6th International Symposium on Recent Advances in Food Analysis
November 5 - 8, 2013 - Prague, Czech Republic

16-EU PAHs IN EDIBLE FATS BY STYRENE DIVINYLBENZENE SPE CLEAN-UP AND SOLVENT VENT PTV GC-MS ANALYSIS

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Introduction

Regulation (EU) 835/2011 identifies benzo(a)pyrene (BaP), benz(a)anthracene (BaA), benzo(b)fluoranthene (BbF) and chrysene (CHR) as indicators for the occurrence of the 16-EU priority Polycyclic Aromatic Hydrocarbons (PAHs) in food, replacing the BaP (Regulation (EU) 1881/2006). Regulation (EU) 836/2011 set specific performances criteria that analytical methods should have before being applied for the official control of PAHs (i.e. LOQ and LOD should be ≤0.9 and ≤0.3 µg/kg respectively). PAHs are highly lipophilic substances thus, in food analysis, their selective isolation from fats is a challenging issue. Analytical methods which use size exclusion chromatography purification are commonly used to isolate PAHs from fat, but there is a limit in respect to the amount of fat loadable on a 1x50 cm GPC-column. Here we present a fast, effective and sensitive method which couples a double clean-up step (styrene-divinylbenzene SPE column and GPC chromatography) with a Large Volume Injection in Programmed Temperature Vaporization (LVI-PTV) GC-MS analysis . The procedure enables the analysis of up to 2 g of fat and oil reaching easily LOQs of 0.9 µg/kg for all the 16-EU priority PAHs.

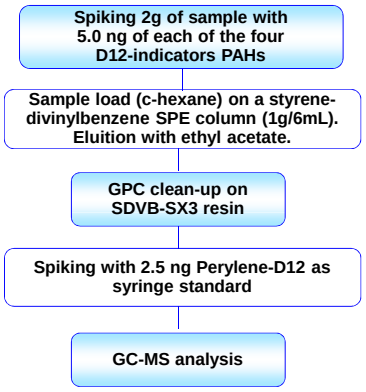

Figure 1 *- Flow diagram of sample preparation*

GC-MS Method

- **Instrument**: Agilent GC-6890N, MS 5973 inert
- **Column**: Agilent DB-17MS 20mx0.18mmx0.18µm.
- **Oven temperature program**: 50°C, ramp to 200°C at 60°C/min, ramp to 220°C at 2°C/min, ramp to 285°C at 3°C/min, ramp to 320°C at 5°C/min hold 13 mins.
- **Injector temperature program**: 50°C, hold 0.25 min, ramp to 320°C at 720°C/min.
- **Temperatures**: transfer line 320°C, source 300°C, quadrupole 150°C
- **Injection mode**: PTV-solvent vent. Vent flow 50 mL, vent pressure 0 psi until 0.2 min.
- **Injection Vol**: 3 µL

Validation study

The method was validated evaluating selectivity, linearity, precision and trueness. The performances were studied doing replicated analysis (n=14) at three different concentration levels (0.9, 2.0, 4.0, µg/kg) in intra-laboratory reproducibility conditions for all the 16-EU priority PAHs.

Table 1 *– GC-MS method: selected ions*

Elution order	PAHs	Monitored Ions(m/z)	
		Target	Qualifiers
1	7H-Benzo[c]fluorene	216	215
2	Benzo[a]anthracene-D12	240	
3	Benzo[a]anthracene	228	226
4	Cyclopenta[cd]pyrene	226	224
5	Chrysene-D12	240	
6	Chrysene	228	226
7	5-Me-Chrysene	242	241
8	Benzo[b]fluoranthene-D12	264	
9, 10, 11	Benzo[b], Benzo[k], Benzo[j]fluoranthene	252	250
12	Benzo[a]pirene-D12	264	
13	Benzo[a]pyrene	252	250
14	Perylene-D12	264	
15	Indeno[1,2,3-cd]pyrene	276	277
16	Dibenzo[a,h]anthracene	278	276
17	Benzo[ghi]perylene	276	277
18, 19, 20, 21	Dibenzo[a,l], Dibenzo[a,e], Dibenzo[a,i], Dibenzo[a,h]pyrene	302	300, 303

Results and Discussion

The GPC chromatography performed on a 1x50 cm column is an efficient clean-up only if low fat matrices are analysed since maximum 100 mg of fat can be loaded on the column. Willing to extend the procedure to highly fatty matrices, a preliminary purification step on a styrene-divinylbenzene (SDVB) SPE has been introduced. SDVB-SPE enable a hundred fold reduction of the fat with good recoveries of the analytes. Afterwards size-exclusion chromatography was performed.

Optimization of the LV-PTV injection parameters was achieved following already published studies [1]. LVI-PTV has shown to be the right approach to enhance the sensitivity of all the 16-PAHs GC-MS analysis, particularly of the last eluting high molecular weights compounds (dibenzopyrenes) which have poor chromatographic responses with broadened peaks (Figure 2).

Instrumental responses were linear in the range 2-100 ng/mL (r^2 > 0.998). With regards to method accuracy, generally good performances were obtained for all the analytes except for Cyclopenta[cd]pyrene(CCP), which shows very high recoveries for all the three spiking levels investigated, probably as a result of not resolved interferences. The Dibenzo[a,i]pyrene (DiP) and Dibenzo[a,h]pyrene (DhP) have too high recoveries only at the LOQ, better performances are obtained at 2 and 4 ng/g. Also good laboratory repeatability and intra-laboratory reproducibility (still estimated only for the LOQ and the LMR) were obtained for all the analytes except for CCP, DiP and DhP (Table 2).

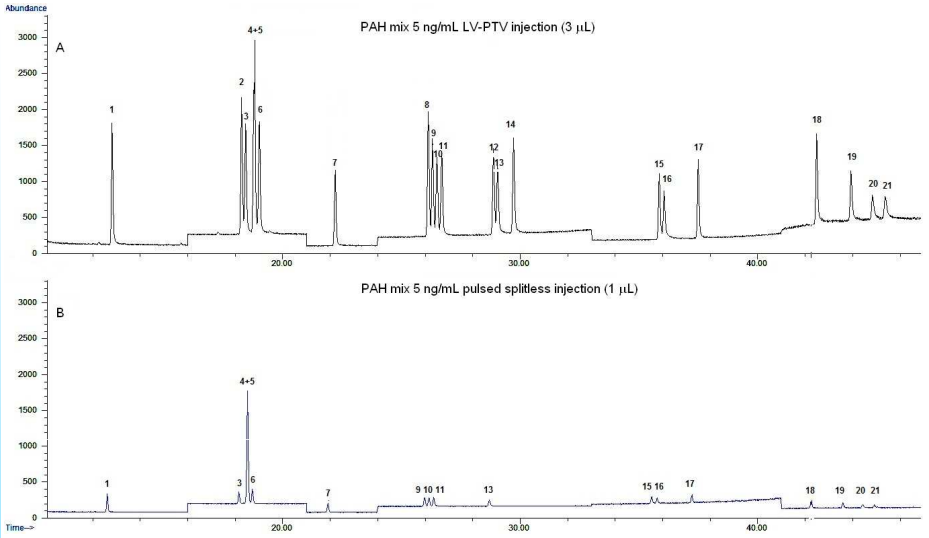

Figure 2 *– TIC Chromatograms (A) of a mixture of the16 priority PAHs (5 ng/mL), the 4 D12-analogues and the D12-Perylene (10 ng/mL) injected (3 µL) in LV-PTV mode and (B) of a mixture of the16 priority PAHs (5 ng/mL) and Chrysene-D_{12} (50 ng/mL) injected (1 µL) in pulsed splitless.*

Table 2 *- Validation study: spiking levels, mean concentrations, recoveries (R %) and coefficients of variation obtained in repeatibility (CVr,%) and inter-laboratory reproducibility (CVR,%) conditions*

PAHs	BcF	BaA	CCP	CRI	5MC	BbF	BkF	BjF	BaP	IcP	DhA	BgP	DlP	DeP	DiP	DhP
Spiking Level (ng/g)	**0.9 *(LOQ)***															
Mean (ng/g fat)	0.9	0.9	1.4	1.0	1.1	0.9	1.0	1.0	0.9	1.0	0.9	0.7	0.8	0.8	1.3	1.5
R%	99	106	158	114	122	103	111	115	99	114	99	81	85	84	145	165
CVr (%)	3.3	3.1	4.5	5.1	5.6	5.3	5.1	5.4	4.0	6.3	18	4.0	4.3	5.1	4.1	6.4
CV_R (%)	18	6.4	36	6.4	5.7	6.8	5.2	5.2	8.2	6.1	16	20	15	14	11	15
Spiking Level (ng/g)	**2.0 *(LMR)***															
Mean (ng/g fat)	1.7	2.0	3.9	2.1	2.4	2.0	2.2	2.2	1.9	1.7	1.4	1.3	1.4	1.3	2.1	2.5
R%	86	100	194	106	120	100	109	113	96	88	72	67	72	66	106	126
CVr (%)	1.7	1.0	5.4	1.3	1.3	1.6	1.8	1.4	1.3	2.2	23	0.8	1.3	1.5	2.3	4.4
CV_R (%)	6.6	12	25	13	13	13	10	13	8.6	4.3	21	12	6.2	7.8	20	36
Spiking Level (ng/g)	**4.0 *(2LMR)***															
Mean (ng/g fat)	3.8	4.4	8.7	4.7	5.4	4.5	4.8	5.0	4.2	3.8	3.6	3.2	3.1	2.9	3.7	4.1
R%	95	112	219	118	136	113	120	125	106	97	91	80	77	73	92	104
CVr (%)	1.3	0.9	3.6	1.6	1.4	1.6	1.5	1.9	0.8	1.5	6.0	0.7	0.6	1.2	1.7	2.1

Conclusions

The use of SDVB-SPE columns before the GPC-chromatography enable to extend the PAHs-analytical procedure to highly fatty matrices reaching performances compliant to legislative requirements. Also LVI-PTV helped to reach the objective with a considerable improvement of the GC-MS response.

The method is easily applicable and enable the simultaneous analysis of several samples in one batch.

References

[1] Gòmez-Ruiz J., Cordiero F., Lòpez P., Wenzl T. Talanta 2009; 80: 643-650.

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Stampato a cura dell'Unità Operativa di Supporto Biblioteca, Informazione, Editoria (2011).