CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

RAPID GC-MS METHOD FOR ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN SEAFOOD: AOAC COLLABORATIVE STUDY

Lucie DRABOVA[1], Jana PULKRABOVA[1], Kamila KALACHOVA[1], Katerina MASTOVSKA[2], Vladimir KOCOUREK[1], Jana HAJSLOVA[1]*

[1]Department of Food Chemistry and Analysis, Institute of Chemical Technology, Prague, Technicka 3, Prague 6, Czech Republic

[2] Covance Laboratories, NCFS, Greenfield, IN, USA

*jana.hajslova@vscht.cz

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Introduction

Following the Mexico Gulf oil spill (April 2010), AOAC INTERNATIONAL launched the call for submitting rapid analytical methods suitable for quantification of polycyclic aromatic hydrocarbons (PAHs) in the raw edible portions of fin fish and seafood. The purpose was to carry out evaluation through the AOAC Official Methods SM program and, supposing the collaborative study is successful, replace existing conventional , time and labour demanding methods, by the new one. Having a rapid method is essential for quick determination of contaminants in food, especially after environmental disasters.

Within the CONffIDENCE project (Contaminants in food and feed: Inexpensive detection for control of exposure) efficient, cheap, rapid and simple multiresidue analytical method for simultaneous determination of PAHs, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in fish and seafood samples was developed.

This method was send to the AOAC and Working Group on Quantitative Methods recommended the ICT Prague method as the most promising candidate.

Aim of the study

- To evaluate the method's intra-laboratory and inter-laboratory performance.

Target analytes

- **19 polycyclic aromatic hydrocarbons (PAHs):** anthracene (Ant), benzo[a]anthracene (BaA), benzo[a] pyrene (BaP), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[g,h,i]perylene (BghiP), chrysene (CHR), dibenzo[a,h]anthracene (DBahA), fluoranthene (Flt), fluorene (Fln), indeno[1,2,3-cd]pyrene (IcdP), naphthalene (Naph), phenanthrene (Phe), pyrene (Pyr), 3-methylchrysene (3-MC), 1-methylnaphthalene (1-MN), 1-methylphenanthrene (1-MP)

Study design

- 3 matrices: mussel, oyster, shrimp
- total of 5 different spiking levels of BaP (2–50 µg/kg), other studied PAHs at varying levels from 2 to 250 µg/kg that mimic typical PAH patterns
- each matrix fortified at 3 different concentration levels in duplicate + one blank for each matrix, total of 7 x 3 = 21 study samples for each lab

Analytical method

Liquid extraction — 10 g sample in PE tube and ^{13}C-PAHs; 5 mL H_2O and 10 mL EtOAc

shaking 1 min

Partition — 4 g $MgSO_4$ and 2 g NaCl

shaking 1 min

centrifuging

evaporating

reconstitution in 1 mL hexane

Clean-up on silica gel SPE column (1g) — Conditioning: 6 mL Hex:DCM (3:1,*v/v*) and 4 mL Hex; Eluting: Hex:DCM (3:1,*v/v*)

evaporating

GC-MS, GC-MS/MS, GC GC TOFMS

Hex – n-hexane, DCM – dichloromethane, EtOAc – ethylacetate

Results and discussion

(i) Laboratory qualification

Qualification steps:

(1) GC separation test
(2) Calibration range test
(3) Solvent evaporation test
(4) PAH and fat elution profiles
(5) Procedure blank test
(6) Low-level spike test
(7) Practice sample analysis

Table I: Participants instrumantation and analytical condition used within the collaborative study

	MS instrumente type	Analytical Column	GC run time (min)	Injection type	Injection volume (µL)	Evaporation technique	Clean-up	Elution volume (mL)
Lab 01	single quadrupole	**DB-EUPAH** (20m x 0.18 mm x 0.14 µm)	31.8	S/SL	1	vacuum evaporation	in-house prapared columns	6
Lab 02	single quadrupole	**Rxi-17 Sil** (30m x 0.25 mm x 0.25 µm)	41.6	S/SL	1	nitrogen blowdown	Enviro Clean, UCT	5
Lab 03	single quadrupole	**Rxi-17 Sil** (30m x 0.25 mm x 0.25 µm)	46.0	PTV	8	nitrogen blowdown	in-house prapared columns	4.5
Lab 04	single quadrupole	**ZB-50** (30m x 0.25 mm x 0.25 µm)	48.3	S/SL	10	nitrogen blowdown	BAKERBOND SPE Columns, J.T. Baker	7
Lab 05	single quadrupole	**DB-EUPAH** (20m x 0.18 mm x 0.14 µm)	36.2	PTV	5	nitrogen blowdown	in-house prapared columns	5
Lab 06	single quadrupole	**DB-17 MS** (30m x 0.25 mm x 0.25 µm)	47.2	S/SL	1	vacuum evaporation	in-house prapared columns	7
Lab 07	triple quadrupole	**TR-50MS** (30m x 0.25 mm x 0.25 µm)	40.0	PTV	8	nitrogen blowdown	Enviro Clean, UCT	5.5
Lab 08	triple quadropole	**Rxi-17 Sil** (30m x 0.25 mm x 0.25 µm)	39.3	PTV	5	nitrogen blowdown	Enviro Clean, UCT	5.5
Lab 09	TOF	**Rxi-17 Sil** (30m x 0.25 mm x 0.25 µm)	50.0	PTV	8	nitrogen blowdown	in-house prapared columns	4.5

(1) GC separation test

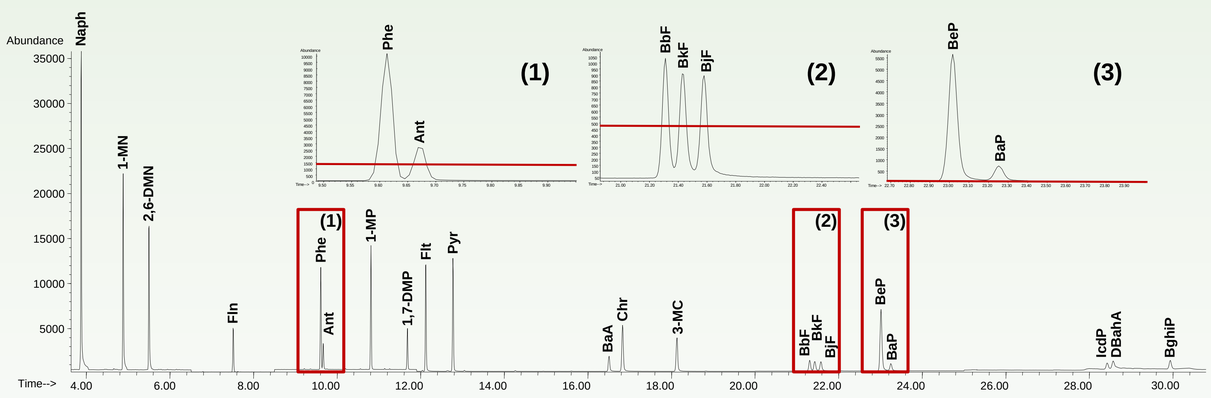

Figure 1: Separation of target PAHs on DB-EUPAH (20 m x 0.18 mm x 0.14 µm) column

(4) PAH and fat elution profiles

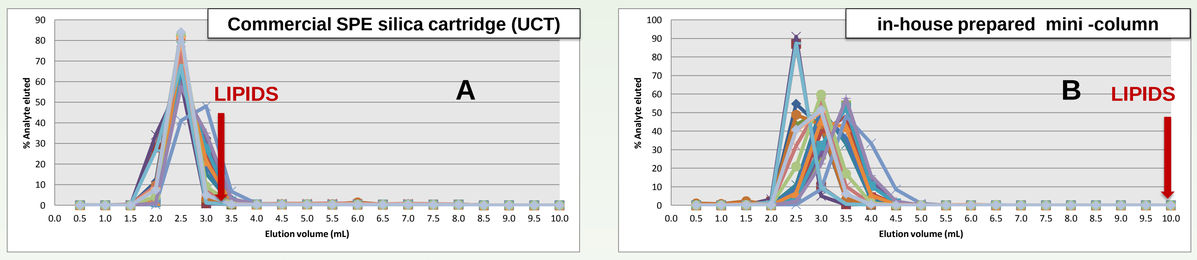

Figure 2: Comparison of PAHs and fat elution profiles on A) commercial SPE silica cartridge (UCT) and B) in-house prepared silica mini-column

(ii) Test sample analysis

Preliminary results:

Oysters (n=8)					
Level I (5 µg.kg^{-1} BaP)		**Level II (10 µg.kg^{-1} BaP)**		**Level III (50 µg.kg^{-1} BaP)**	
Rec %	***RSD %***	***Rec %***	***RSD %***	***Rec %***	***RSD %***
69–112	4–23	64–111	4–24	64–111	2–23
Shrimps (n=8)					
Level I (2 µg.kg^{-1} BaP)		**Level II (5 µg.kg^{-1} BaP)**		**Level III (25 µg.kg^{-1} BaP)**	
Rec %	***RSD %***	***Rec %***	***RSD %***	***Rec %***	***RSD %***
101–120	8–24	102–123	6–17	95–106	4–18
Mussels (n=6)					
Level I (2 µg.kg^{-1} BaP)		**Level II (10 µg.kg^{-1} BaP)**		**Level III (25 µg.kg^{-1} BaP)**	
Rec %	***RSD %***	***Rec %***	***RSD %***	***Rec %***	***RSD %***
80–114	6–21	86–115	3–14	79–107	2–17

Conslusions

(1) Laboratory qualification

- **15 laboratories started** the collaboration study
- **9 laboratories** have been **qualified** and started the actual study (analysis of the test samples)

(2) Test sample analysis

- **Preliminary results:**

Mussels, oysters and shrimps: ***Recovery:*** 64–123%
Repeatability: 2–24%

Lower recoveries of BaP and Ant in oyster samples when stored at -20 C

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