

Determination of the four EU marker Polycyclic Aromatic Hydrocarbons (PAHs) in Cocoa, Milk-based Infant formula and Cereal-based Baby Foods



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Abstract

A high sensitivity method for the determination of the four EU marker polycyclic aromatic hydrocarbons (PAHs) benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene in cocoa, milk-based infant formula and cereal-based baby foods with relative fat content levels from 5% to 35% was developed successfully. It is a method that employs internal standard (benzo[b]chrysene), the sample preparation is based on saponification, liquid-liquid extraction and solid phase extraction clean-up (C18 and Florisil) followed by high-performance liquid chromatography with fluorescence detection (HPLC-FLD). In the case of milk-based infant formula, severe matrix interferences were tackled with the addition of α -amylase enzyme that provided improved chromatography and higher selectivity. Moreover, validation of the proposed method was performed for cocoa according to the Second edition (2014) of the Eurachem guide and fulfilled the required performance criteria as far as it concerns the precision, selectivity, trueness and linearity. The developed method was calibrated for the linear range of $1 \mu\text{g kg}^{-1}$ to $10 \mu\text{g kg}^{-1}$. Recovery values of more than 60% were achieved for all the target process contaminants in three different types of food matrices with expanded relative uncertainties below 28%. In cocoa, the achieved limits of detection were $0.21 \mu\text{g kg}^{-1}$ for benzo[b]fluoranthene, $0.17 \mu\text{g kg}^{-1}$ benzo[a]pyrene and $1 \mu\text{g kg}^{-1}$ for benz[a]anthracene and chrysene. The performance of the analytical method fulfilled the requirements set by Commission Regulation (EC) No 333/2007, modified by Commission Regulation No 836/2011, for cocoa.

Method Protocol



Samples Commercial Infant Formula (Sanilac), Cereal-based Baby food and Cocoa of YIOTIS S.A.

Injection volume: 50 μL
Mobile Phase: A: MeCN 100 %
 B: MeCN / H₂O 50:50

Pump Gradient:
 0 min A 50 %, B 50 %
 8 min A 50 %, B 50% Isocratic
 25min A 65 %, B 35 % Linear Gradient
 40 min A 65 %, B 35 % Isocratic
 50 min A 100%, B 0% Linear Gradient
 80 min A 100 %, B 0 %

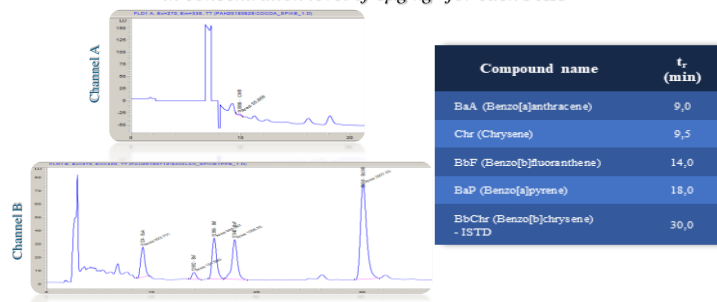
Post Run: 10 min
Flow Rate: 1 ml/min
Column Temperature: 25°C
Column: Vydac PAH 250X4.6 mm, 5 μm

FLD
Time program of Excitation:
 0 min 270 nm,
 8 min 290 nm,
 29.5 min 270 nm

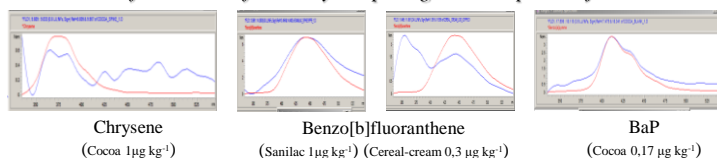
Time program of Emission:
 Channel A:
 0 min 335 nm
 8min 400nm,
 29.5 min 385 nm
 Channel B: 420 nm

Chromatographic Conditions

Representative Chromatograms of a sample spiked in concentration level of $1 \mu\text{g kg}^{-1}$ for each PAH



Overlaid spectra acquired on-line from analysis of different matrices to verify absence of matrix interference by comparing with the spectra of standards



Validation

Compound	Equation	r ²
BaA	y=0,0312x-0,0024	0,999
CHR	y=0,0058x-0,0008	0,998
BbF	y=0,0118x-0,0013	0,999
BaP	y=0,0653x-0,0033	0,999

Cocoa Compound	Recovery (%)	RSD _r (%) N=4	RSD _d (%) N=3	LOD ($\mu\text{g kg}^{-1}$)	u < U _f
BaA	108	12	11	1	0,21
CHR	91	18	17	1	0,27
BbF	89	10	9	0,21	0,16
BaP	100	10	8	0,17	0,16

Conclusions

- The determination of PAHs is a complex procedure and demands extended clean-up steps to overcome matrix interferences.
- LOD for each PAH was defined as the concentration in samples that its acquired spectra had satisfactory match (over 800) when compared with the respective template spectra registered in the HPLC software (spectra library) – LODs would be even lower if calculation was based in the Signal to Noise way.
- Milk-based infant formula demands the use of α -amylase in order to clearly isolate PAHs from starchy components.
- The use of selective SPE columns (+NH₂) was tested without satisfactory results
- Future work: - screening of 15 EU priority PAHs
 - extension to GC-MS as confirmative analysis
 - application to chocolate and other types of baby foods