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POLYCYCLIC AROMATIC HYDROCARBONS IN FATTY FOOD USING HRGC-HRMS: FAPAS RESULTS

B. Angelone[1], M. Gasparini[1], E. Ferretti[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna " Bruno Ubertini"*

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are a large group of molecules, about 660[1], containing two or more condensed aromatic rings. They are produced by incomplete combustion of organic matter or released into the environment by industrial processes[2]. Toxicological studies confirmed the carcinogenicity of some PAHs[3]. Their lipophilic properties cause accumulation in adipose tissue and their hydrolysed derivatives are able to bind to the DNA resulting in genetic mutation processes[4]. To ensure public health, the European Commission regulated some of these molecules and defined indicators for the presence of PAHs in food: standard methods for food sampling and analysis are established in Reg. (EU) 333/2007[5] and maximum limits for Benzo (a) Pyrene and the sum of 4 PAHs (Benzo (a) Pyrene, Benzo (a) Anthracene, Benzo (b) Fluoranthene and Chrysene) are set in Reg. (EU) 1881/2006[6].
Recently, Pöhlmann et al. (2013) introduced the use of gas chromatography coupled to high resolution mass spectrometry to selectively detect PAHs in smoked meats and in traditional products from north-east Europe[7]. The use of a very selective mass spectrometric detection method, associated to a stereo-specific chromatographic system, allows unique identification of PAHs.
The aim of this work was to develop an analytical method suitable to quantify 9 polycyclic aromatic hydrocarbons (Figure 1) in different fatty foods, both of animal and vegetable origin, using extraction and purification procedures able to isolate the molecules of interest from interfering substances and selective instrumental method to separate different structural isomers, according with performance criteria indicated by the current regulations.

Materials and methods

Before the analysis, all matrices, except vegetable oils, were homogenized and freeze-dried. Food samples (1-2 g of powder) were mixed with diatomaceous earth and spiked with a mixture of ^{2}H-labeled PAHs. The extraction was performed with Accelerated Solvent Extractor (ASE), samples were subjected to two extraction cycles at 100°C and 1500 psi using n-Hexane. The solvent was filtered through anhydrous sodium sulfate and concentrated to 0.5 ml in a TurboVap evaporator at 35°C with a gentle stream of Nitrogen. The lipid fraction was solubilized with ethyl acetate/cyclohexane solution (1:1, v:v) to a volume of 10 ml and injected in a GPC system (Gel Permeation Chromatography). The vegetable oil sample was directly injected into the GPC system. The purification program provided a flow of 5 ml/min with a mixture of ethyl acetate/cyclohexane (1:1, v:v), the collection fraction ranged from 32 to 65 minutes. The sample was concentrated to 0.5 ml in a TurboVap evaporator. The second purification step provided a filtration on a pre-packed silica column, conditioned with 3 ml of cyclohexane. The column was washed with 10 ml of cyclohexane and the filtrated was evaporated to dryness in a TurboVap evaporator. PAHs fraction was dissolved in 50 µl of ^{13}C-labeled US EPA 16 PAH Cocktail solution. Instrumental analysis of food samples was carried out with TRAGE GC ULTRA gas chromatographs coupled with DFS high resolution magnetic scan system (Thermo Fisher Scientific, USA). Chromatographic separation was performed using PAHs Select Agilent Technologies capillary column (10 m x 0,15 mm x 0,10 µm).

Results and discussions

The oven program and the column used led to a great chromatographic separation of critical pairs and triplets. Figure 2 shows the separation between Benzo [b] Fluoranthene, Benzo [k] Fluoranthene Benzo [j] Fluoranthene in the processed milk sample, often not solved with less selective columns.
Complete validation studies were conducted on matrices sampled in the majority from routine analysis, such as vegetable oils and milk. For these products, parameters required by the legislation were studied, such as linearity, specificity, repeatability, reproducibility, limit of detection (LOD) and limit of quantitation (LOQ), processing fortified samples with a mixture of native to the interesting levels.

According to the ISO/IEC/17025[8], measurement uncertainty was also estimated with the Bottom-up approach, taking into account the parameters that most affected the analytical determination.
Furthermore, the method was tested on other matrices of interest using materials belonging to FAPAS circuits with already known results. The applicability of the method was evaluated studying the repeatability and accuracy on different types of matrices. Through reports of results, z-scores were calculated using the following formula (Figure 3):
The results obtained are reported in Figure 4 and Table 2.
The validation study confirmed that the developed analytical method is applicable to different types of matrices. The excellent chromatographic separation through highly selective column and the identification given by a high-resolution detection system, allowed an unequivocal determination of critical pairs and triplets. The use of isotope dilution and response factors led to an independent quantification from different matrix effects.

References:

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[4]B. Veyrand, A. Brosseaud, L. Sarcher, V. Varlet, F. Monteau, P. Marchand, F. Andre, B. Le Bizec: Innovative method for determination of 19 polycyclic aromatic hydrocarbons in food and oil using chromatography coupled to tandem mass spectrometry based on an isotope dilution approach. Journal of Chromatography A, 1149 (2007) 333-344.

[5]Commission Regulation (EC) N. 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs and subsequent amendments

[6]Commission Regulation (EC) N. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs and subsequent amendments

[7]M. Pöhlmann, A. Hitzel, F. Schwägele, K. Speer, W. Jira, Polycyclic aromatic hydrocarbons (PAH) and phenolic substances in smoked Frankfurter-type sausages depending on type of casing and fat content. Food Control 31 (2013) 136-144

[8]ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories

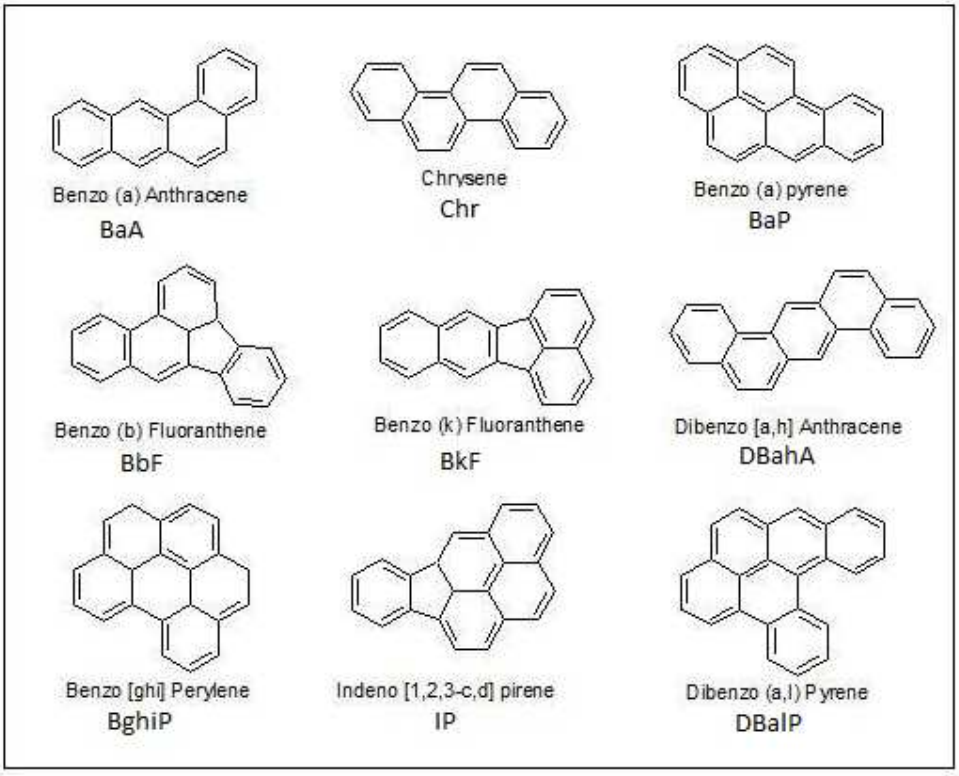

Figure 1: PAHs validated in this study

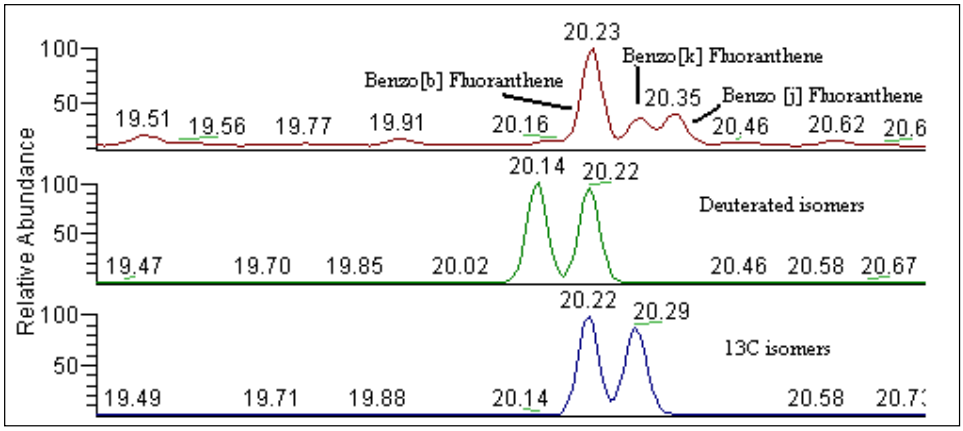

Figure 2: Chromatographic separation of critical triplet in a milk sample

$$\text{Z-scores} = \frac{(x - x_a)}{\sigma_p}$$

x= participant's reported result
x_a= assigned value
σ_p= standard deviation for proficiency

Figure 3: Z-score formula

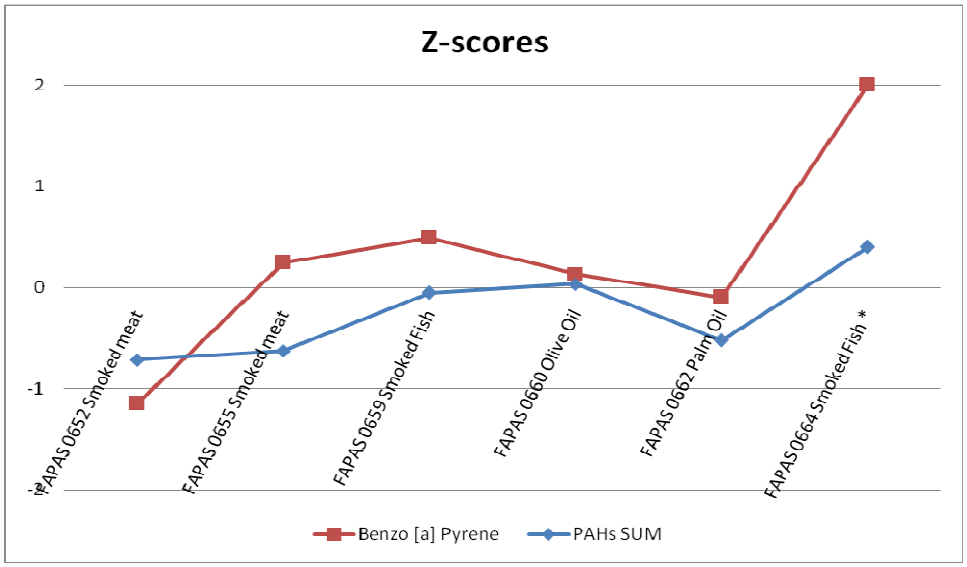

Figure 4: Z-scores calculated and assigned (*) in different matrices

	FAPAS 0652 Smoked meat	FAPAS 0655 Smoked meat	FAPAS 0659 Smoked Fish	FAPAS 0660 Olive Oil	FAPAS 0662 Palm Oil	FAPAS 0664 Smoked Fish *
Benzo[a] Anthracene	-0.57	0.01	0.74	0.32	0.00	0.70
Chrysene	-0.73	-1.11	-0.28	0.15	-0.80	-0.70
Benzo [b] Fluoranthene		0.30	-0.26	-0.07	-0.82	0.50
Benzo [k] Fluoranthene				-0.03		
Benazo [a] Pirene	-1.14	0.24	0.49	0.13	0.10	2.00
Indeno (1,2,3-c,d) Pyrene	-1.73	-0.54	-0.77	-0.10	-1.06	0.00
Dibenzo [a,h] Anthracene				0.89		
Benzo[ghi] Perylene	-1.19	0.89	0.48	0.25	0.76	0.50
Dibenzo [a,l] Pyrene				-0.06		
PAHs Sum	-0.71	-0.62	-0.05	0.03	-0.52	0.40

Table 2: Z-score calculated and assigned (*) in different matrices