example, in food packaging, non-stick coatings, fireproof foams, paper coatings and fabrics and personal care products [1]. Over the past decade, PFASs have proven to be ubiquitous in water, air, food, wildlife and humans thanks to their high resistance to typical environmental degradation processes. Although studies on their toxicity are not definitive, their action as endocrine disruptors is now clear. The aim of this work was the development and validation of an analytical method for the determination of a considerable number of these pollutants (33) in animal liver samples.

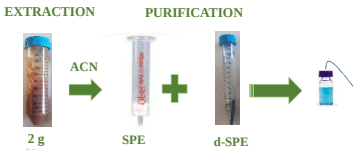

Figure 1- Sample preparation steps

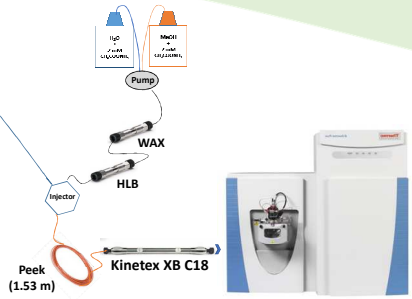

Figure 2- Instrumental settings

Results and Discussion

One of the major drawback of PFAS analysis is the possible laboratory contamination due to the extensive use of these substances in industrial products and, therefore, also in labware and equipments. For example, during the preliminary experiments it was noticed that the LC system released some PFASs. Two cartridges (2.1 x 20 mm) packed with weak anion exchange (WAX) and hydrophilic-lipophilic balance (HLB) stationary phase, respectively, were therefore installed between the pumping system and the injector device to minimize PFAS emission (Figure 2). Furthermore, even the SPE WAX cartridges used to purify the sample extracts (Figure 1) must be preliminary washed with MeOH. With regard to the optimization of chromatographic conditions, the high number of analytes (33) with very different polarities prevented the achievement of acceptable peak shapes for all the compounds. In order to dilute the high percentage of "strong" phase (80% MeOH) contained in the dissolution mixture of the final extracts, a peek tube was installed between the injector and the analytical column, improving peak symmetry and reducing broadening. This arrangement also allowed to increase the injection volume (from 5 to 20 µL). The method was validated according to Regulation 2017/644 requirements [3] starting from 2 ng/kg to 1000 ng/kg on wet weight basis. The results were satisfactory with intra-laboratory reproducibility CVs lower than 20% and trueness from 80 to 110%. Detection and quantification limits were from 2 to 50 ng/kg. Finally, real liver samples belonging to pig, bovine and poultry species were collected at local slaughterhouses and analysed. In Figure 3, the LC-Q-Orbitrap chromatograms of a pig liver are shown. The sample was analysed with spiking (10 ng/kg) and without (blank). Some of the determined PFASs such as PFOA, PFOS, PFUdA, PFDoA were "naturally" present in the animal tissue, confirming the ubiquity of perfluoroalkylated substances also in food producing species [4].

Thirty-three analytes were included in the method scope together with twenty-one labelled compounds used as internal standards (Table 1). An amount of 2 g each homogenized liver was purified following the protocol suggested by Kärrman et al. [2] with slight modifications (Figure 1). The quantification was performed by liquid-chromatography coupled to Q-Orbitrap analyser (LC-Q Exactive, Thermoscientific, San Jose, California, USA) using ESI negative ionization mode and full scan/SIM acquisition. The resolving power was set at 17500, 35000 or 70000 (FWHM at *m/z* 200) depending on the reachable number of acquisition points (minimum 10). The mobile phases were water and MeOH both containing ammonium acetate. The analyte separation was achieved on a LC column Kinetex XB-C18 100 Å (100 mm x 3 mm, 2.6 µm, Phenomenex). The validation study was performed spiking liver samples of different animal species (bovine, pig and poultry) at eight concentrations: 2, 5, 10, 25, 50, 100, 500 and 1000 ng/kg. For each level, four replicates in three different days were carried out, except at 500 and 1000 ng/kg (one validation session).

Experimental

Table 1- List of the 33 analytes and related labelled compounds

N	Name	Acronym	Labelled compounds
1	Perfluoro-n-butanoic acid	PFBA	[13C4]PFBA
2	Perfluoro-n-pentanoic acid	PFPeA	[13C5]PFPeA
3	Perfluoro-n-hexanoic acid	PFHxA	[13C5]PFHxA
4	Perfluoro-n-heptanoic acid	PFHpA	[13C4]PFHpA
5	Perfluoro-n-octanoic acid	PFOA	[13C8]PFOA
6	Perfluoro-n-nonanoic acid	PFNA	[13C9]PFNA
7	Perfluoro-n-decanoic acid	PFDA	[13C6]PFDA
8	Perfluoro-n-undecanoic acid	PFUdA	[13C7]PFUdA
9	Perfluoro-n-dodecanoic acid	PFDoA	[13C2]PFDoA
10	Perfluoro-n-tridecanoic acid	PFTrDA	[13C2]PFTeDA
11	Perfluoro-n-tetradecanoic acid	PFTeDA	[13C2]PFTeDA
12	Perfluoro-n-hexadecanoic acid	PFHxDA	[13C2]PFHxDA
13	Perfluoro-n-octadecanoic acid	PFODA	[13C2]PFHxDA
14	Potassium perfluoro-1-butanesulfonate	L-PFBS	[13C3]L-PFBS
15	Sodium perfluoro-1-pentanesulfonate	L-PFPeS	[13C3]L-PFHxS
16	Sodium perfluoro-1-hexanesulfonate	L-PFHxS	[13C3]L-PFHxS
17	Sodium perfluoro-1-heptanesulfonate	L-PFHpS	[13C3]L-PFHxS
18	Sodium perfluoro-1-octanesulfonate	L-PFOS	[13C3]L-PFOS
19	Sodium perfluoro-1-nonanesulfonate	L-PFNS	[13C6]PFDA
20	Sodium perfluoro-1-decanesulfonate	L-PFDS	[13C7]PFUdA
21	Sodium perfluoro-1-dodecanesulfonate	L-PFDoS	[13C7]PFUdA
22	Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	9Cl-PF3ONS	[13C7]PFUdA
23	Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUdS	[13C7]PFUdA
24	Sodium 1H,1H,2H,2H-perfluorooctanesulfonate	6:2FTS	[13C2]M2-6:2FTS
25	Sodium 1H,1H,2H,2H-perfluorodecanesulfonate	8:2FTS	[13C2]M2-8:2FTS
26	3-Perfluoroheptylpropanoic acid	FHpPA	d3-N-MeFOSAA
27	2-Perfluorooctylethanoic acid	FOEA	[13C2]MFOEA
28	2H-Perfluoro-2-decenoic acid	FOUEA	[13C2]MFOUEA
29	2,3,3,3-tetrfluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid	HFPO-DA	[13C3]M3HFPO-DA
30	Sodium dodecafluoro-3H-4,8-dioxanonanoate	NaDONA	[13C5]PFHxA
31	N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	d5-N-EtFOSAA
32	N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	d3-N-MeFOSAA
33	Potassium perfluoro-4-ethylcyclohexanesulfonate	PFECHS	[13C5]PFHxA

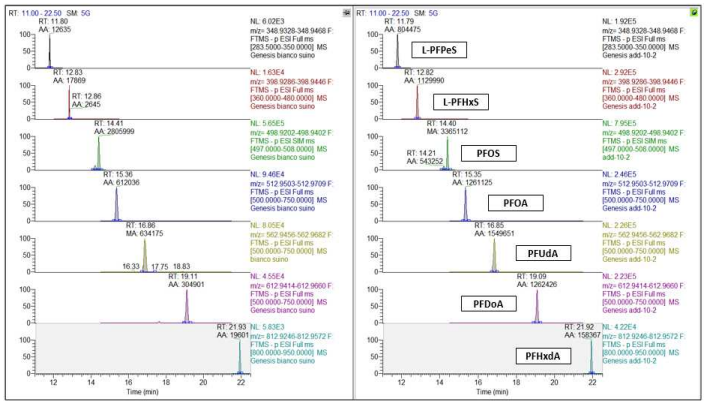

Figure 3- LC-Q-Orbitrap chromatograms of a blank pig liver (left) and pig liver fortified at 10 ng/kg (right). The endogenous levels (left side) of PFOS, PFOA, PFUdA, PFDoA were 90, 12, 5 and 4 ng/kg, respectively.

Conclusions

The measures put in place during the method development allowed to minimize and keep the PFAS laboratory contamination under control. One of the consequences was the possibility of reaching limits of detection and quantification lower than 100 ng/kg. This work demonstrated that the achievement of satisfactory performances in PFAS analysis not only depends on the available instrumentation, but also on the implementation of rigorous quality assurance practices including the availability of a wide set of labelled compounds to quantify PFASs (isotope dilution).

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Acknowledgements

This work was supported by the Italian Health Ministry (Ricerca Corrente IZSSA01/16 "Development and validation of non/multi-target methods for identification and quantification of per-and poly-fluorinated chemicals (PFAS) in the food chain, to support risk assessment").
Thanks to the DSM-SCI for the fellowship to support 6th MS FOOD DAY participation.