FAST SCREENING OF EDIBLE OILS FOR POLYAROMATIC HYDROCARBONS USING A WATER/METHANOL GRADIENT WITH THE ACQUITY UPLC H-CLASS SYSTEM

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INTRODUCTION

PolyAromatic Hydrocarbons (PAHs) are commonly found in the environment. Formed by the incomplete combustion of hydrocarbons from such diverse processes as forest fires, industrial manufacturing, auto combustion and even food preparation, these compounds can be found in air, water, and various products of human consumption. Of particular concern is their presence in edible seed oils. This can occur due to inadequate drying processes such as overheating or excess smoking.

Because of their prevalence and carcinogenic properties, their analysis is mandated by several U.S. and international agencies. The U.S. Environmental Protection Agency (USEPA) has promulgated several methods such as 550, 610, and 8310 for their detection.

These methods describe HPLC procedures for 16 priority pollutants using several sample preparation procedures followed by separation with a water/acetonitrile gradient and detection by UV and fluorescence. The European Union (EU) targets 15 analytes, 8 of which are common to the USEPA list. One of these analytes, Benzo(a)pyrene (BaP) is used as a marker for the occurrence of PAHs in food and is regulated both in the US[1] and EU[2]. In specific reference to edible oils, the EU have set a maximum residue limit of 2.0 ppb.[2]

This poster will demonstrate rapid screening of several oils for these compounds utilizing dilution with isopropyl alcohol (IPA) followed by injection. A quantitative determination of BaP at the maximum residue limit (MRL) is also shown.

Figure 1 – Waters ACQUITY UPLC H-Class System.

METHODS

Chromatographic Conditions:

System: ACQUITY UPLC® H-Class
with eλ PDA / FLR-LVFC detection
Column: Waters PAH 4.6 X 50mm, 3µ @ 35°C
Injection Volume: 10 µL
Sampling Rate: 20 pts/sec (both detectors)
Filter Response: 0.1 (both detectors)
Detection: PDA (200-500 nm)
FLR (programmed wavelength changes)
Software: Empower™ 2
Mobile Phase A: Water
Mobile Phase B: Methanol
Mobile Phase C: Acetonitrile
Mobile Phase D: Isopropanol

Gradient Table

	Time (min)	Flow Rate (mL/min)	%A	%B	%C	%D	Curve
1	0.00	2.000	30.0	70.0	0.0	0.0	
2	2.25	2.000	0.0	70.0	30.0	0.0	6
3	3.50	2.000	0.0	0.0	100.0	0.0	6
4	6.50	2.000	0.0	0.0	100.0	0.0	6
5	6.60	1.000	0.0	0.0	0.0	100.0	6

Each analyte was scanned to determine the most sensitive excitation and emission wavelengths. The results along with the UV λ max are shown in Table 1. Acenaphthalene and Cyclopenta(c,d)pyrene are non– fluorescent while Benzo(j)fluoranthene exhibits weak fluorescence.[3]

Samples of olive, walnut, and hazelnut oil were spiked at a 10 ppb level with a USEPA 16 component mixture. Using a modification of the method of Lee[4] for oil analysis, each sample along with a blank was diluted 1:10 with IPA and injected. No other sample preparation was employed. The quaternary capability of the ACQUITY UPLC H-Class System was used to clean the column with IPA between each injection. A 10 ppb mix in acetonitrile of the EPA 16 analytes was run for retention time confirmation.

Separate samples of olive oil were spiked at the MRL with Benzo(a)pyrene, carried through the above procedure and injected 7 times each for a reproducibility and recovery study.

Analyte	λ max UV (nm)	Excitation (nm)	Emission (nm)
Naphthalene (N)[1]	220	276	331
Acenaphthalene(Ac)[1]	229	-	-
Acenaphthene (Ace)[1]	226	298	329
Fluorene (F)[1]	261	293	306
Phenanthrene (Pa)[1]	250	248	363
Anthracene (A)[1]	251	248	400
Fluoranthene (Fl)[1]	235	246	488
Pyrene (P)[1]	240	312	390
Cyclopenta(c,d)pyrene (CPP)[2]	223	-	-
Benzo(a)anthracene (BaA)[1,2]	287	282	392
Chrysene (ch) [1,2]	266	267	367
5-Methylchrysene (5-MeCh)[2]	268	268	377
Benzo(j)fluoranthene (BjF)[2]	223	239	511
Benzo(b)fluoranthene (BbF) [1,2]	256	298	437
Benzo(k)fluoranthene (BkF) [1,2]	307	301	407
Benzo(a)pyrene (BaP) [1,2]	296	364	408
Dibenzo(a,l)pyrene (DBalP)[2]	314	314	427
Dibenzo(a,h)anthracene (DBahA) [1,2]	296	297	395
Benzo(g,h,i)perylene (BghiP) [1,2]	299	298	422
Indeno(1,2,3-cd)pyrene (IP) [1,2]	249	305	500
Dibenzo(a,e)pyrene (DBaeP)[2]	302	271	398
Dibenzo(a,i)pyrene (DBaiP)[2]	240	370	436
Dibenzo(a,h)pyrene (DBahP)[2]	309	309	453

Table 1– Spectral Data for PAH Analytes
1– USEPA analyte, 2– EU analyte

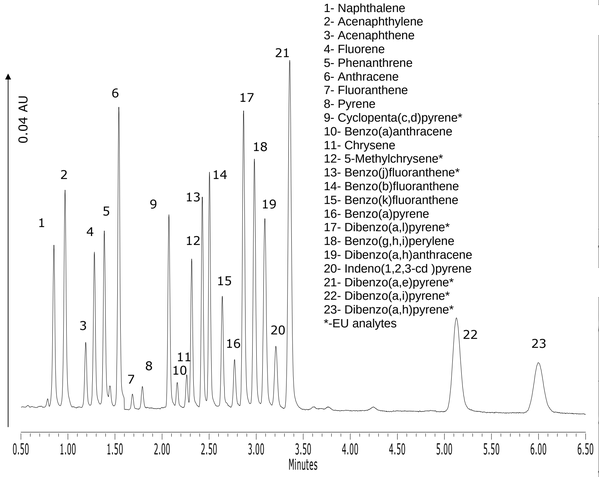

Figure 2 – UV separation of 23 PAH analytes, 1 ppm
Peaks 1-8 @ 254 nm, 9-23 @ 300 nm

RESULTS

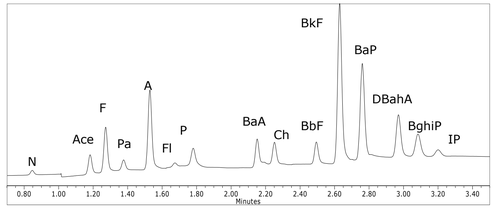

Figure 3 – USEPA 16 PAH analytes– 10 ppb level

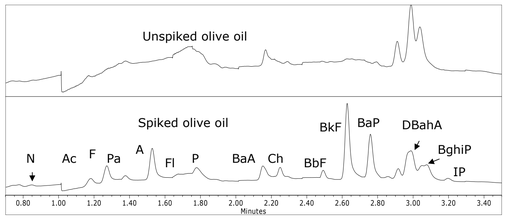

Figure 4 – Unspiked and spiked olive oil

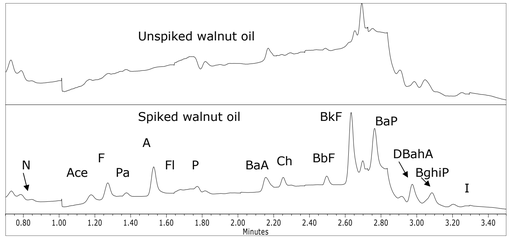

Figure 5 – Unspiked and spiked walnut oil

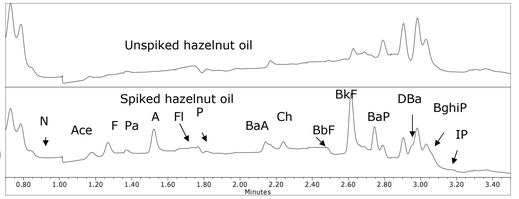

Figure 6 – Unspiked and spiked hazelnut oil

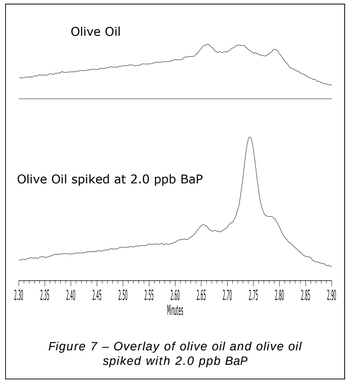

Figure 7 – Overlay of olive oil and olive oil spiked with 2.0 ppb BaP

Spike amount	2.0 ppb
Recovery	1.764 ppb
% Recovery	88.2
% RSD Amount	0.803

Table 2 – Recovery data for 7 injections of olive oil spiked with 2.0 ppb BaP

DISCUSSION

Figure 2 is a UV separation of 23 EPA and EU analytes (1 ppm). Figure 3 is a fluorescence trace of the 16 EPA analytes (10 ppb). Time programmed wavelengths were used for maximum sensitivity. Figures 4-6 are overlays of unspiked and spiked (10 ppb EPA 16 mixture) olive, walnut, and hazelnut oils, respectively. Individual differences in the oil blanks are observed, however the spiked analytes are easily discerned. Acenaphthalene is non-fluorescent and would have to be enriched to a level where it could be determined by UV @ 229nm. The regulated BaP is quite evident in all samples. Figure 7 is an overlay of unspiked and BaP spiked olive oil at 2.0 ppb (MRL).

CONCLUSION

- **A rapid screening method has been developed for PAHs in edible oils using the ACQUITY UPLC H-Class System.**
- **The method meets the MRL set for BaP in edible oil.**
- **The quaternary blending capability of ACQUITY UPLC H-Class System allows a column cleaning step with IPA to be programmed between injections.**
- **Ternary gradient programming allows improved selectivity for the PAH separation.**
- **The analysis of PAHs in edible oils can be achieved in less than 6 minutes without the need for sample preparation.**

References
1. USEPA " National Primary Drinking water Regulations " # 811-F-95-003-T, October 1995 pp.13
2. 2006 R 1881EN 01.07.2007 pp. 22
3. Windal I. et al Validation of the Analysis of the 15+1 European-priority Polycyclic Aromatic Hydrocarbons by Donor-acceptor Complex Chromatography and High Performance Liquid Chromatography-Ultraviolet/Fluorescence Detection" Journal of Chromatography A 1212 (2008) pp16-22
4. Lee, P. et al " ACQUITY UPLC Analysis of Seed Oils (Part 1) Olive Oil Quality and Aldulteration Waters Application Note # 720002025en

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