Fast and Efficient Analysis of Polynuclear Aromatic Hydrocarbons in Edible Oils Using Dual-Layer SPE

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Introduction

Polynuclear aromatic hydrocarbons (PAHs) can contaminate edible oils through environmental exposure and processes used in the production of the oil itself. Since some PAHs are suspected carcinogens, this has lead to several countries adopting regulations to limit their content in edible oils. In the European Union, EU Commission Regulation No. 835/2011 sets maximum levels for PAH contamination in oils intended for human consumption (1). This has led to a growing need for simpler and faster testing methodologies.

Traditional Methods for Analysis of PAHs in Edible Oils

Iso Methods

- 15302:
  - Benzo[a]pyrene only, uses large alumina column (30 cm x 1.5 cm) for extraction
- 15753:
  - 16 PAHs (light to heavy), uses liquid-liquid extraction (LLE) and 2-step cleanup with C18 and Florisil® solid phase extraction (SPE)

Other Methods

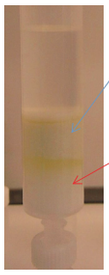
- LLE followed by Gel Permeation Chromatography (GPC)
  - GPC is expensive, time consuming
- Silica gel or Florisil SPE using large, glass columns
  - Expensive, inadequate cleanup for GC
- Molecularly Imprinted Polymer (MIP) SPE
  - Poor recoveries of lighter PAHs

Analytical techniques used include HPLC with fluorimetric detection, GC/MS, and GC/MS/MS.

A New Approach: Dual-layer SPE Cartridge

Supelclean™ EZ-POP NP: Dual-layer SPE cartridge containing Florisil and Z-Sep/C18 mix.

Figure 1. EZ-POP NP Dual-layer SPE during Extraction of PAHs from Edible Oil Sample



Florisil layer: retains background constituents with polar functionality such as fatty acids.

Z-Sep/C18 layer: retains fatty matrix through both Lewis acid/base and hydrophobic interactions.

General Sample Prep Procedure:

1. Condition SPE cartridge with acetone, and dry cartridge.
2. Add oil sample and internal standard onto the cartridge.
3. Elute sample with acetonitrile.
4. Concentrate eluent and analyze by HPLC or GC.

Experimental

- Samples: Canola and soybean oils spiked at 10 ng/g with 15 different PAHs, containing 2 to 6 rings in their structures.
- Extraction: EZ-POP NP, per procedure in Table 1.
- Analysis: HPLC/FLD & GC/MS-SIM, conditions in Tables 2 & 3.
- Quantitation: 5-point calibration curves
  - In solvent for HPLC/FLD
  - In unspiked canola oil extract for GC/MS

Table 1. SPE Method using EZ-POP NP

Condition	• 10 mL acetone (gravity). Dry using vacuum (10-15" Hg) for 10 min.
Load	• 0.5 mL oil weighed directly onto SPE cartridge. Add internal std.
Elute	• 2 x 7.5 mL acetonitrile
Concentrate	• 40 °C, under N2, FV 0.5 mL • do not allow to go dry
Analyze	• HPLC/FLD & GC/MS-SIM

Table 2. HPLC Conditions

column:	SUPELCOSIL™ LC-PAH, 25 cm x 4.6 mm I.D., 5 µm
mobile phase:	(A) water; (B) acetonitrile
gradient:	40% B for 5 min; to 100% B in 15 min; held at 100% B for 12 min
flow rate:	1.4 mL/min
pressure:	2790 psi at start
temp.:	25 °C
det.:	FLD, programmed naphthalene, acenaphthene, fluorene: 225 nm/320 nm phenanthrene, anthracene: 250 nm/368 nm. fluoranthene, pyrene: 237 nm/440 nm benzo[a]anthracene, chrysene: 265 nm/380 nm. benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene: 280 nm/420 nm. benzo[g,h,i]perylene, indeno[1,2,3-cd]pyrene, benzo[b]chrysene: 300 nm/466 nm.
injection:	20 µL

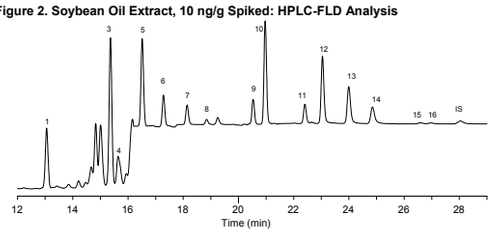
Table 3. GC/MS Conditions

column:	SLB®-35ms, 30 m x 0.25 mm I.D. x 0.25 µm
MS temps:	interface = 330 °C, source = 250 °C, quads = 200 °C
inj. temp.:	300 °C
oven:	60 °C (1 min), 20 °C/min. to 340 °C (10 min)
carrier gas:	helium, 1 mL/min constant flow
injection:	0.5 µL pulsed splitless (60 psi/0.75 min), splitter open at 0.75 min.
liner:	2 mm I.D. FocusLiner™ w/taper

Results and Discussion

- All PAHs were detected free of background by GC/MS. By HPLC-FLD, only fluorene could not be quantitated due to matrix interference. (Soybean oil shown in Figures 2 and 3.)
- Average recoveries for 3 spiked replicates after blank subtraction, as determined by GC/MS-SIM, are reported in Table 4.
  - Most recoveries were >80%, with RSDs <20%; PAHs designated in EU Commission Regulation No. 835/2011 noted in blue.
  - Evaporative losses of lighter PAHs (<4 rings) during concentration step, especially in canola oil. These extracts were concentrated at a faster rate than the soybean oil extracts.
- GC/MS data is compared directly with HPLC-FLD analysis of the same extracts in Figures 4 and 5.
  - Good correlation of recovery results between the two analytical techniques for most PAHs.
  - Acenaphthylene does not fluoresce and could not be analyzed by HPLC-FLD.
  - Coeluting background prevented accurate quantitation of fluorene by HPLC-FLD.
  - Recovery data for chrysene was higher by HPLC-FLD.

Figure 2. Soybean Oil Extract, 10 ng/g Spiked: HPLC-FLD Analysis



1. Naphthalene
2. Acenaphthylene (GC/MS only)
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benzo[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Benzo[a]pyrene
14. Dibenzo[a,h]anthracene
15. Benzo[g,h,i]perylene
16. Indeno [1,2,3-cd]pyrene

IS. Benzo[b]chrysene

Figure 3. Soybean Oil Extract, 10 ng/g Spiked: GC/MS-SIM Analysis

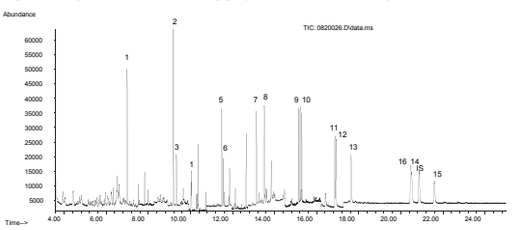


Table 4. Average Recoveries of Spiked Replicates, GC/MS-SIM Analysis

n=3	Soybean Oil		Canola Oil		
	Avg. % Rec.	%RSD	Avg. % Rec.	%RSD	
Naphthalene	24%	34%	5%	30%	Evaporative losses
Acenaphthylene	93%	23%	50%	9%	
Acenaphthene	115%	30%	40%	8%	
Fluorene	106%	25%	38%	8%	
Phenanthrene	112%	19%	41%	15%	
Anthracene	122%	15%	58%	5%	
Fluoranthene	143%	8%	87%	6%	
Pyrene	146%	8%	90%	6%	
Benzo[a]anthracene	145%	9%	105%	4%	
Chrysene	87%	9%	67%	3%	
Benzo[b]fluoranthene	134%	8%	102%	3%	
Benzo[k]fluoranthene	136%	9%	103%	3%	
Benzo[a]pyrene	116%	9%	102%	1%	
Dibenzo[a,h]anthracene	123%	7%	109%	2%	
Benzo[g,h,i]perylene	103%	21%	88%	3%	
Indeno[1,2,3-cd]pyrene	111%	14%	93%	3%	

PAHs designation in EU Commission Regulation No. 835/2011

Figure 4. Analyses of Spiked Soybean Oil Extracts; Comparison of GC and HPLC Data

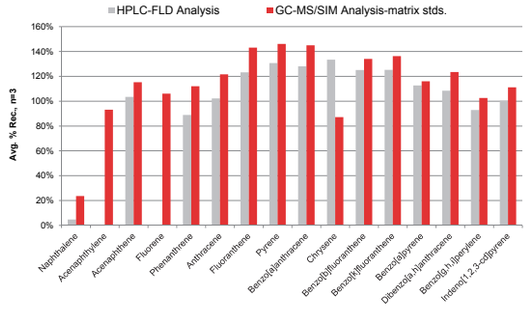
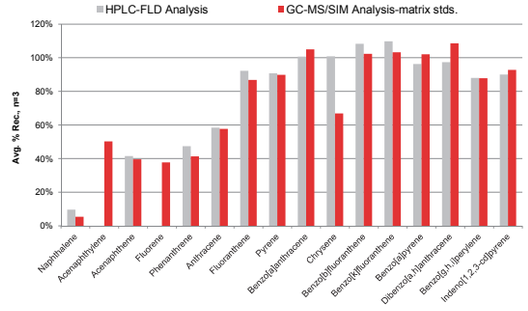


Figure 5. Analyses of Spiked Canola Oil Extracts; Comparison of GC and HPLC Data



Conclusions

- Dual-layer SPE containing Florisil and Z-Sep/C18 sorbents can be used to extract PAHs from canola and soybean oil samples.
  - Adequate recoveries & reproducibility
- The resulting extract can be analyzed by HPLC-FLD or GC/MS.
  - The extract is clean enough for analysis on a single quadrupole GC/MS system.
  - Good correlation in data between two analytical techniques for most PAHs.
  - Fluorene could not be analyzed by HPLC-FLD due to a coeluting interference.
  - The difference in chrysene recovery by GC/MS and HPLC-FLD is not known at this time, but may be matrix-related.

References

1. European Union (EU) Commission Recommendation No 835/2011/EC, Off. J. Eur. Union. L215 (2011) 4.

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