

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 $\mu\text{g}/\text{kg}$ 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 $\mu\text{g}/\text{kg}$ for all the 16-EU priority PAHs.

GC-MS Method

- Instrument: Agilent GC-6890N, MS 5973 inert
- Column: Agilent DB-17MS 20mx0.18mmx0.18 μm .
- 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, $\mu\text{g}/\text{kg}$) 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,i], Dibenzo[a,e], Dibenzo[a,h]pyrene | 302 | 300, 303 |

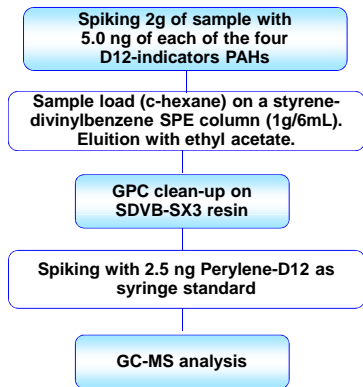


Figure 1 - Flow diagram of sample preparation

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 LVI-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).

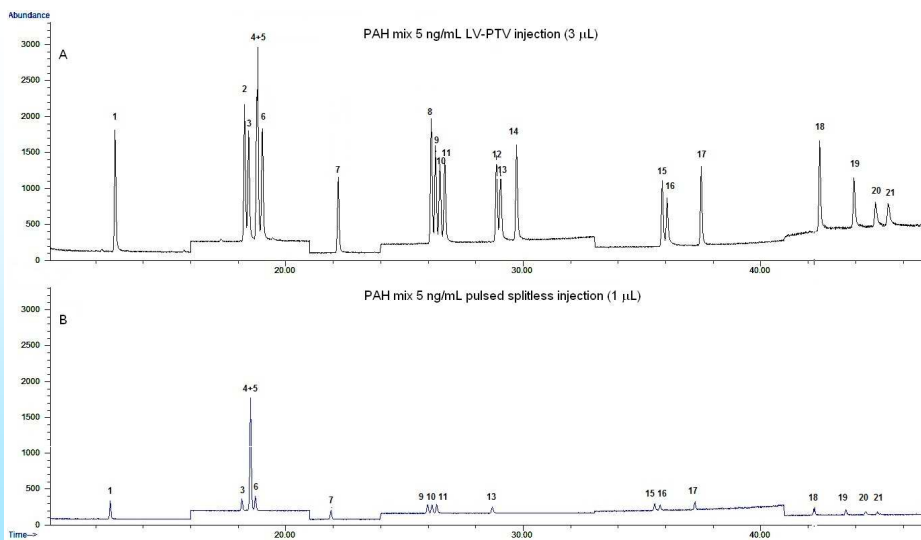


Figure 2 – TIC Chromatograms (A) of a mixture of the 16 priority PAHs (5 ng/mL), the 4 D12-analogues and the D12-Perylene (10 ng/mL) injected (3 μL) in LV-PTV mode and (B) of a mixture of the 16 priority PAHs (5 ng/mL) and Chrysene-D₁₂ (50 ng/mL) injected (1 μL) in pulsed splitless.

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

| PAHs | BcF | BaA | CCP | CRI | 5MC | BbF | BkF | BjF | BaP | IcP | DhA | BgP | DIP | 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 |
| CVR (%) | 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 |
| CVR (%) | 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] Gómez-Ruiz J., Cordiero F., López P., Wenzl T. Talanta 2009; 80: 643-650.