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Determination of PAHs in vegetable oils by on-line gel permeation chromatography-gas chromatography/mass spectrometry

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1: Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 different chemicals that are formed during the incomplete burning of coal and oil, or other organic substances like tobacco or charbroiled meat. Since the genotoxic and carcinogenic properties of most of the PAHs, the maximum levels for PAHs, specifically benzo[a]pyrene, in certain foods were set by the governments or some international organizations. For PAHs determination in foods, HPLC with Fluorescence detector or GCMS were commonly used. This method employed on-line gel permeation chromatography-gas chromatography/mass spectrometry（GPC-GCMS）to determinate 16 PAHs in vegetable oils. The oil samples were solvent extracted with acetonitrile and dispersive SPE purified by PSA and C18 sorbent. Then the final extract was injected and detected by on-line GPC-GCMS instrument. Comparing with normal GCMS for PAHs determination in vegetable oils, much simpler and quicker sample pretreatment was adopted and higher sensitivity was obtained by using on-line GPC-GCMS.

2: Materials and methods

2-1: Reagents and Instruments

Reagents: Acetonitrile, Hexane, Acetone, Cyclohexane, HPLC grade, purchased from Merk (Germany); PAHs standard solution were purchased from; Accustandard (US); Primary second amine (PSA) and C18 sorbent were purchase from CNW technologies GmbH (Germany)   .
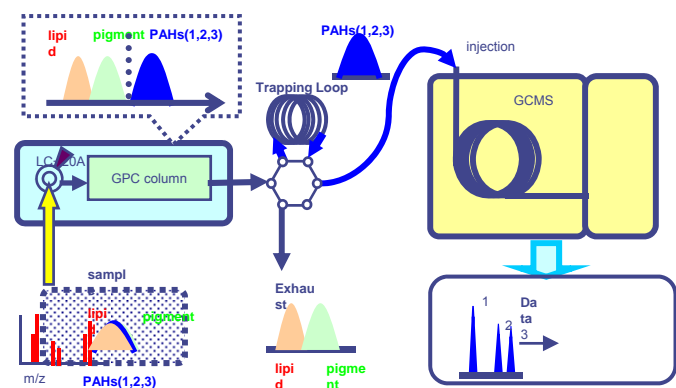Instruments: On-line GPC-GCMS, Shimadzu cooperation(Japan)

Figure 1 Scheme of On-line GPC-GCMS

2-2: methods

Sample Pretreatment method
Accurately weight 0.5 g vegetable oils, dilute by adding 5 mL hexane. Add 5 mL acetonitrile which is saturated by hexane to extract the PAHs, repeat 3 times and combine the acetonitrile filtrate. Concentrate the filtrate to less than 1 mL by rotary evaporator at 40℃. Transfer the residue and volume to 1mL. Add 50 mg PAS and 50 mg C18 sorbent. Vortex mixed for 1 min and centrifuge for 20 min at 10000 rpm, take the supernatant for injection.

GPC parameters
Column: Shodex CLNpak EV-200 2.0 mmid × 150 mm, 16 μm
Mobile phase: Acetone and cyclohexane mixture (3:7)
Flow rate: 0.1 mL/min
Oven temp: 40℃
Injection volume: 10 μL.

GCMS parameters
Column: Inert empty column (5 m × 0.53 mm)
       Pre-column (5 m × 0.25 mmid × 0.25 μm df, Rtx-5 MS)
       Analytical column (25 m × 0.25 mmid × 0.25 μm df, Rtx-5 MS)
PTV injector temp program:120 ℃ (4.5 min)_ 80 ℃/min_ 280 ℃(33 min)
Carrier gas pressure program:
120 kPa (0 min)_ 100 kPa/min _180 kPa (4.4 min) _49.8 kPa/min _120 kPa (33 min)
Column oven temp program:
 82 ℃ (5 min)_ 15 ℃/min_ 200 ℃(0 min))_ 5 ℃/min_ 300 ℃(7 min)
Injection mode: Splitless (Sampling time: 7 min)
Interface Temp: 280 ℃
Ion source Temp: 260 ℃
Acquisition mode: SIM
Quantitation: External standard

3: Results and Discussion
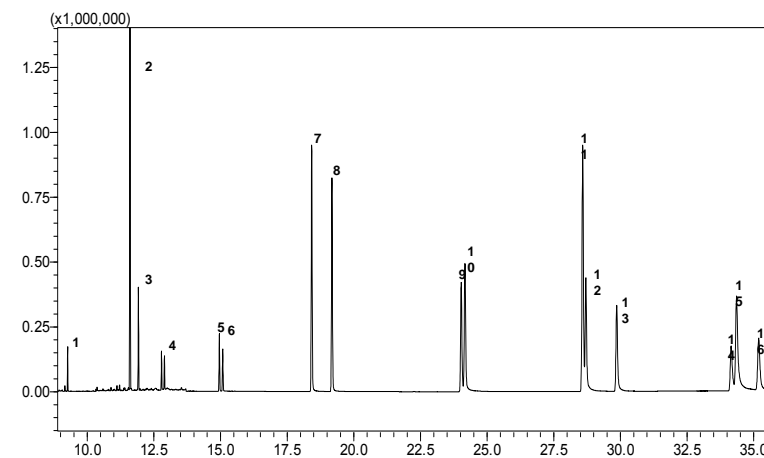
3-1: Standards Chromatogram

Figure 2 TIC of 16 PAHs standard solution

1. Naphthalene   2. Acenaphthylene   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. Indeno[1,2,3-cd]pyrene   15. Dibenz[a,h]anthracene   16. Benzo[g,h,i]perylene

3-2: Calibration Curves of some PAHs

Five concentration of 16 PAHs standard solution from 1 to 100 μg/L were injected and the calibration curve were established. For most of the PAHs compounds, the correlation coefficient(r) of calibration curve was more than 0.999 listed in table 1. Some of the calibration curves are showed as following.

Naphthalene   Anthracene   Pyrene

Chrysene   .Benzo[a]pyrene   Benzo[g,h,i]perylene
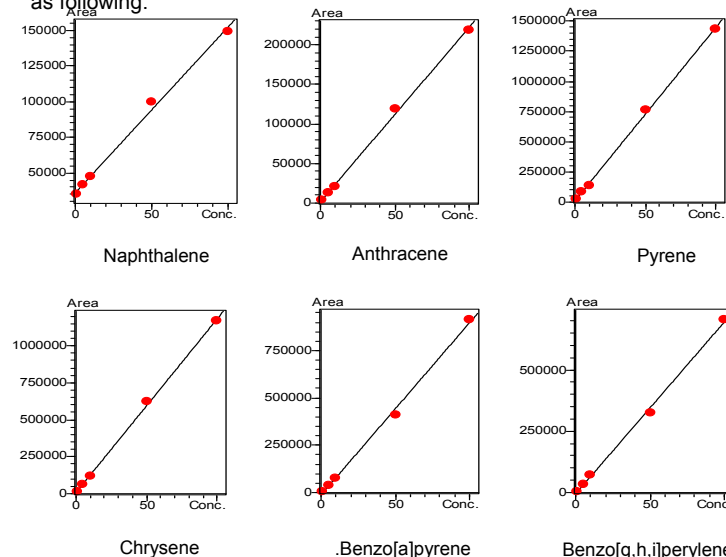
3-3: Method recovery, repeatability and MDL

Certain amount of PAHs standard solution were spiked into the blank oil sample and pretreated same as the oil sample, the average recoveries ranged from 70 to 120% and the RSD of 6 parallel tests were below 10%. The LODs of the 16 PAHs were 0.01~1.2 μg/kg.

Table 1 correlation coefficient , Recovery , Repeatability and Minimal detection limit

No.	Name	RT (min)	r	Recovery %	RSD%	MDL(μg/kg)
1	Naphthalene	9.275	0.9977	74.13	8.24	0.117
2	Acenaphthylene	11.608	0.9999	79.05	9.03	0.774
3	Acenaphthene	11.925	0.9994	79.47	7.22	1.029
4	Fluorene	12.900	0.9999	76.32	6.06	0.099
5	Phenanthrene	14.958	0.9991	79.61	6.69	0.007
6	Anthracene	15.083	0.9990	86.23	7.40	0.054
7	Fluoranthene	18.417	0.9991	89.67	2.46	0.016
8	Pyrene	19.175	0.9995	100.64	1.32	0.008
9	Benzo[a]anthracene	24.025	0.9996	96.51	1.12	0.020
10	Chrysene	24.158	0.9996	101.71	1.68	0.014
11	Benzo[b]fluoranthene	28.575	0.9996	105.16	2.31	0.020
12	Benzo[k]fluoranthene	28.700	0.9996	104.51	1.85	0.020
13	Benzo[a]pyrene	29.850	0.9986	105.81	2.75	0.048
14	Indeno[1,2,3-cd]pyrene	34.142	0.9986	109.78	7.19	0.094
15	Dibenz[a,h]anthracene	34.342	0.9995	113.25	6.92	0.027
16	Benzo[g,h,i]perylene	35.183	0.9991	108.40	6.32	0.091

4: Conclusion

A method for determination of 16 polycyclic aromatic hydrocarbons(PAHs) in vegetable oils was developed using on-line gel permeation chromatography-gas chromatography/mass spectrometry（GPC-GCMS）By using on-line GPC cleanup technique, high molecular weight interferences such as the lipids and pigments in the final extract were further removed automatically, so the background brought about by the matrix in the samples were effectively reduced and higher sensitivities of the target compounds were obtained. The method is simple, rapid and characterized with acceptable sensitivity and accuracy to meet the requirements for the analysis of PAHs in vegetable oils..

-**Reference**
[1] Xiao-min Xu, Cun yu, Jian-long Han, et al. *J. Sep. Sci.* 2011, 34, 210–216.
[2] GB/T 23213-2008 Determination of polycyclic aromatic hydrocarbons in vegetable oils GCMS method