Effective Extraction Method for the Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Environmental Samples

SORNNARIN BANGKEDPHOL

Contents

Objectives

1. To study the optimum conditions for extraction of PAHs from sediment samples between microwave extraction and sonication

2. To compare efficiency of extraction methods for extraction of PAHs from sediment samples between microwave extraction, sonication and Soxhlet extraction

3. To develop the spectrofluorometric method for qualitative and quantitative analysis of PAHs from extraction

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs)

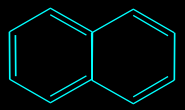
- PAHs consist of two or more fused benzene rings
- PAHs are produced naturally by combustion processes, industrial processes and transport etc.
- PAHs are pollutants in environment
- PAHs can be polluted in many kinds of environmental samples

soil & sediment river plant

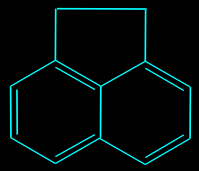
rain ground water

Toxicity of PAHs

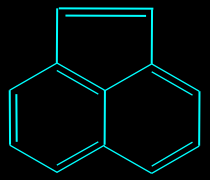
- Chronic health effect and carcinogenicity
- High bioaccumulation potential
- Low removal efficiency in treatment process



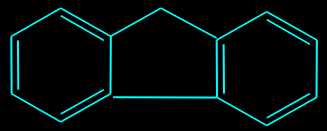
Naphthalene



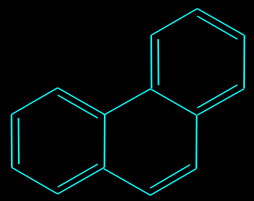
Acenaphthene



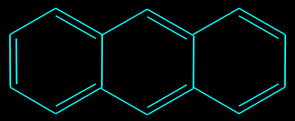
Acenaphthylene



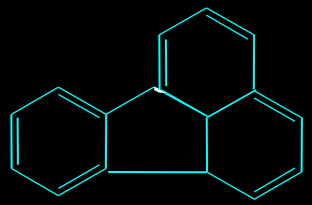
Flourene



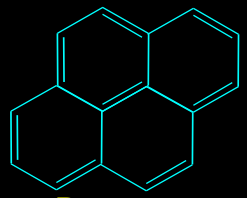
Phenanthene



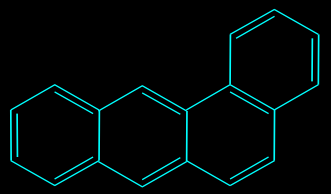
Anthracene



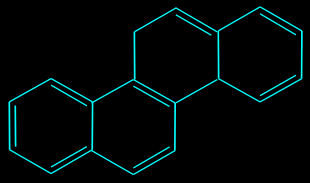
Fluoranthene



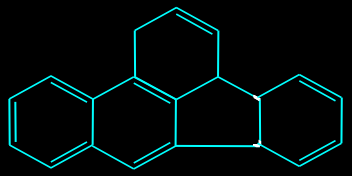
Pyrene



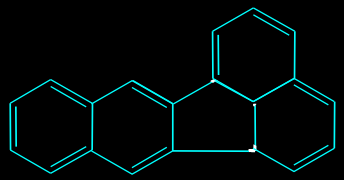
Benzo(a)anthracene



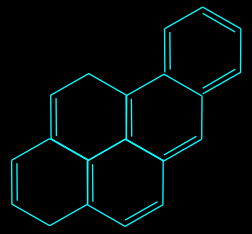
Chrysene



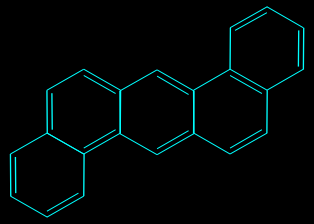
Benzo(b)fluoranthene



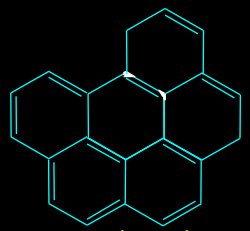
Benzo(k)fluoranthene



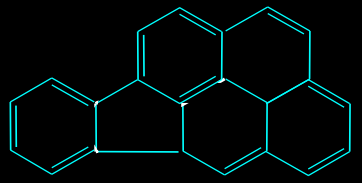
Benzo(a)pyrene



Dibenz(a,h)anthracene



Benzo(g,h,i)perylene

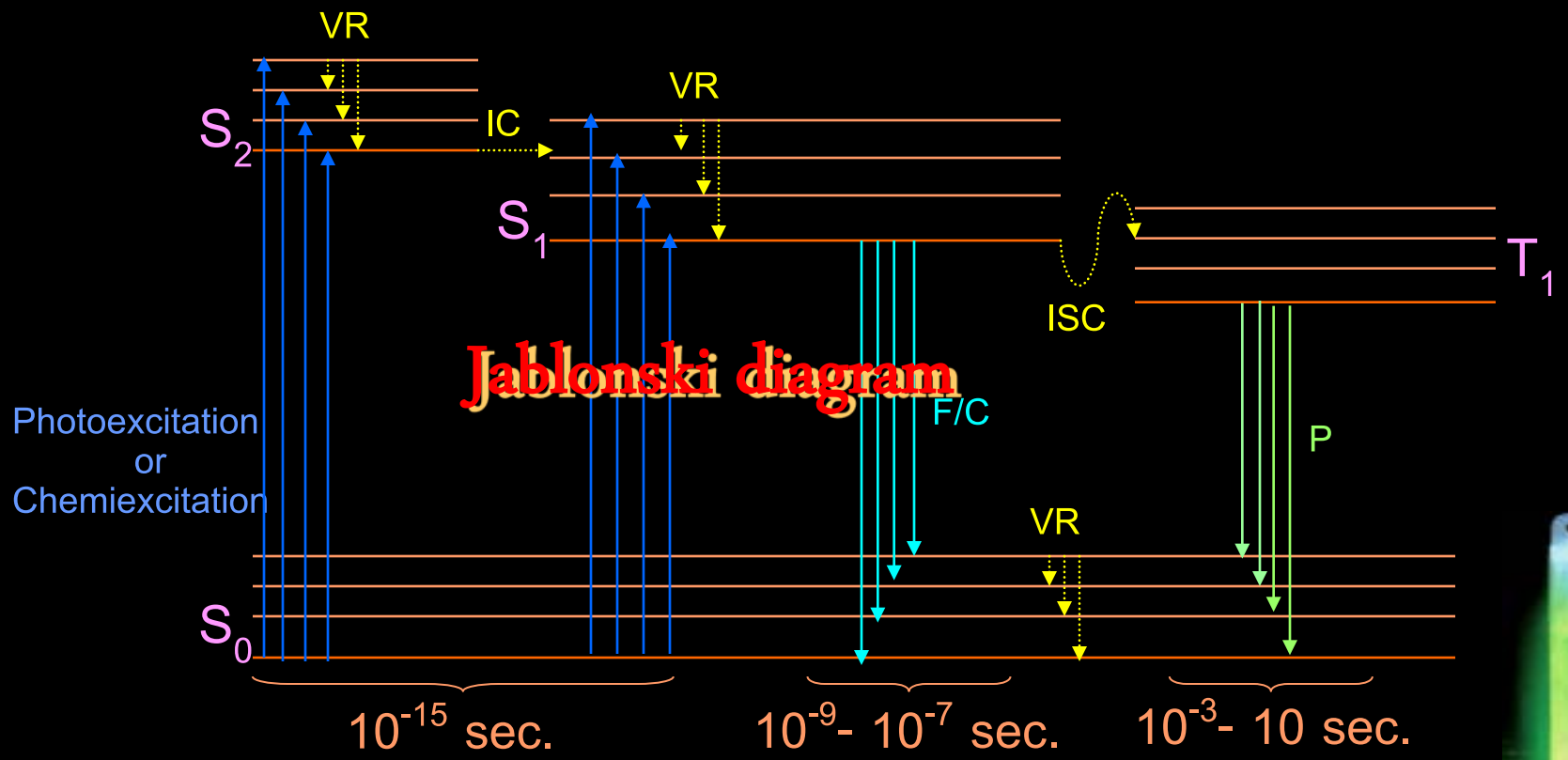


Indeno(1,2,3,c,d)pyrene

Chemical structure of the sixteen PAHs, which were identified by The Environmental Protection Agency (EPA) as priority pollutant

Method for determination of PAHs in samples

1. Spectrofluorometric method



S_0 : ground singlet state

S_1,S_2 : excited singlet state

T_1 : excited triplet state

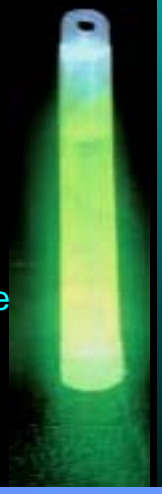
IC : internal conversion

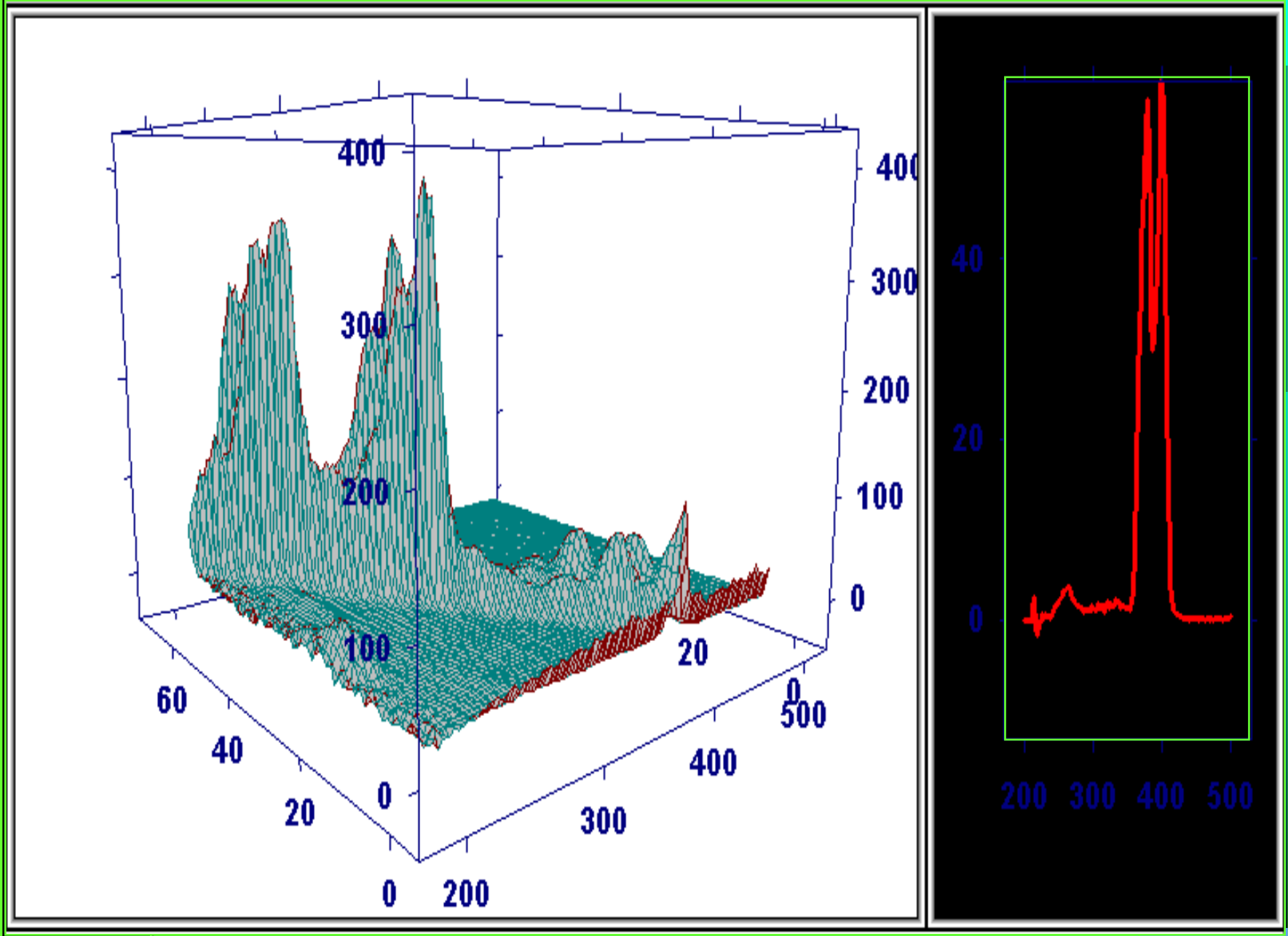
ISC : intersystem crossing

F : fluorescence

C : chemiluminescence

P : phosphorescence

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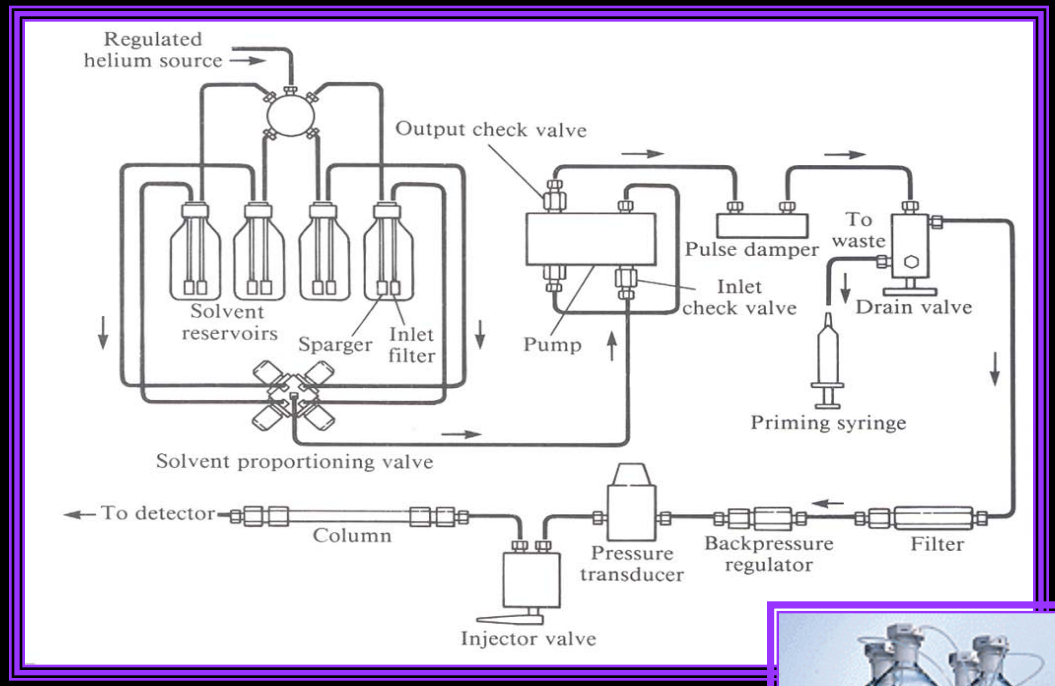
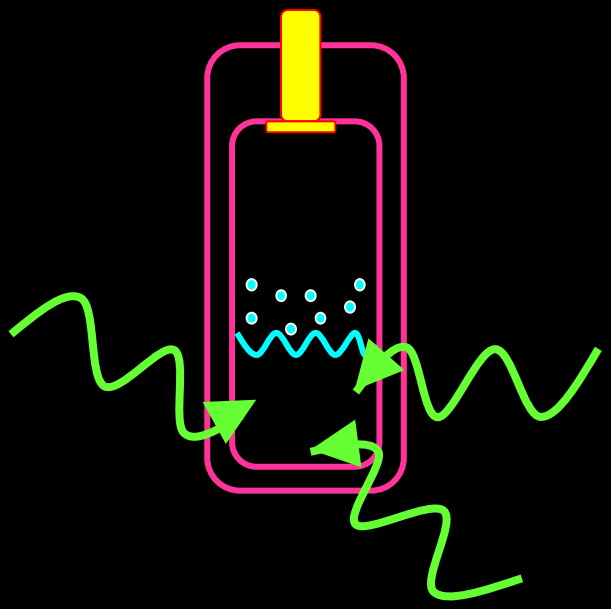


Condition of HPLC (EPA 8310)

- The injection volume: 40 µl
- Gradient elution: A = water, B = acetonitrile (50-100%B; 0-25 min, 100%B; 25-50 min)
- Analytical column: ChromSpher PAH
- Detector: photodiode array detector at 254 nm
- Column temperature: 25°C

Method for extraction

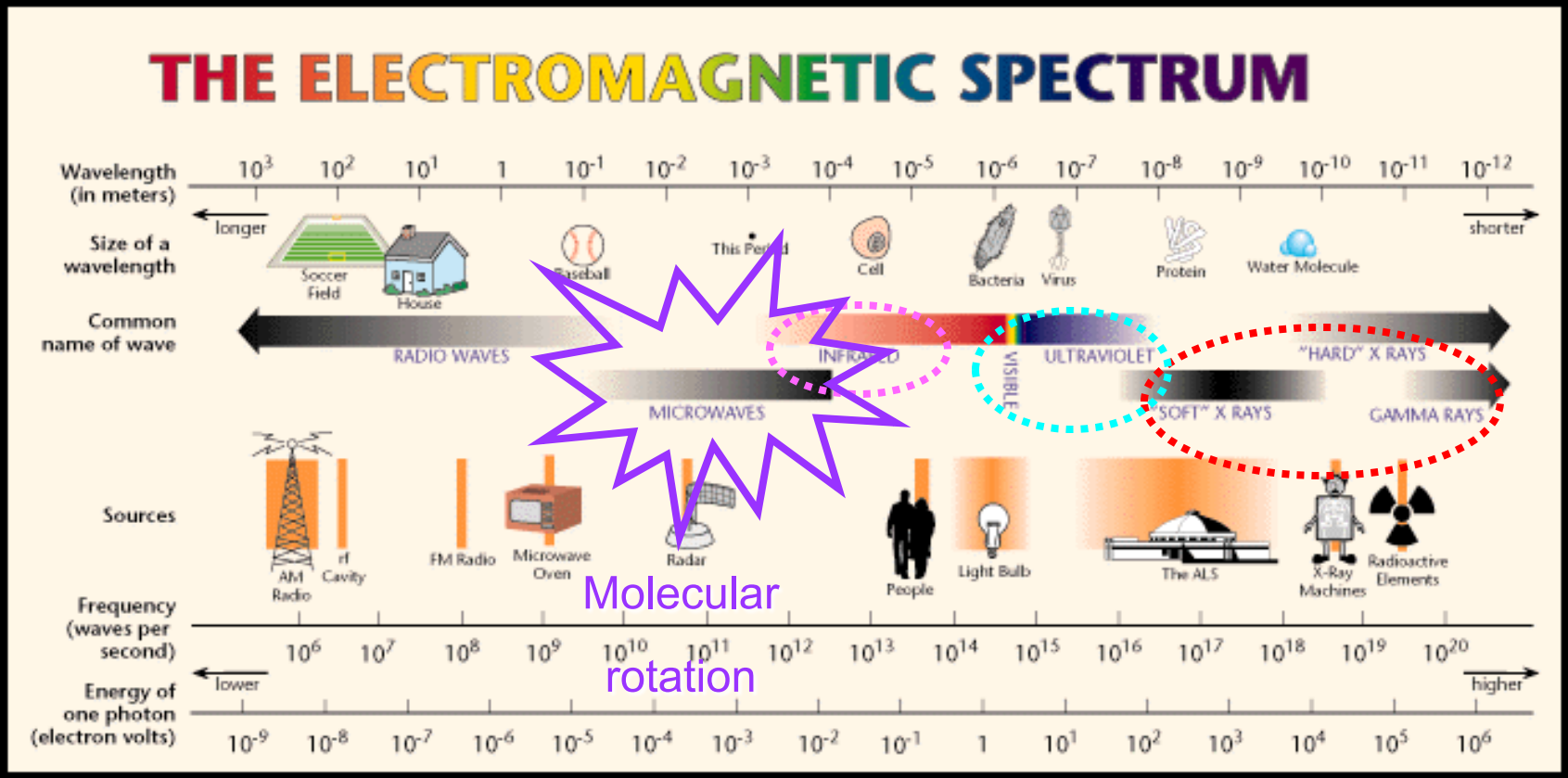
Microwave Extraction



Soxhlet Extraction

Sonication

1. Microwave Extraction



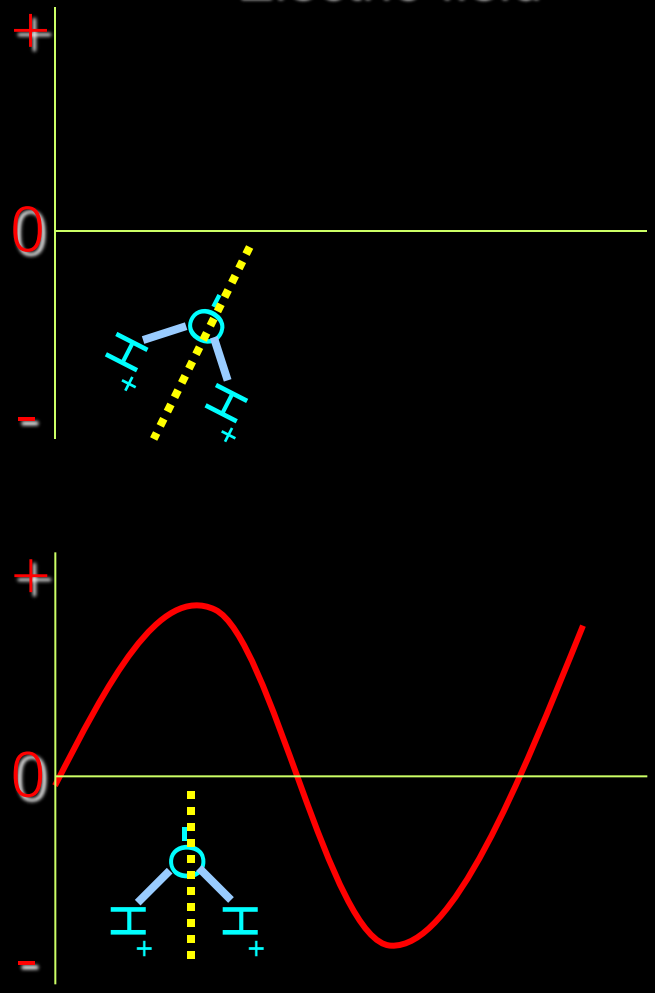
Molecular vibration

Outer-shell electron

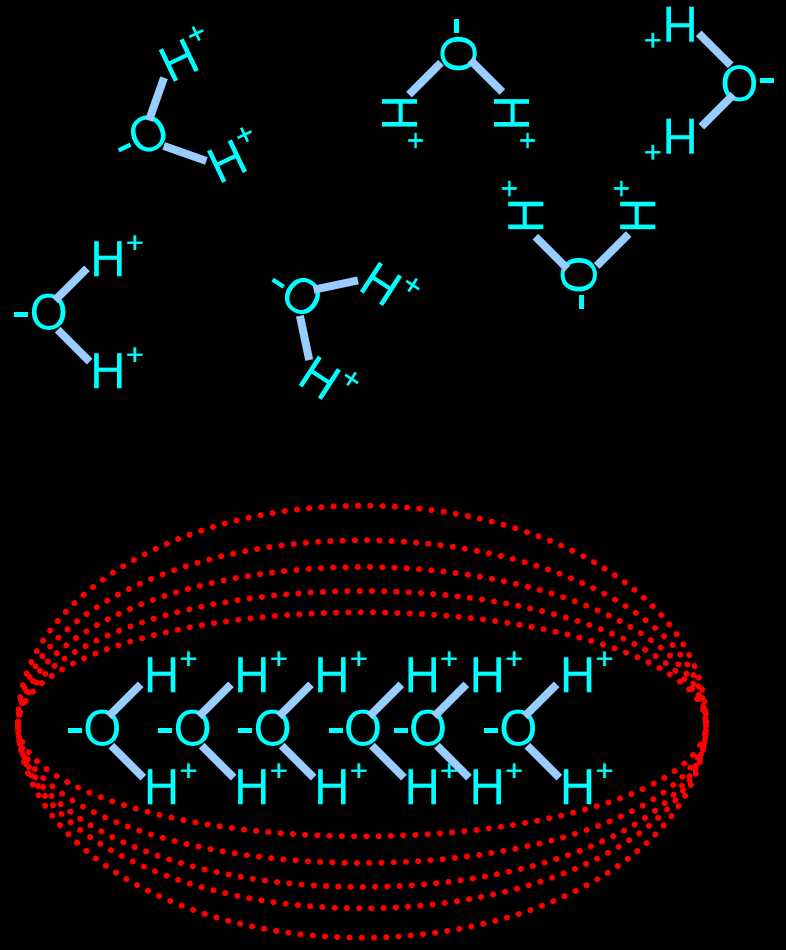
Inner-shell electron

Dipole Rotation

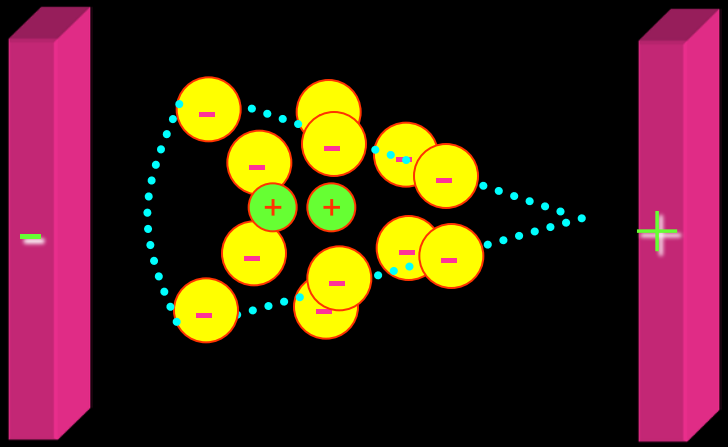
Electric field



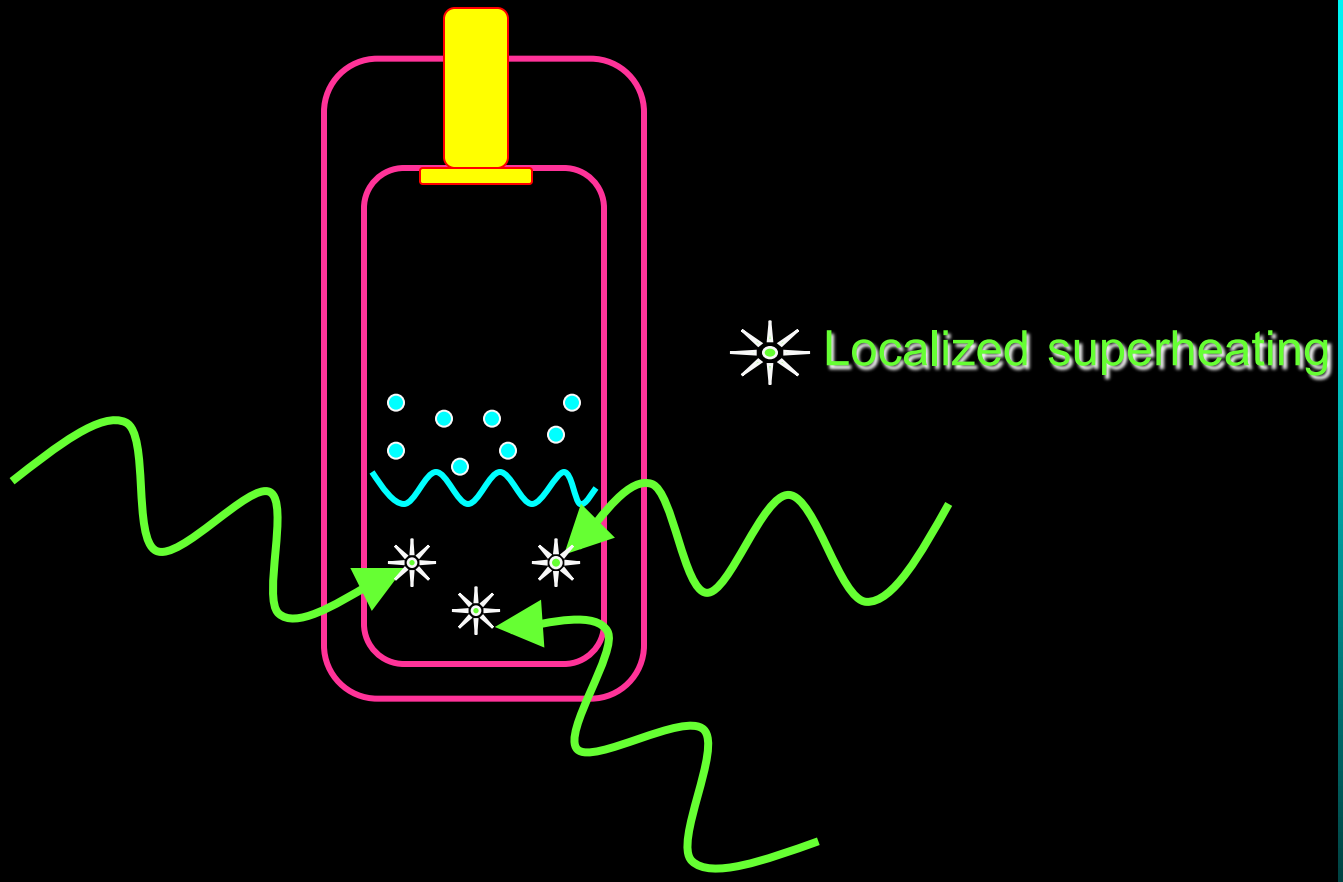
Magnetic field



Ionic Conduction



Pressurized microwave-assisted extraction : closed-system



Schematic of sample heating by microwaves

2. Soxhlet Extraction

Extraction Thimbles

3. Ultrasonic Extraction: Sonication

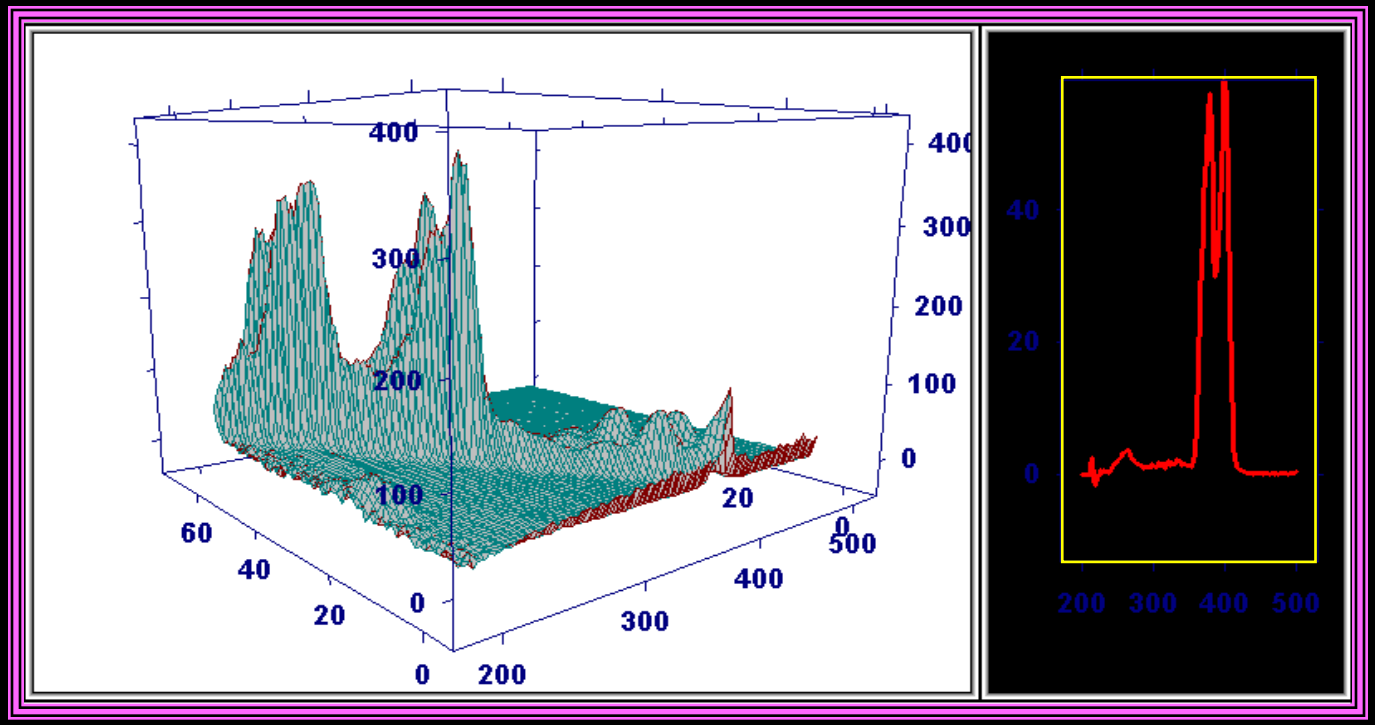
Operation frequency 30-40 KHz

Part I

Determination of PAHs in ethanolic samples by spectrofluorometric method

Part I/I

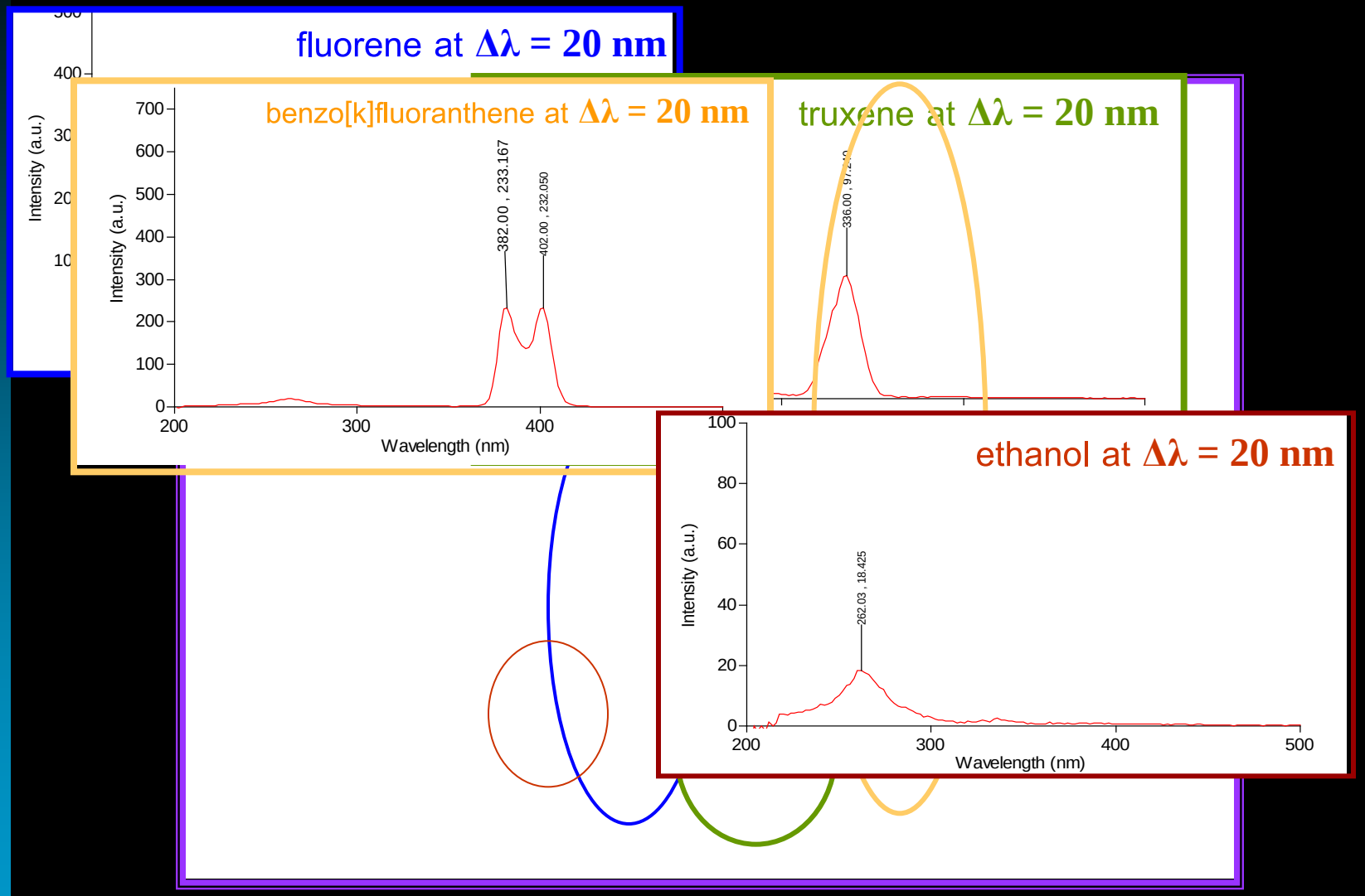
Synchronous Fluorescence Spectroscopic Technique: The Tool for Rapid Identification of PAHs in Liquid Samples



Songsasen, A. et al., *Kasetsart J.*, 2002, 36 , 301-311.

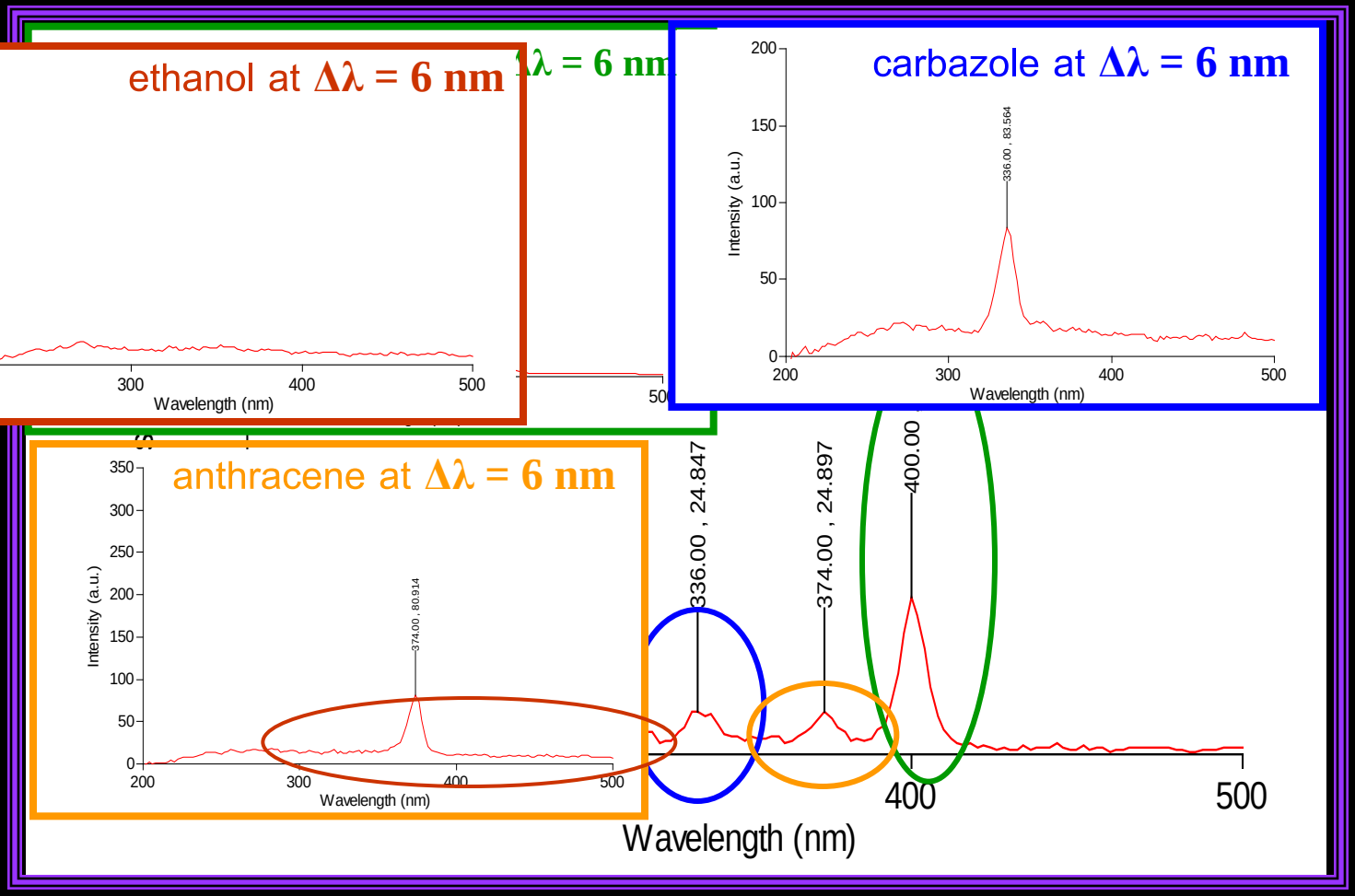
RESULTS
EXPERIMENT
DISCUSSION

Synthetic mixture of benzo[k]fluoranthene, fluorene and truxene



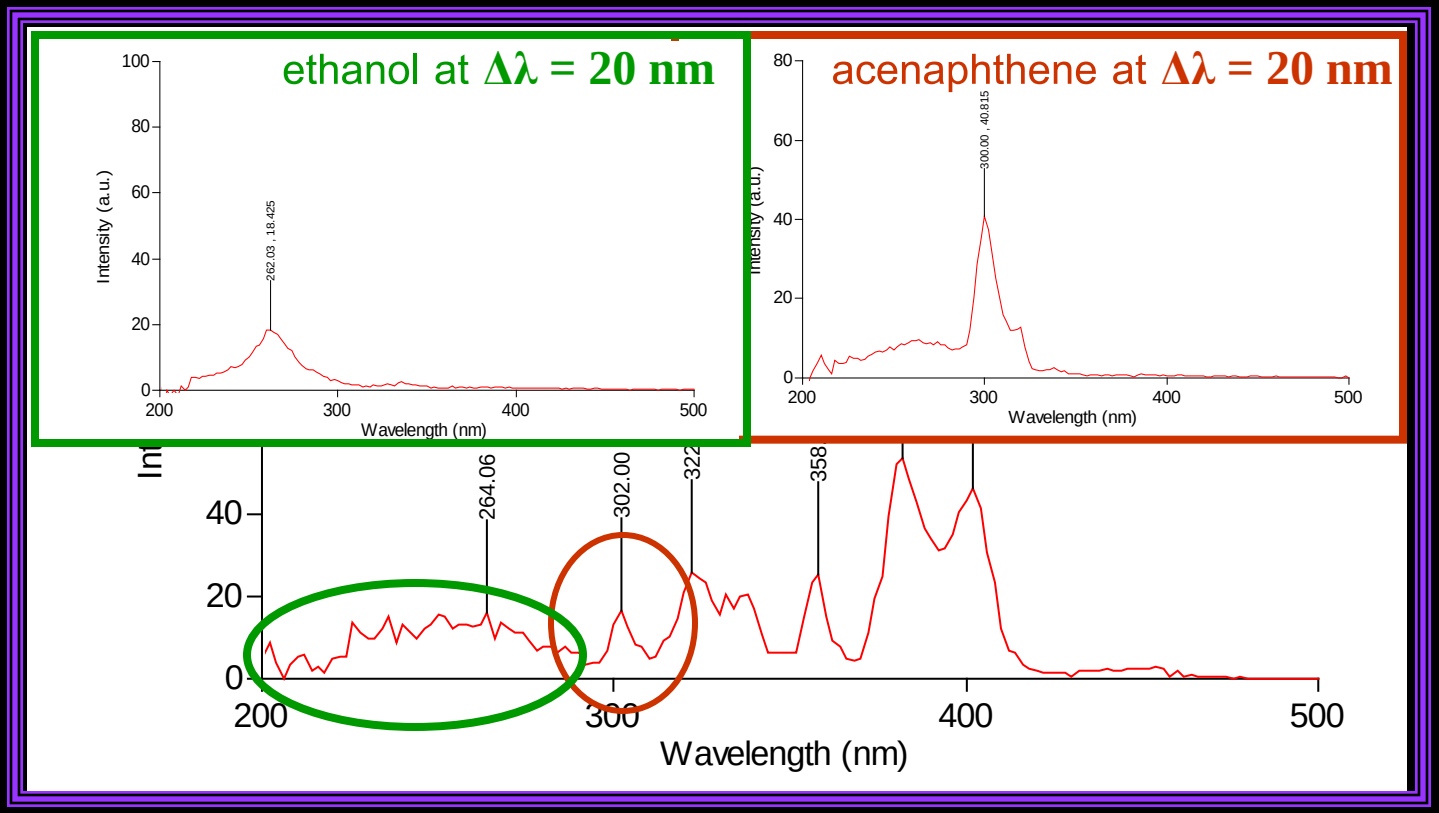
The synchronous spectrum of benzo[k]fluoranthene, fluorene and truxene at $\Delta\lambda = 20$ nm

Synthetic mixture of benzo[k]fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno[1,2,3,cd]pyrene



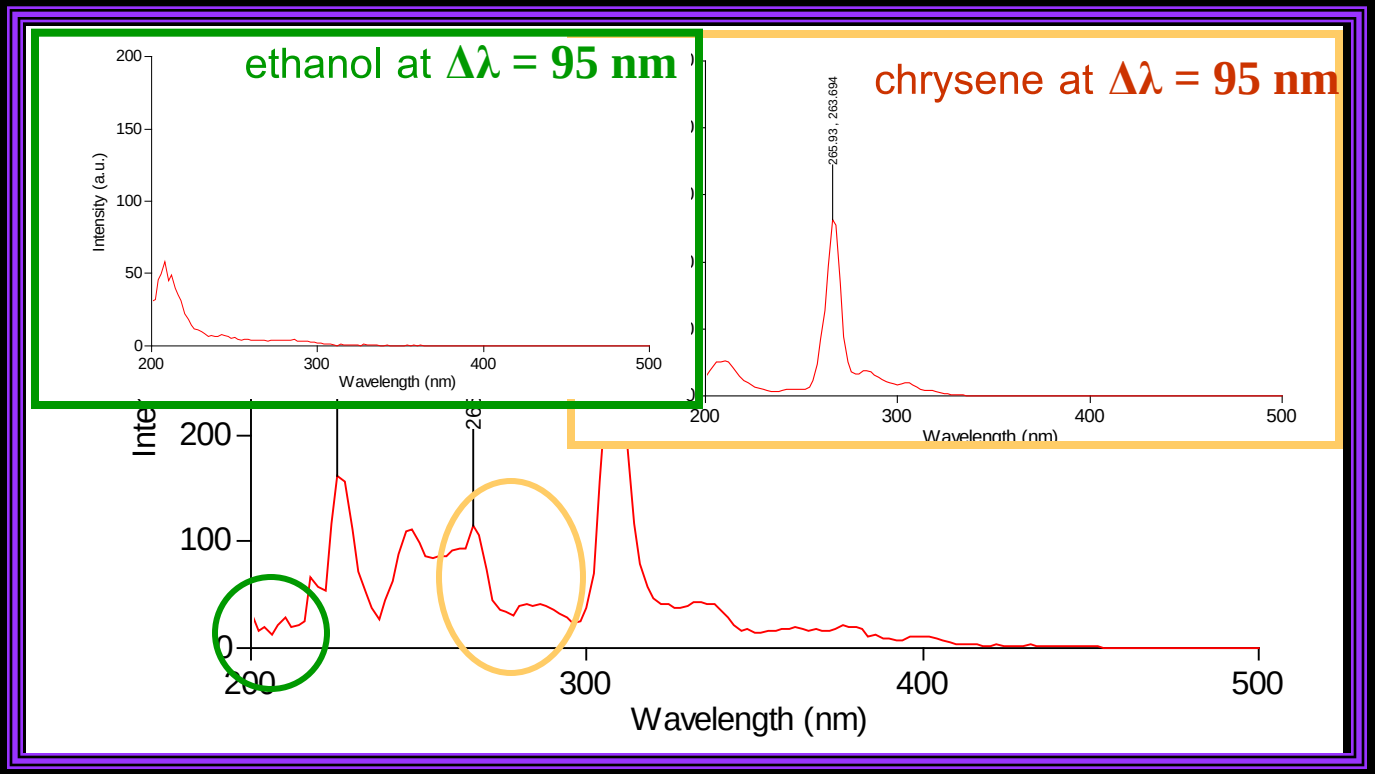
The synchronous spectrum of benzo[k]fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno[1,2,3,cd]pyrene at $\Delta\lambda = 6$ nm

Synthetic mixture of benzo[k]fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno[1,2,3,cd]pyrene



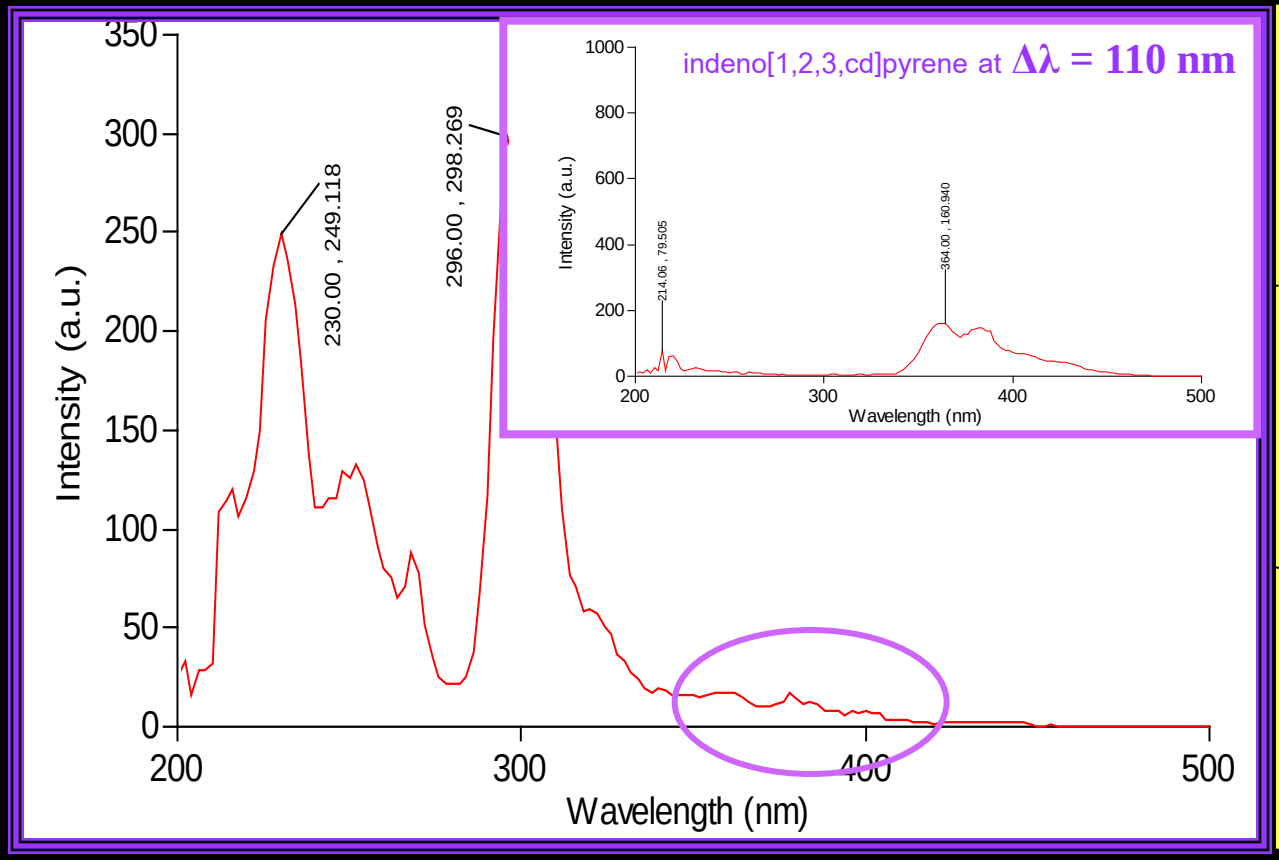
The synchronous spectrum of benzo[k]fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno[1,2,3,cd]pyrene at Δλ = 20 nm

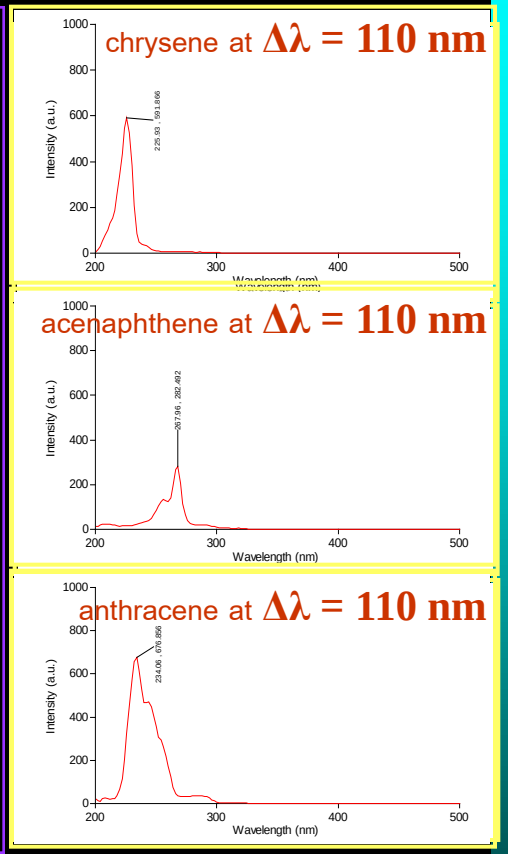
Synthetic mixture of benzo[k]fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno[1,2,3,cd]pyrene



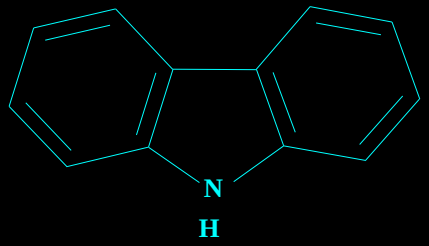
The synchronous spectrum of benzo[k]fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno[1,2,3,cd]pyrene at $\Delta\lambda = 95$ nm

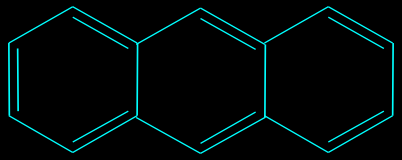
Synthetic mixture of benzo[k]fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno[1,2,3,cd]pyrene

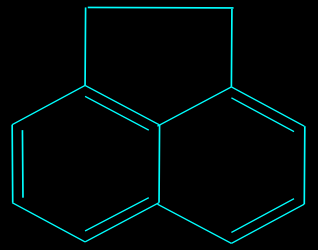




The synchronous spectrum of benzo[k]fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno[1,2,3,cd]pyrene at $\Delta\lambda = 110$ nm

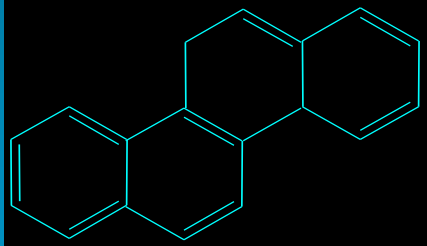


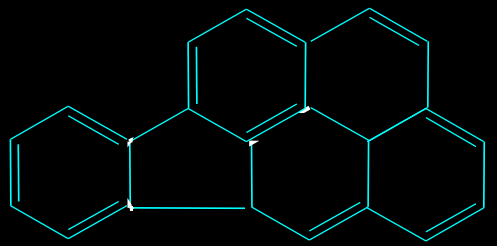


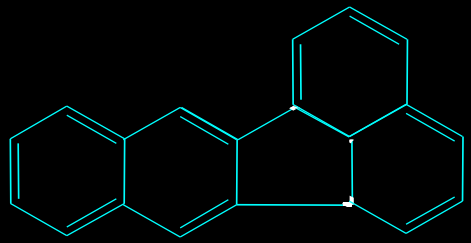


Part I/II

Quantification of PAHs in synthetic sample by spectrofluorometric method







RESULTS
EXPERIMENT
DISCUSSION

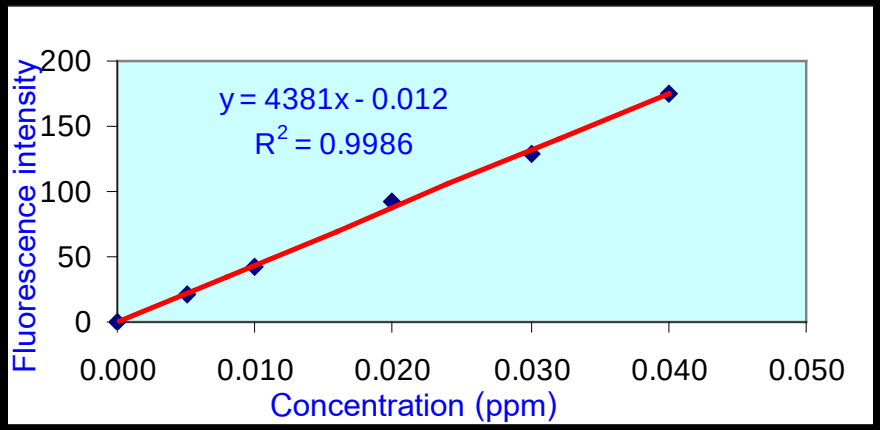
Quantitative Analysis by Spectrofluorometric Method

- Calibration graph : range 0.005-0.040 ppm

- Scanning : Excitation wavelength (Ex), Emission wavelength (Em)

	Ex (nm)	Em (nm)
chrysene	267	380
carbazole	233	356
acenaphthene	227	321
indeno(1,2,3-cd)pyrene	250	510
benzo(k)fluoranthene	307	407
anthracene	252	372

Calibration graph of chrysene



Limit of detection (LOD): $y - y_B = 3S_B$

Concentration. (ppm)	Intensity,y_i	$[\hat{y}]$, y=4381x-0.012	$[y_i - y\hat{}]^2$
0.000	0.017	0.012	$2.421X10^{-5}$
0.005	21.626	21.893	0.071
0.010	41.656	43.798	4.588
0.020	92.319	87.608	22.1948
0.030	129.370	131.418	4.194
0.040	174.975	175.228	0.064
			31.111

Miller, J. N., *Statistics and chemometrics for analytical chemistry.*, 2000.

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0.020	92.319	87.608	22.1948
0.030	129.370	131.418	4.194
0.040	174.975	175.228	0.064
			31.111

Signal of LOD = a + 3Sy/x

a = Intercept = -0.012

Sy/x = {sum $(yi - \hat{y})^2$ / n-2} $^{1/2}$, n = 6 = 2.789

Signal of LOD = -0.012 + 3(2.789) = 8.355

Therefore: LOD = (8.355+0.012)/4381 = 0.0019 ppm

LOD of chrysene = 0.0019 ppm

PAHs	Limit of detection (LOD), ppm
chrysene	0.0019
carbazole	0.0021
acenaphthene	0.0021
indeno(1,2,3-cd)pyrene	0.0017
benzo(k)fluoranthene	0.0012
anthracene	0.0024

Quantitative Analysis

	Amount of PAHs (mg/l), 1st replicate	Amount of PAHs (mg/l), 2nd replicate	Amount of PAHs (mg/l), 3rd replicate	Average the amount of PAHs (mg/l)	SD
chrysene	0.020	0.020	0.020	0.020	0.000
carbazole	0.021	0.021	0.020	0.021	$5.773X10^{-4}$
acenaphthene	0.020	0.020	0.020	0.020	0.000
indeno[1,2,3-cd]pyrene	0.136	0.134	0.135	0.135	0.001
benzo[k]fluoranthene	0.020	0.020	0.020	0.020	0.000
anthracene	0.031	0.030	0.031	0.031	$5.773X10^{-4}$

Hypothesis testing

$n < 30$: t-test

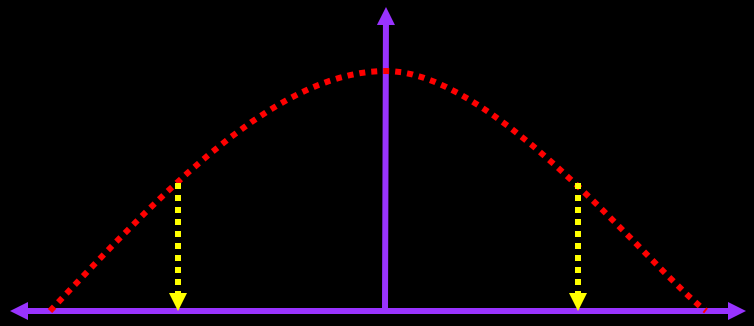
$n \geq 30$: Z-test

	Amount of PAHs (mg/l), 1st replicate	Amount of PAHs (mg/l), 2nd replicate	Amount of PAHs (mg/l), 3rd replicate	Average the amount of PAHs (mg/l)	SD
carbazole	0.021	0.021	0.020	0.021	5.773×10^{-4}

$H_0: \mu = 0.02$

$H_1: \mu \neq 0.02$

95% confidence limit: $\alpha = 0.05$



The critical values of [t] for a two-tailed test = 4.30

$$t = \frac{(\bar{X} - \mu)\, n^{1/2}}{SD}$$

carbazole $t = \dfrac{(0.021-0.02)(3)^{1/2}}{5.773 \times 10^{-4}} = 2.000$ ➡ Accept H_o

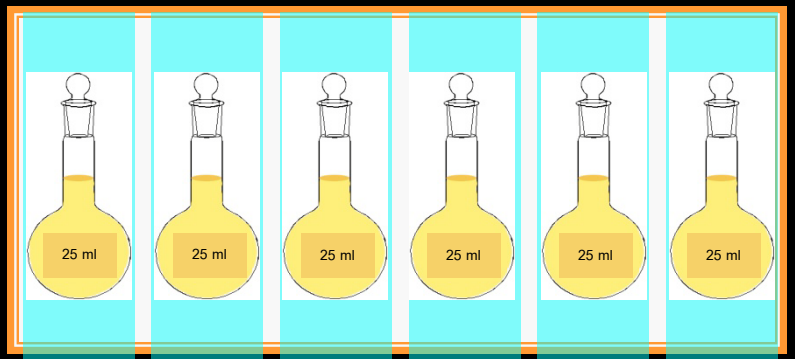
indeno(1,2,3-cd)pyrene $t = 199.180$ ➡ Reject H_o

anthracene $t = 31.999$ ➡ Reject H_o

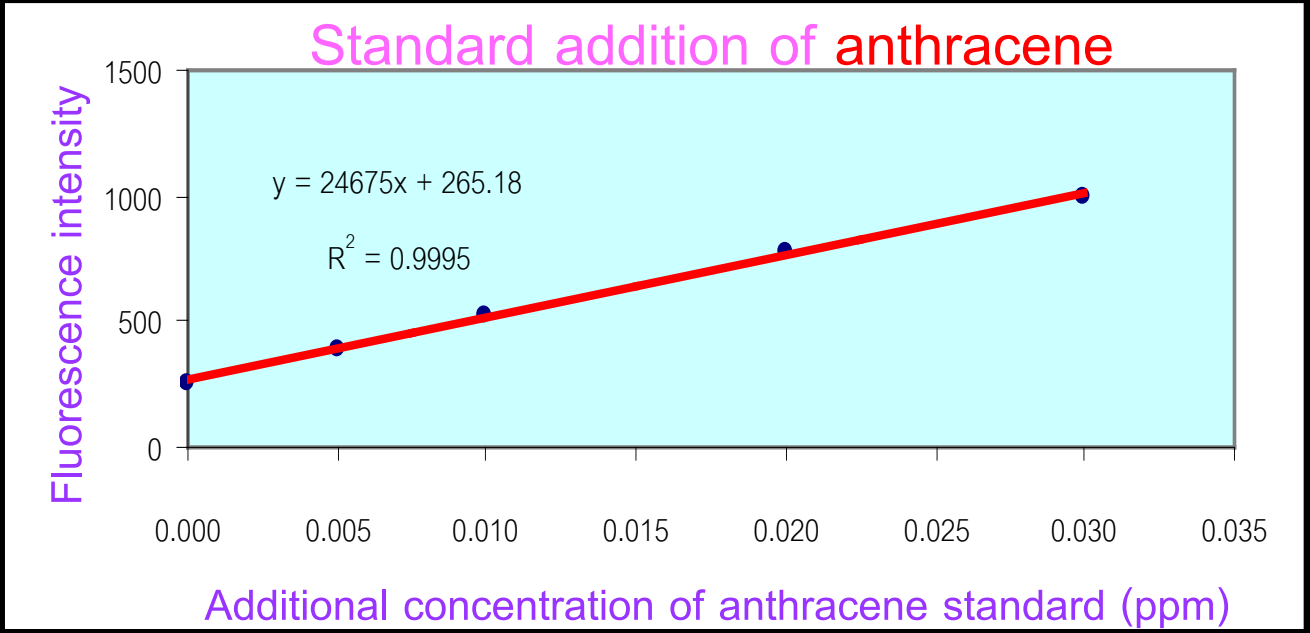
Standard addition

indeno(1,2,3-cd)pyrene

anthracene



Synthetic sample	1.0	1.0	1.0	1.0	1.0	1.0	ml
Standard PAHs (5 ppm of anthracene or indeno[1,2,3,cd]pyrene)	0.0	20.0	30.0	40.0	50.0	60.0	µl

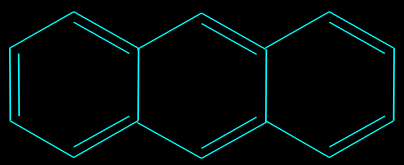

Standard addition of anthracene
Fluorescence intensity
1500
1000
500
0
y = 24675x + 265.18
R2 = 0.9995
0.000
0.005
0.010
0.015
0.020
0.025
0.030
0.035
Additional concentration of anthracene standard (ppm)

	equation	R^2	Amount of PAHs, mg/l	real conc.
anthracene	Y = 24675X + 265.180	0.9995	0.011	
	Y = 24727X + 264.350	0.9996	0.010	0.010
	Y = 24709X + 262.770	0.9996	0.010	
indeno(1,2,3-cd)pyrene	Y = 1265.2X + 25.303	0.9997	0.020	
	Y = 1294.6X + 25.343	0.9996	0.020	0.020
	Y = 1264.3X + 25.264	0.9987	0.021	

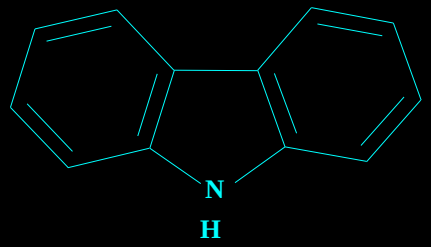
Part II

Optimization of extraction methods using the spiked sediment sample

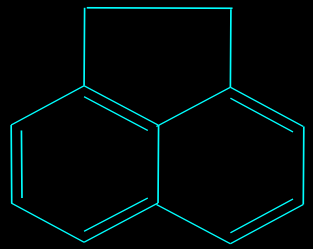
RESULTS
EXPERIMENT
DISCUSSION



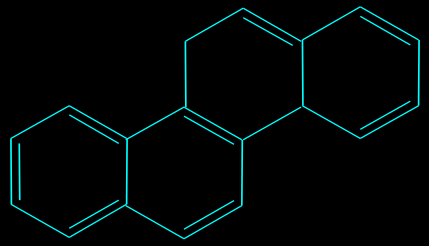
Anthracene



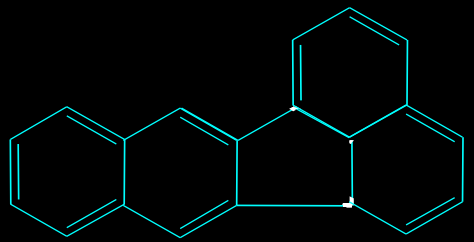
Carbazole



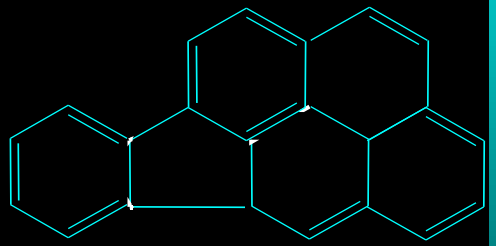
Acenaphthene



Chrysene



Benzo(k)fluoranthene



Indeno(1,2,3,c,d)pyrene

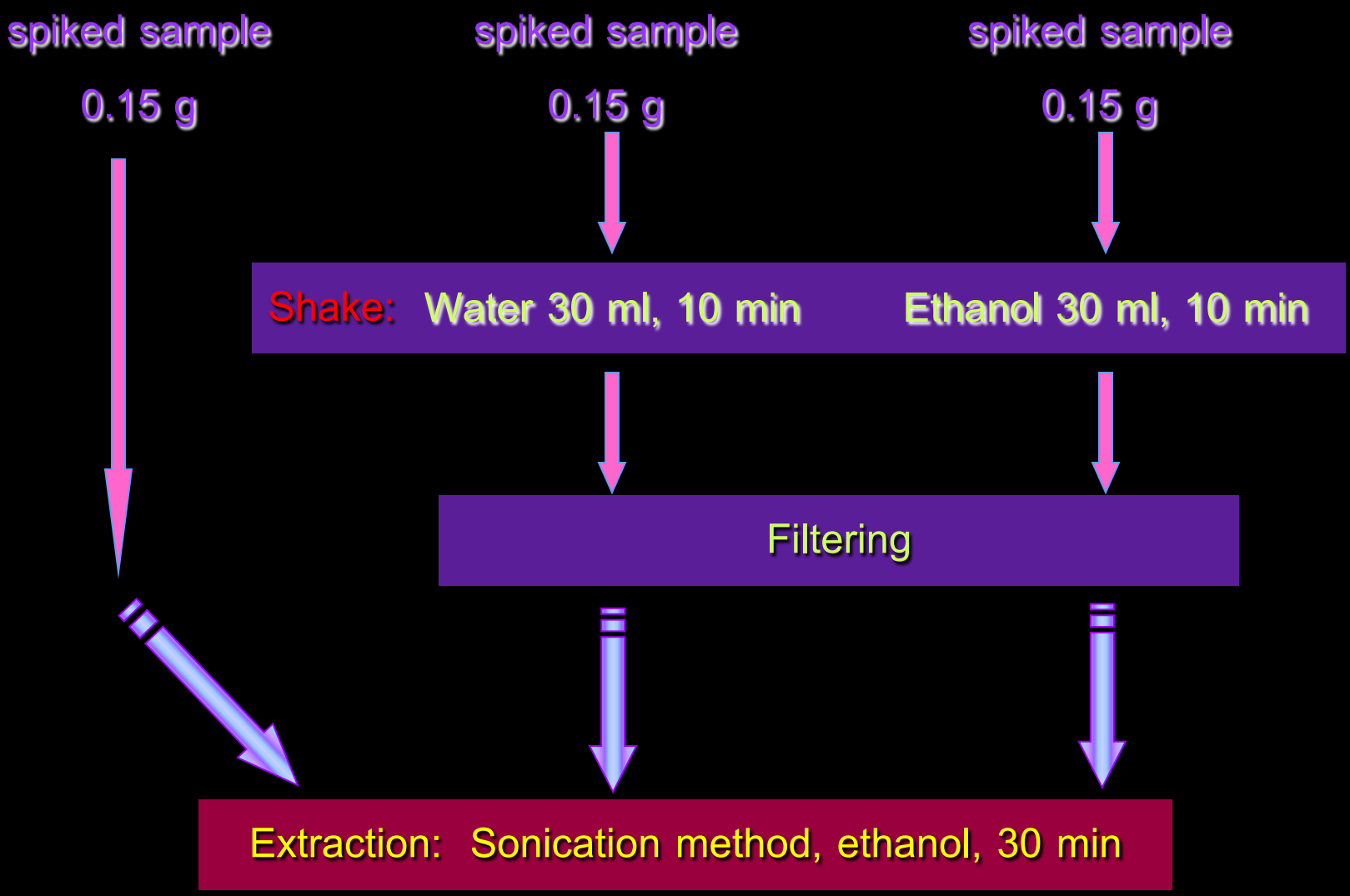
Preparation of spiked sample

soil → grind → sieve (100 mesh) → burn → store in desicator

weight 100 g → add 6 PAHs → shake 2 days → air-dried

Shake at room temperature for 2 days

Testing on PAHs adsorption on the spiked sediment sample



Amount of PAHs: mg/g (n=3) equivalent of chrysene Gaines et al. (2000)

unwashed	water	ethanol	
0.0045	0.0045	0.0022	
0.0045	0.0044	0.0021	
0.0045	0.0044	0.0021	
0.0045	0.0044	0.0021	average
$1.89X10^{-5}$	$2.15X10^{-5}$	$3.95X10^{-5}$	SD
0.42%	0.49%	1.86%	RSD
0.0045±0.42%	0.0044±0.49%	0.0021±1.86%	

Extraction Methods

Microwave Extraction

- Extraction Solvent
- Extraction Time
- Temperature of Irradiation

Soxhlet Extraction

(Conventional Method)

- Extraction Time

Sonication

- Extraction Solvent
- Extraction Time

Microwave Extraction

- Extraction Solvent : methanol, acetonitrile, acetone, dichloromethane, hexane:acetone (3:2), cyclohexane:acetone (3:2)
- Extraction Time : 3, 5, 10, 15, 20, 25 min
- Temperature of Irradiation : 60%, 80%, 100%, 120%, 140% ,160% of boiling point

- Fixed : weight of synthetic sample ~0.25 g, extraction solvent = 20 ml

↓

centrifuge 5,500 RPM (15 min)

↓

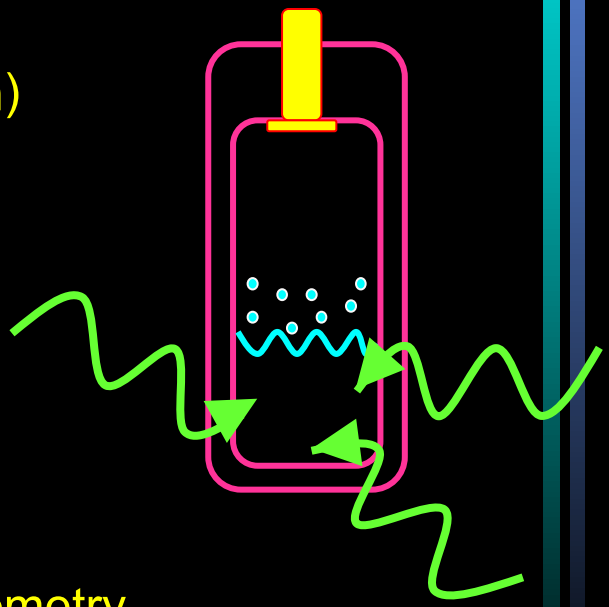
pipette 10 ml

↓

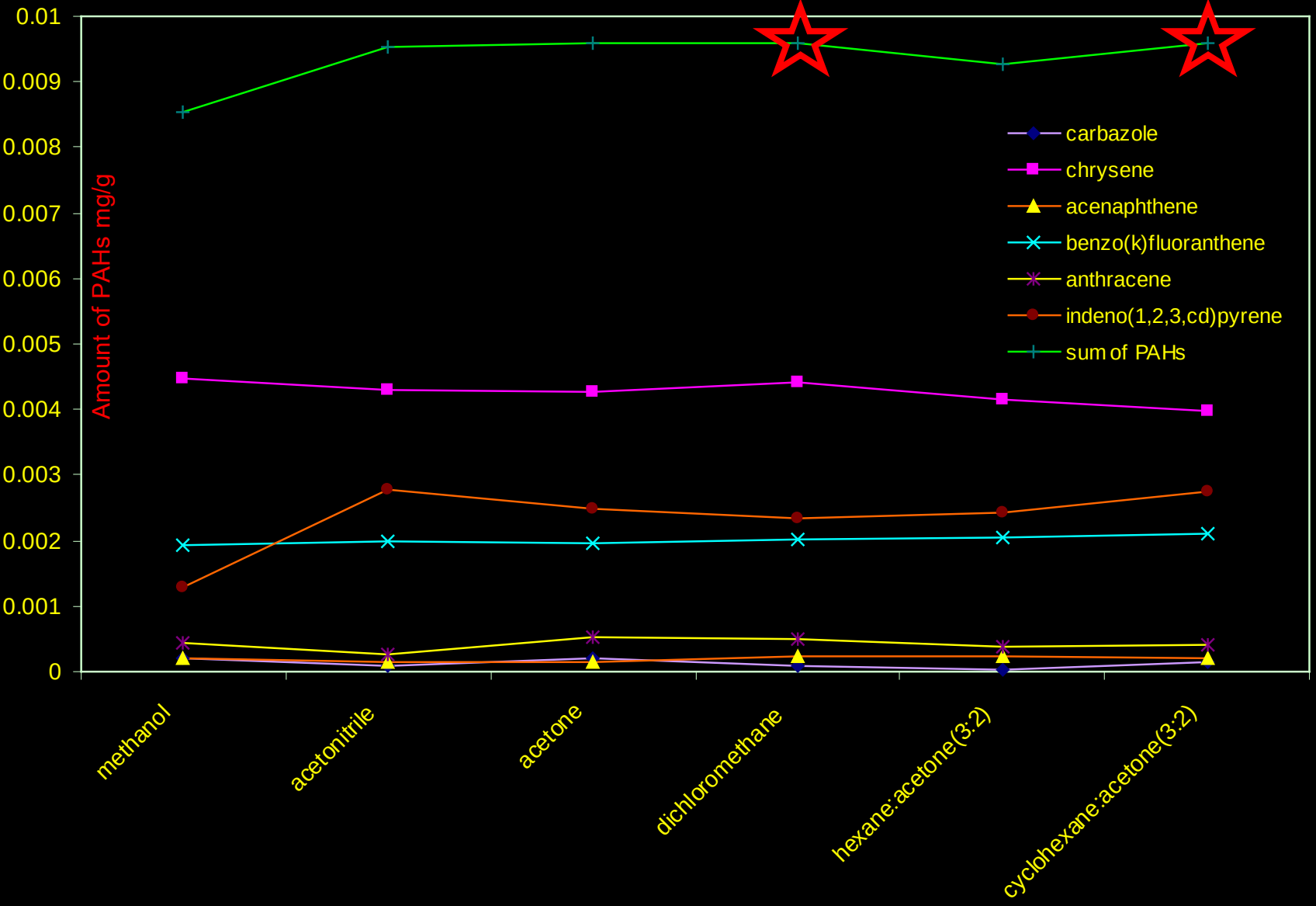
evaporate to 25 ml of ethanol

↓

Quantitative analysis by spectrofluorometry

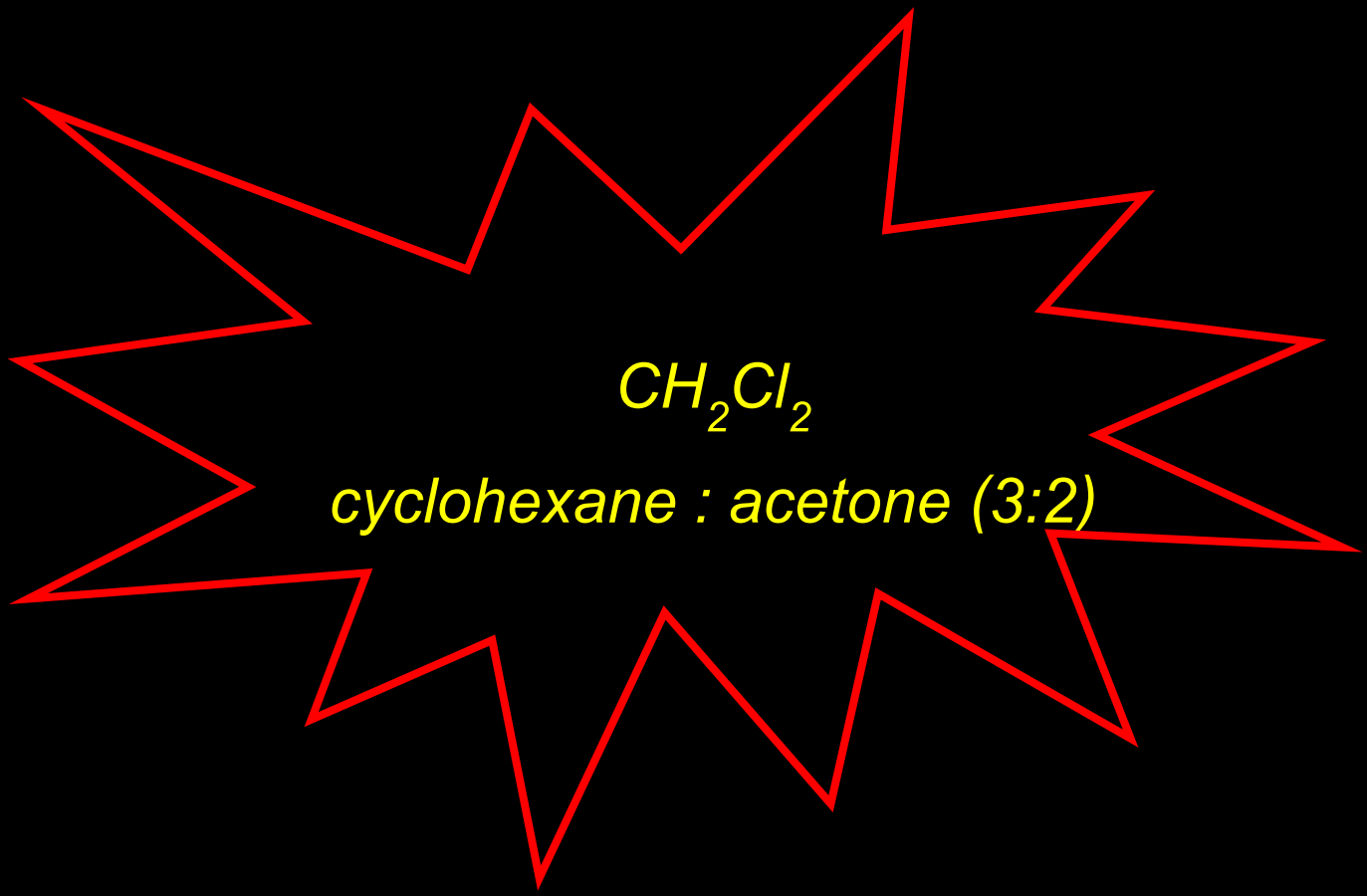


Extraction solvent

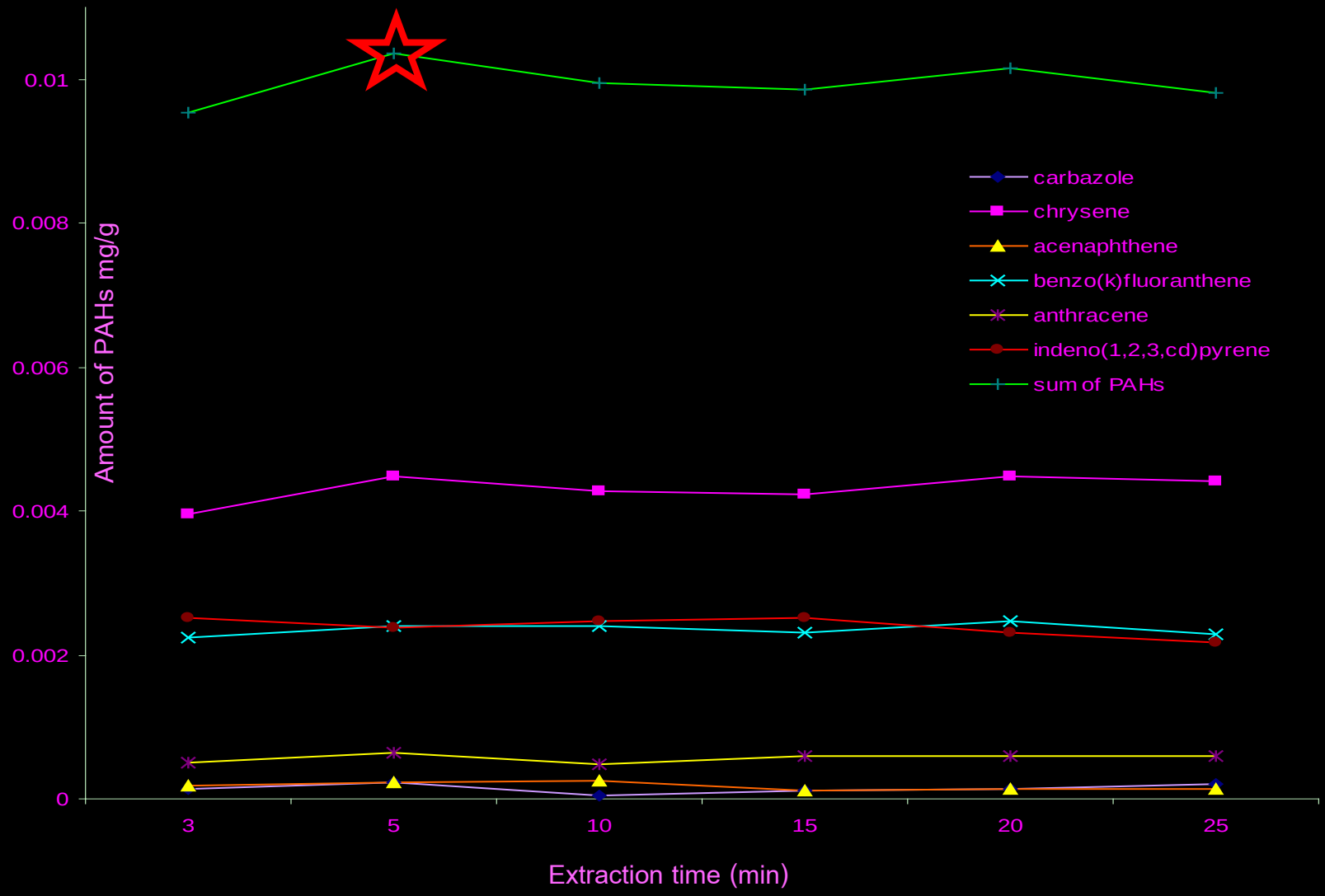


- Fixed: extraction time = 10 min, temp. of irradiation = 80%BP for each solvent

Extraction solvent for microwave extraction

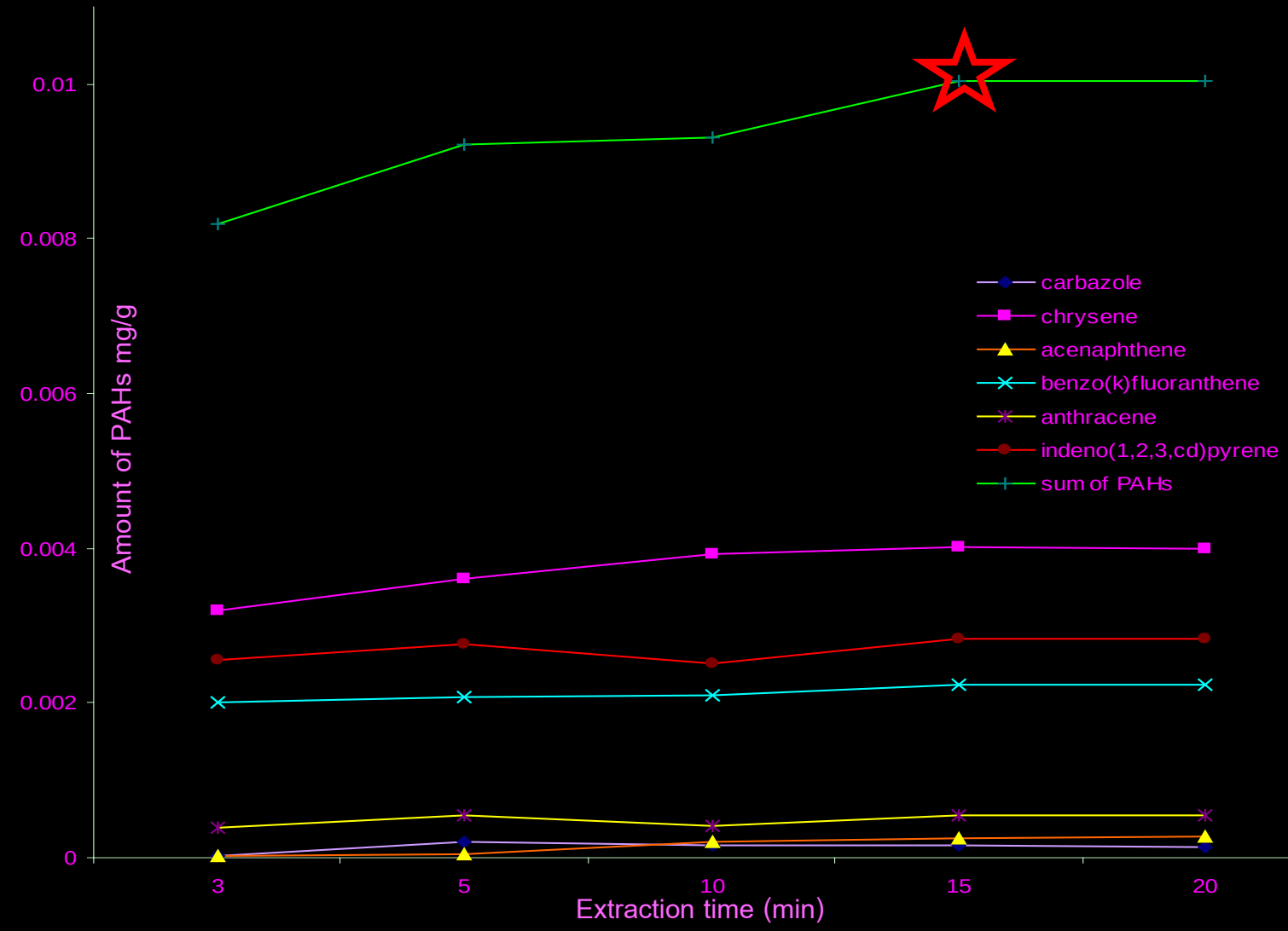


Extraction time of dichloromethane



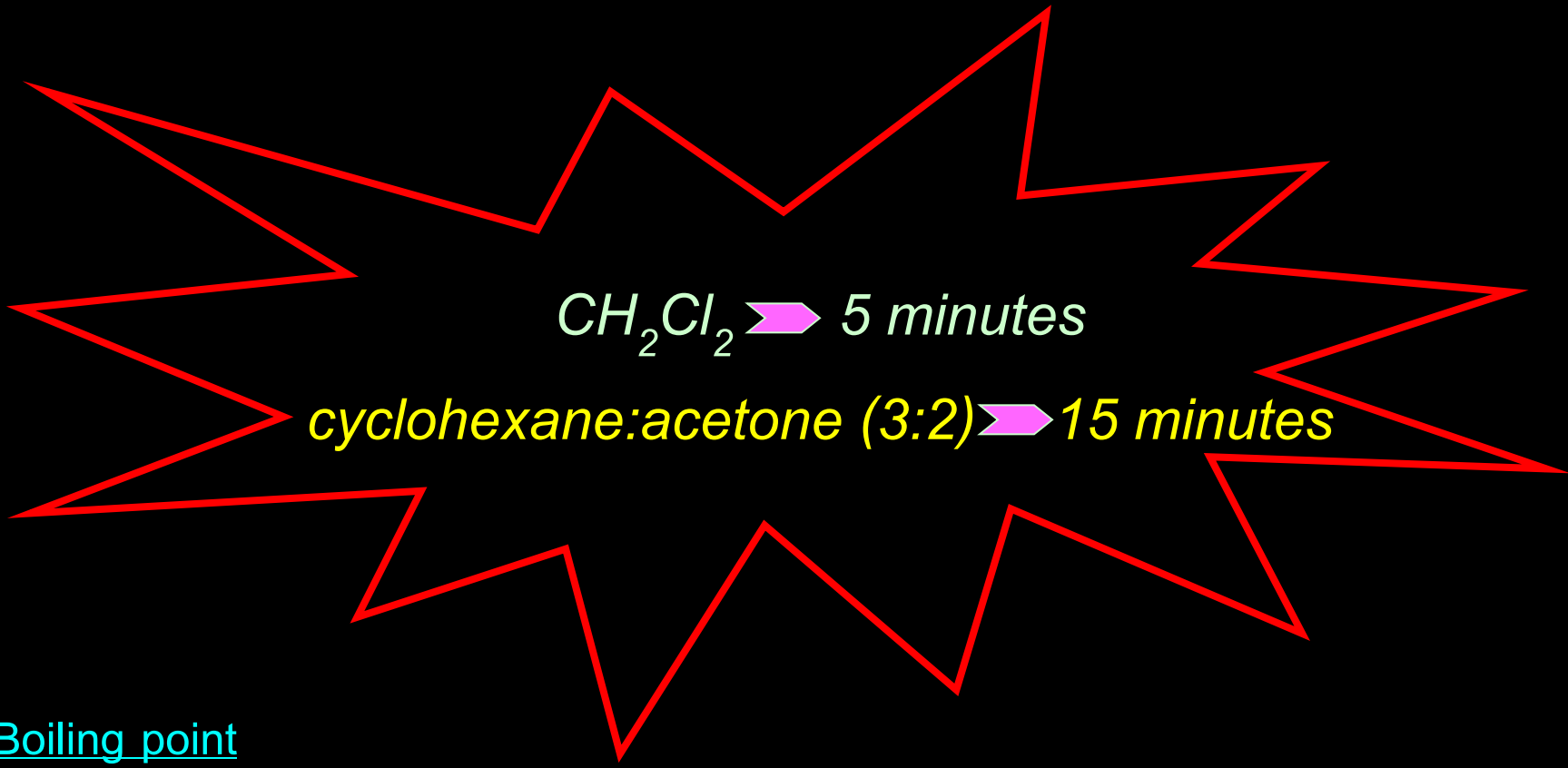
- Fixed: extraction solvent, temp. of irradiation = 80%BP = 32 ^{o}C

Extraction time of cyclohexane:acetone (3:2)



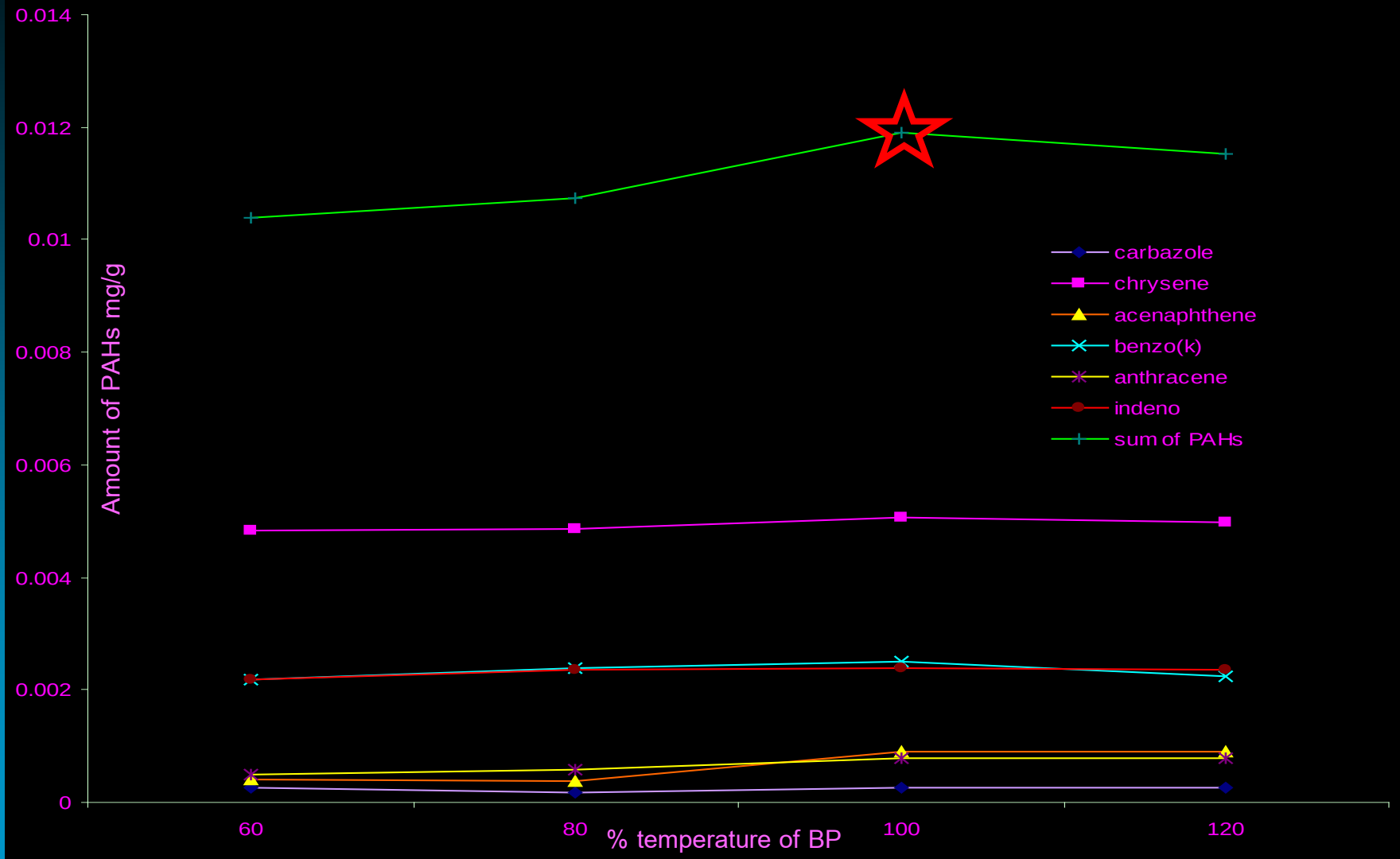
- Fixed: extraction solvent, temp. of irradiation = 80%BP(acetone) = 45°C

Extraction solvent & time for microwave extraction



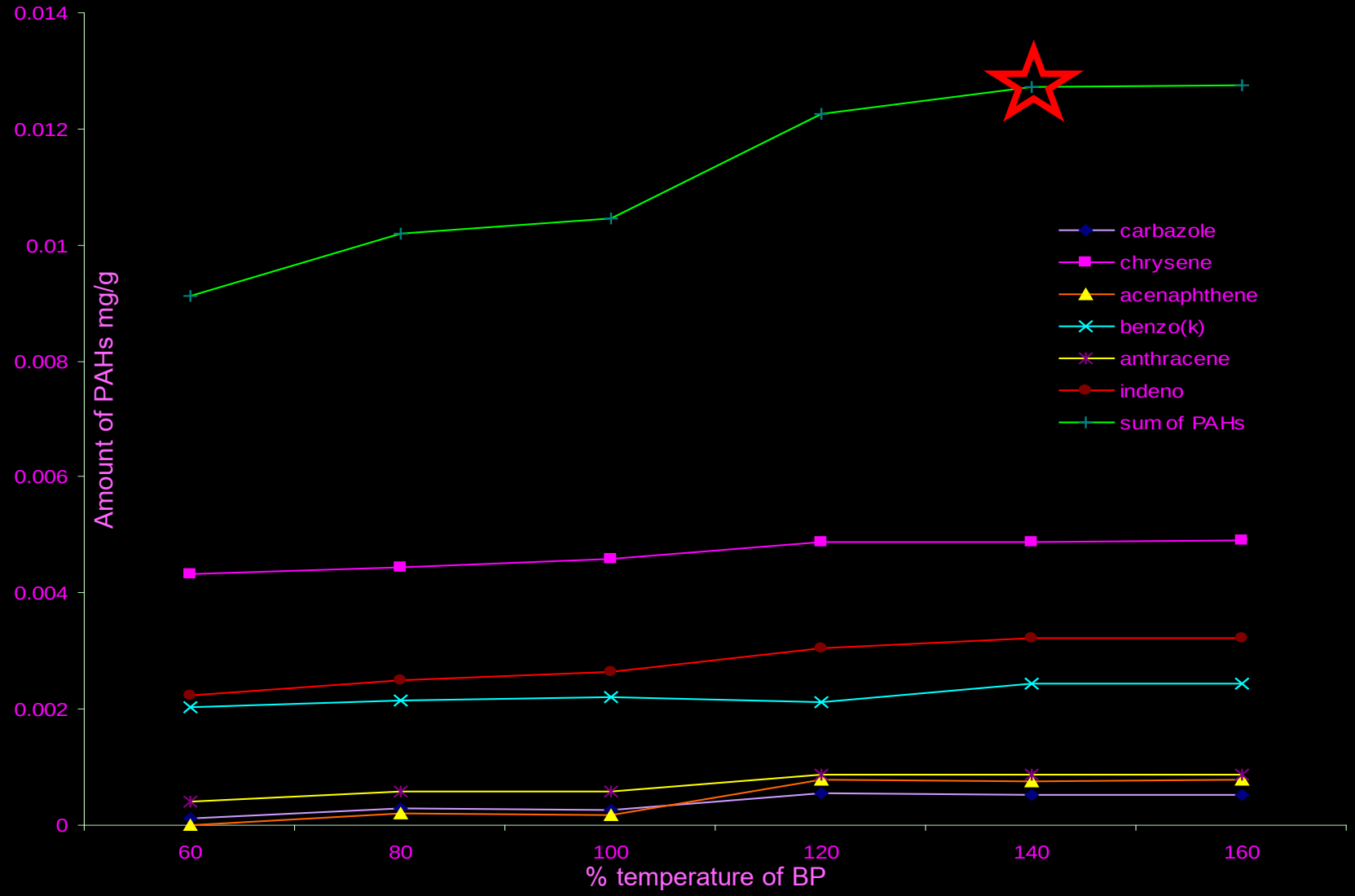
Boiling point	
CH_2Cl_2	39.6°C
cyclohexane	80.7°C
acetone	56.1°C

Temperature of irradiation for dichloromethane



- Fixed: extraction solvent, extraction time = 5 minutes

Temperature of irradiation for cyclohexane:acetone (3:2)



- Fixed: extraction solvent, extraction time = 15 minutes

The optimum condition for microwave extraction

CH_2Cl_2 5 minutes 100% BP.

cyclohexane:acetone 15 minutes 140 % BP.

Boiling point	
CH_2Cl_2	39.6°C
cyclohexane	80.7°C
acetone	56.1°C
acetone(140% BP.)	79.0°C

Sonication

- Extraction Solvent : methanol, acetonitrile, acetone, dichloromethane, hexane:acetone (3:2), cyclohexane:acetone (3:2)
- Extraction Time : 10, 20, 30, 40, 50 min
- Temperature of Irradiation : No

- Fixed : weight of synthetic sample ~0.25 g, extraction solvent = 20 ml

↓

centrifuge 5,500 RPM (15 min)

↓

pipette 10 ml

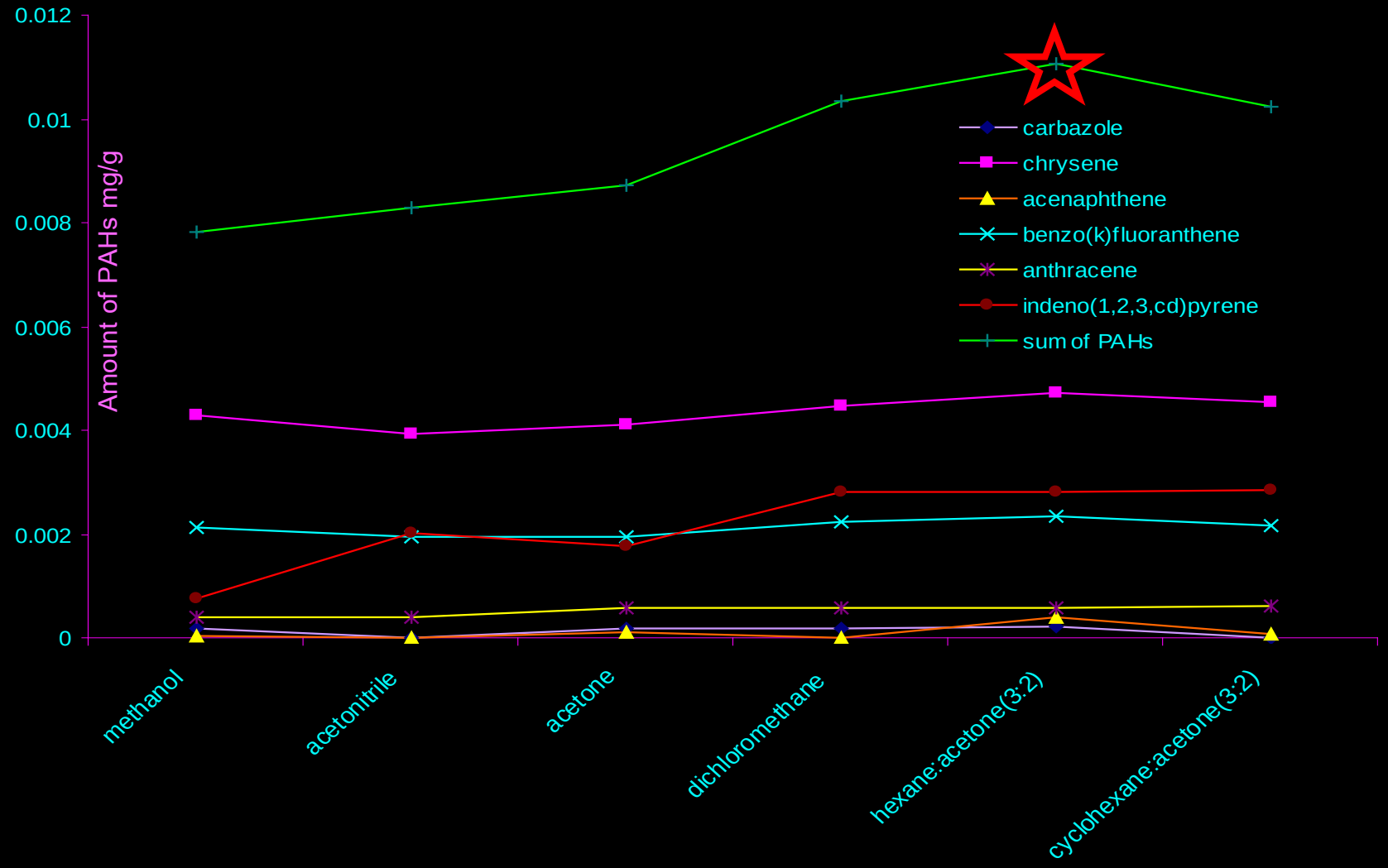
↓

evaporate to 25 ml of ethanol

↓

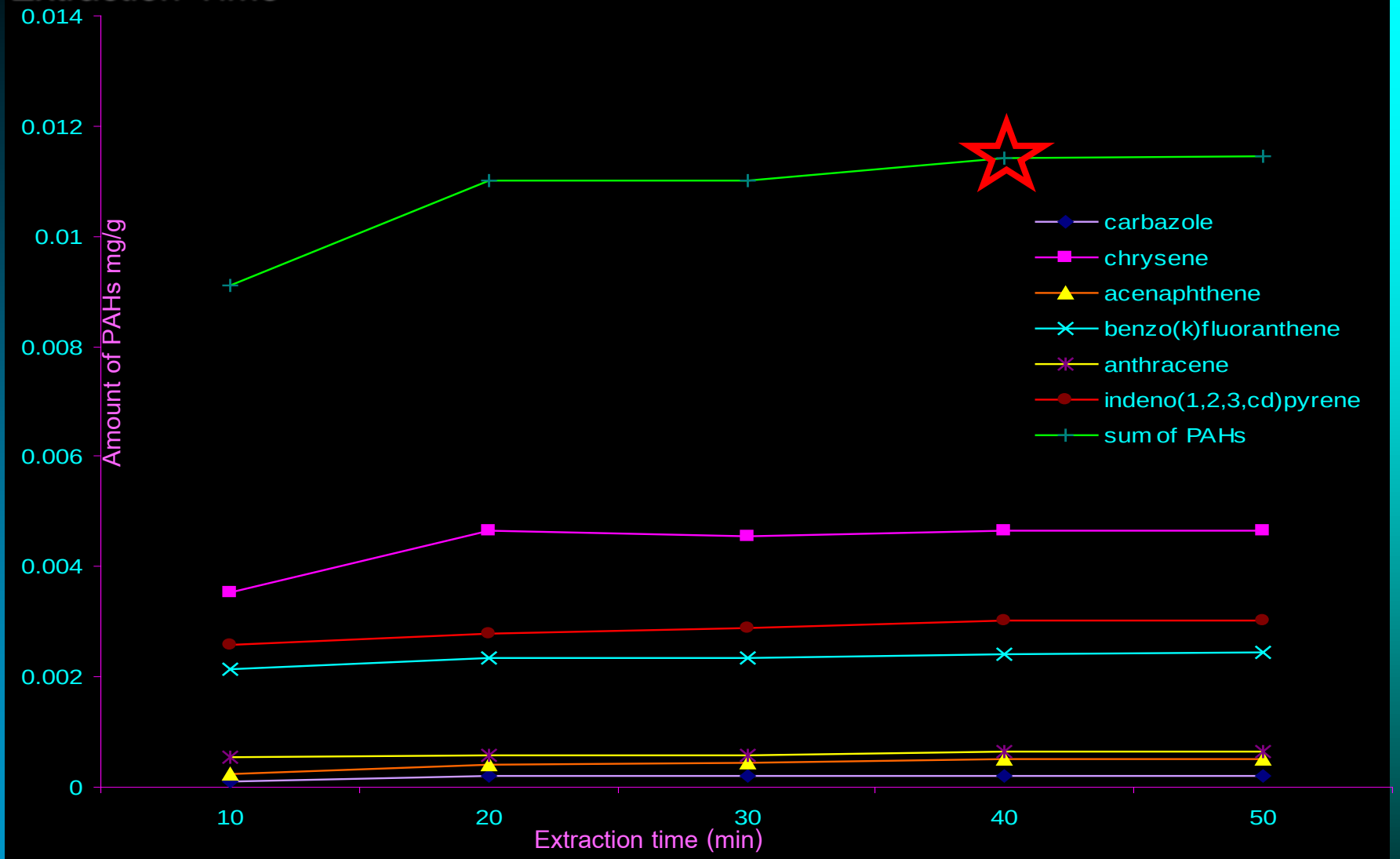
Quantitative analysis by spectrofluorometry

Extraction Solvent



- Fixed: extraction time = 20 minutes, temperature (measure = 41 ^{o}C)

Extraction Time



- Fixed: extraction time = 20 min, temp. (measure = 33, 40.5, 43, 46, 51 ^{o}C)

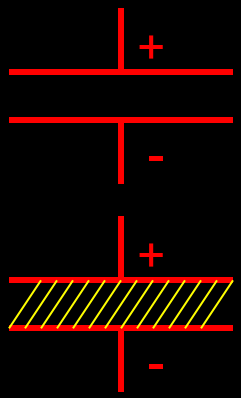
BP of acetone = 56.1^{o}C

The optimum condition for sonication

hexane:acetone (3:2) at 40 minutes

Dielectric constant

Dielectric constant = E_o/E



E : electric field strength

Solvent	Dielectric constant at 25 °C
methanol	32.66
acetone	20.56
acetonitrile	35.94
dichloromethane	8.93
hexane	1.88
cyclohexane	2.02
water	78.3
sulfuric acid	100

Chipperfield, J. R., *Non-aqueous solvents.*, oxford chemistry primers, 1998, 6-10.

Soxhlet Extraction

- Extraction Time: 20 min, 40 min, 1, 3, 6, 12 and 24 hours

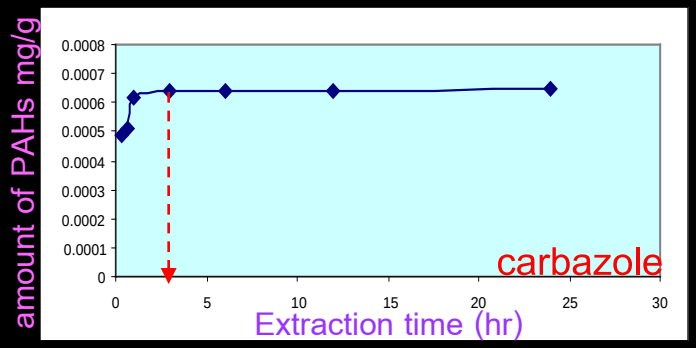
(1 cycle time = 20 min)

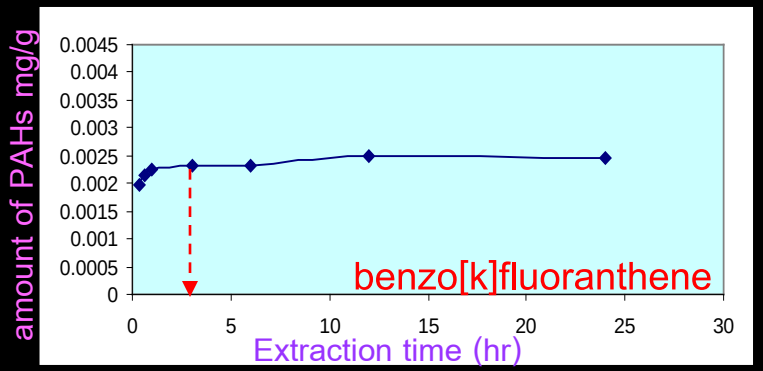
- Fixed : weight of synthetic sample ~0.25 g, extraction solvent = CH_2Cl_2 100 ml

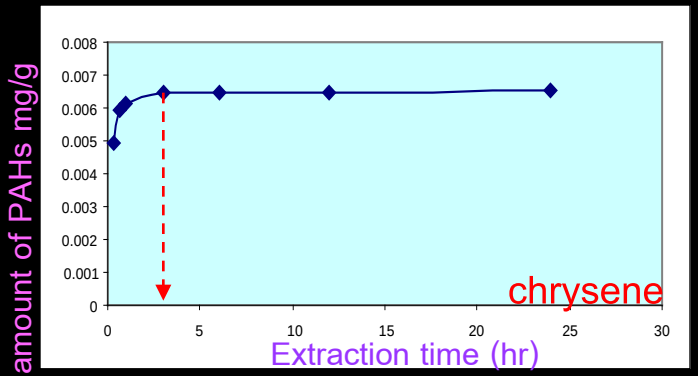
(Budzinski et al., 1993)

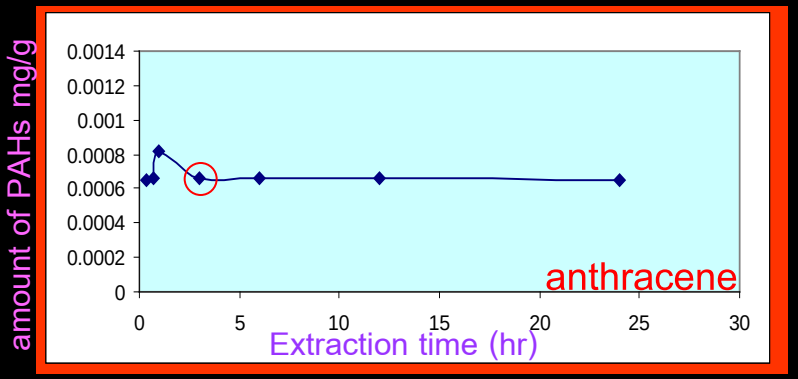
Evaporate to 25 ml of ethanol

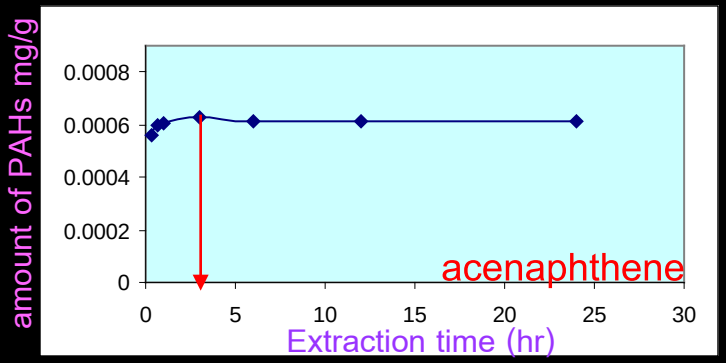
Quantitative analysis by spectrofluorometry

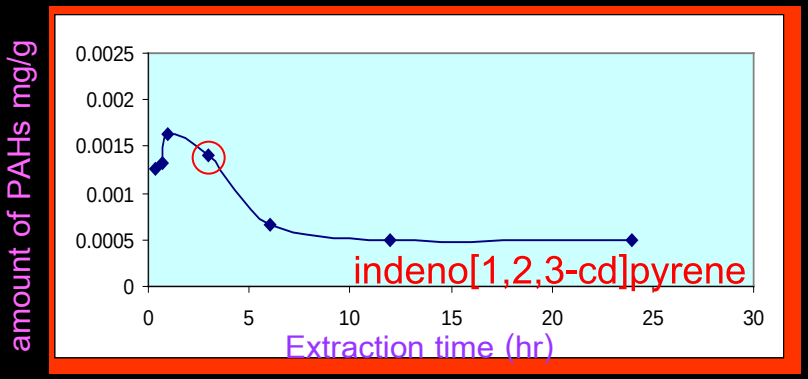

amount of PAHs mg/g
0.0008
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0
0 5 10 15 20 25 30
Extraction time (hr)
carbazole


amount of PAHs mg/g
0.0045
0.004
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
0 5 10 15 20 25 30
Extraction time (hr)
benzo[k]fluoranthene


amount of PAHs mg/g
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
0 5 10 15 20 25 30
Extraction time (hr)
chrysene


amount of PAHs mg/g
0.0014
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
0
0 5 10 15 20 25 30
Extraction time (hr)
anthracene


amount of PAHs mg/g
0.0008
0.0006
0.0004
0.0002
0
0 5 10 15 20 25 30
Extraction time (hr)
acenaphthene

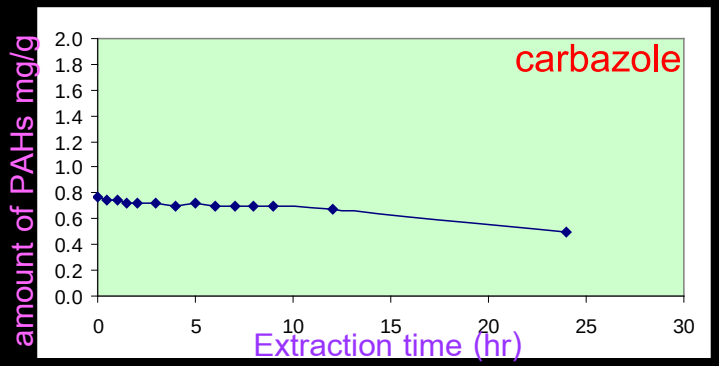

amount of PAHs mg/g
0.0025
0.002
0.0015
0.001
0.0005
0
0 5 10 15 20 25 30
Extraction time (hr)
indeno[1,2,3-cd]pyrene

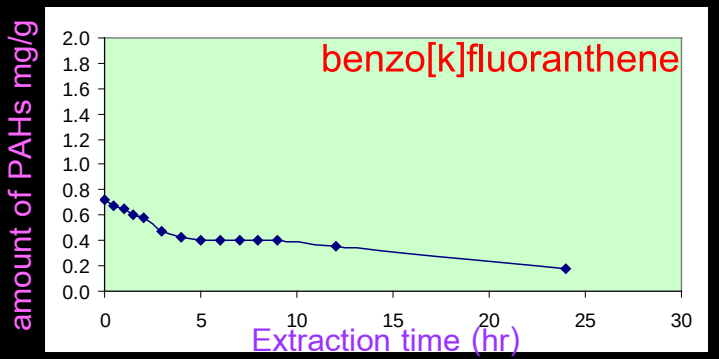
The optimum condition for soxhlet extraction

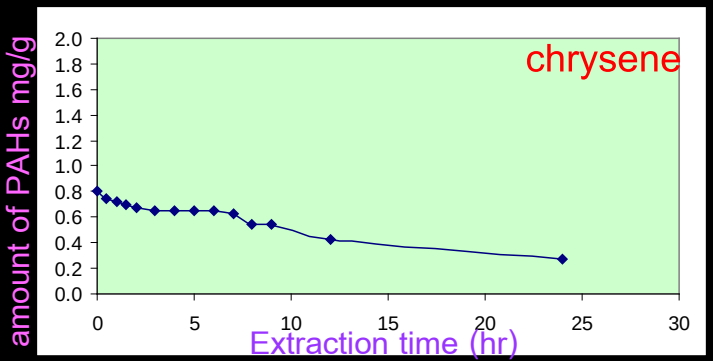
Type of solvent : CH_2Cl_2

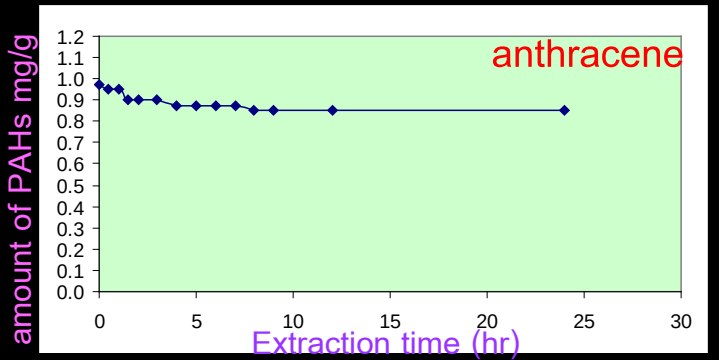
Extraction time : 3 hr (24 hr)

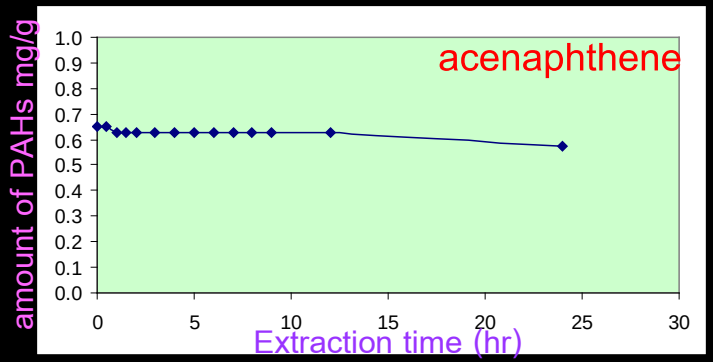
Decomposition of PAHs on Soxhlet Extraction System

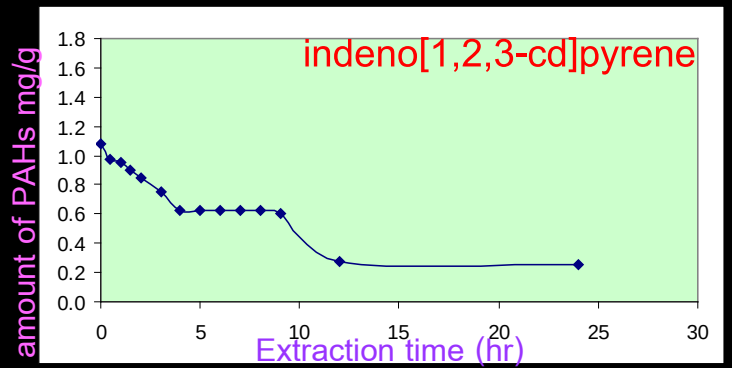

carbazole
amount of PAHs mg/g
Extraction time (hr)


benzo[k]fluoranthene
amount of PAHs mg/g
Extraction time (hr)


chrysene
amount of PAHs mg/g
Extraction time (hr)


anthracene
amount of PAHs mg/g
Extraction time (hr)


acenaphthene
amount of PAHs mg/g
Extraction time (hr)


indeno[1,2,3-cd]pyrene
amount of PAHs mg/g
Extraction time (hr)

Part III

Recovery of the extraction methods

Optimum Conditions

Microwave Extraction

-Extraction Solvent

CH_2Cl_2 cyclohexane:acetone

-Extraction Time

5 minutes 15 minutes

-Temp of Irradiation

100%BP 140%BP

Soxhlet Extraction

CH_2Cl_2 3 & 24 hours

CRM : LGC 6188

Sonication

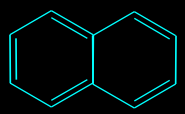
-Extraction Solvent

hexane:acetone

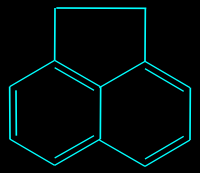
-Extraction Time

40 minutes

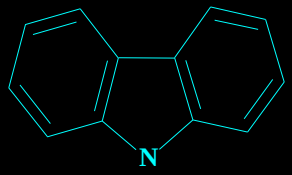
LGC : Laboratory of the Government Chemist



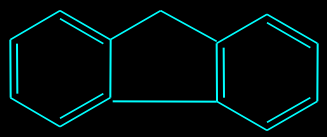
Naphthalene



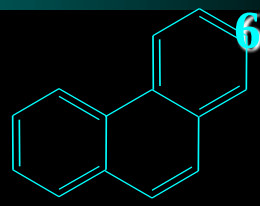
Acenaphthene



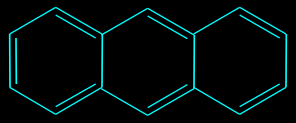
Carbazole



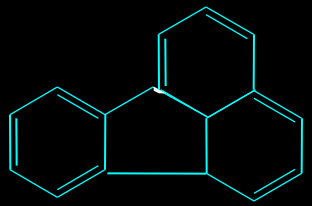
Flourene



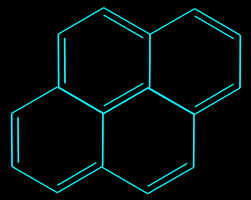
Phenanthene



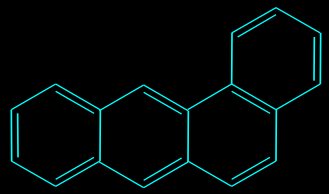
Anthracene



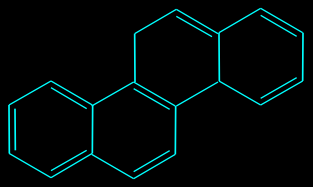
Fluoranthene



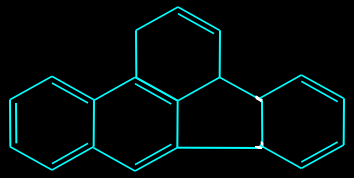
Pyrene



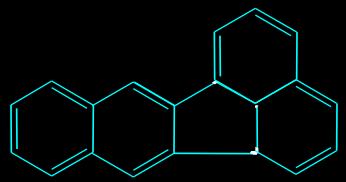
Benzo(a)anthracene



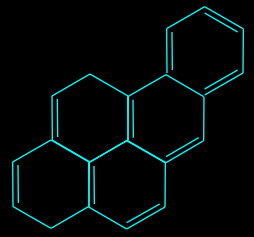
Chrysene



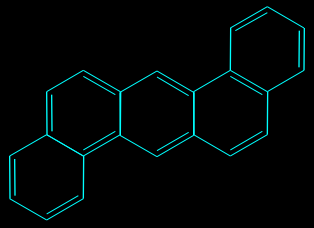
Benzo(b)fluoranthene



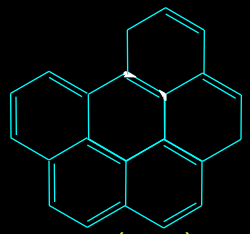
Benzo(k)fluoranthene



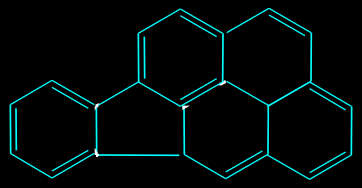
Benzo(a)pyrene



Dibenz(a,h)anthracene



Benzo(g,h,i)perylene



Indeno(1,2,3,c,d)pyrene

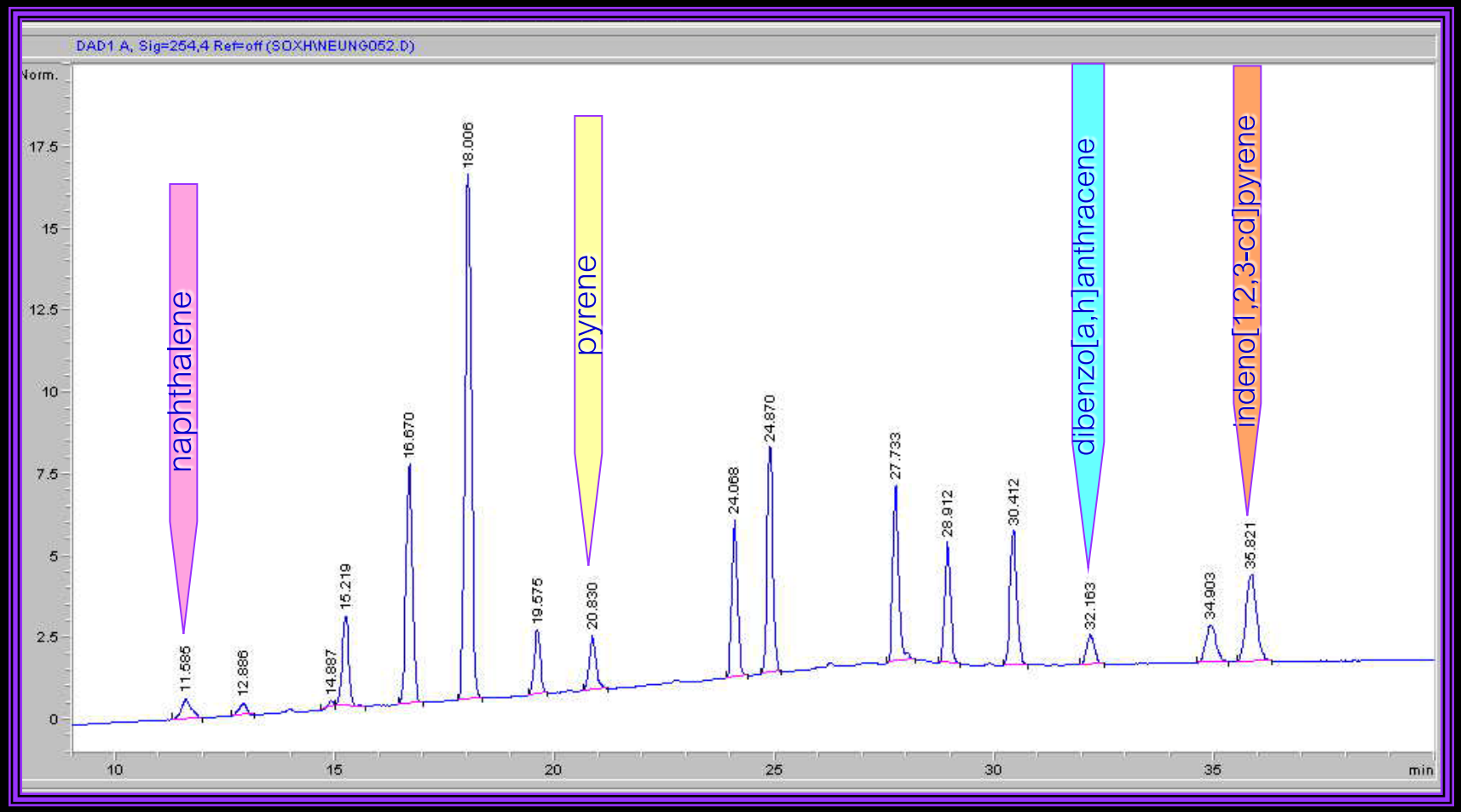
Chemical structure of PAHs, which were studied

Conditions of HPLC (EPA method 8310)

- Column : Chromopher PAHs
- Dimension : 250 X 4.6 mm
- Particle size : 5 μm
- Pore size : 120 A°
- Mobile phases : solvent A = water, solvent B = acetonitrile

  50-100%B:0-25 min, 100%B:25-50 min
- Temperature : 25 °C
- Flow rate : 1 ml/min
- Photodiode array detector : 254 nm

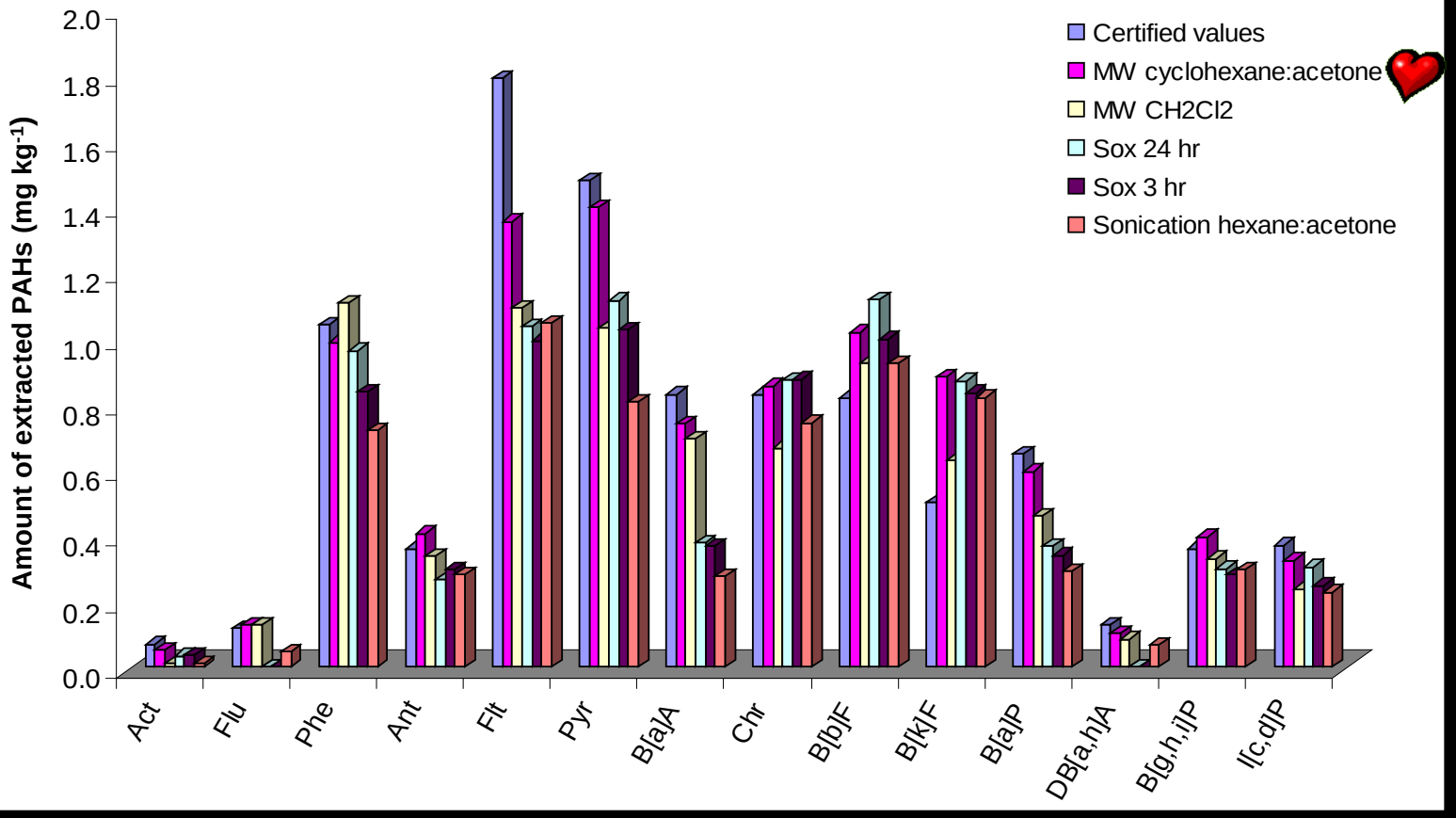
Chromatogram of standard PAHs



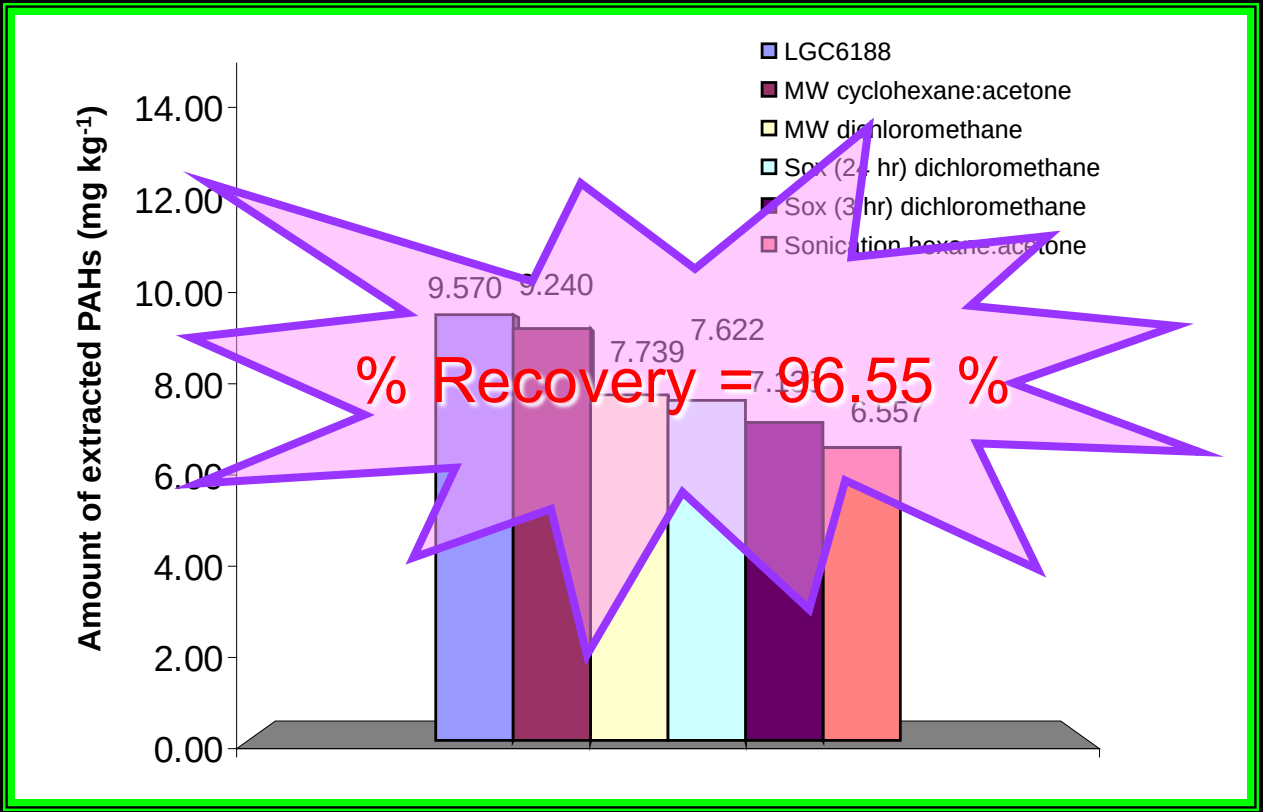
% Recovery of extracted PAHs in CRM of each extraction method by optimized condition

PAHs	%Recovery: based on certified value of LGC6188 (average ± RSD)		
	PMAE	Soxhlet extraction	Sonication
	cyclohexane:acetone (3:2)		
acenaphthene	75.59±0.71		
fluorene	107.52±11.01		
phenanthrene	94.91±4.99		
anthracene	112.89±2.50		
fluoranthene	75.62±1.30		
pyrene	94.35±0.67		
benzo(a)anthracene	89.25±1.17		
chrysene	102.95±2.01		
benzo(b)fluoranthene	124.11±5.91		
benzo(k)fluoranthene	176.71±1.55		
benzo(a)pyrene	91.59±0.21		
dibenzo(a,h)anthracene	78.22±2.69		
benzo(g,h,i)perylene	109.92±3.39		
indeno(1,2,3,cd)pyrene	87.81±1.50		

Amount of individual extracted PAHs by each extraction method using selected condition



Total amount of extracted PAHs by each extraction method using selected condition



CONCLUSION

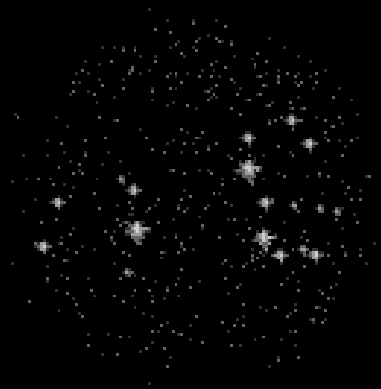
The synchronous scanning fluorescence technique can be used as a rapid method for qualitative analysis of PAHs. Furthermore, PAHs in samples can also be quantitatively analyzed using external calibration and standard addition method.

The suitable conditions of microwave assisted extraction, sonication and Soxhlet extraction were studied and optimized. The certified reference material was extracted by using optimum condition of each extraction method for recovery testing.

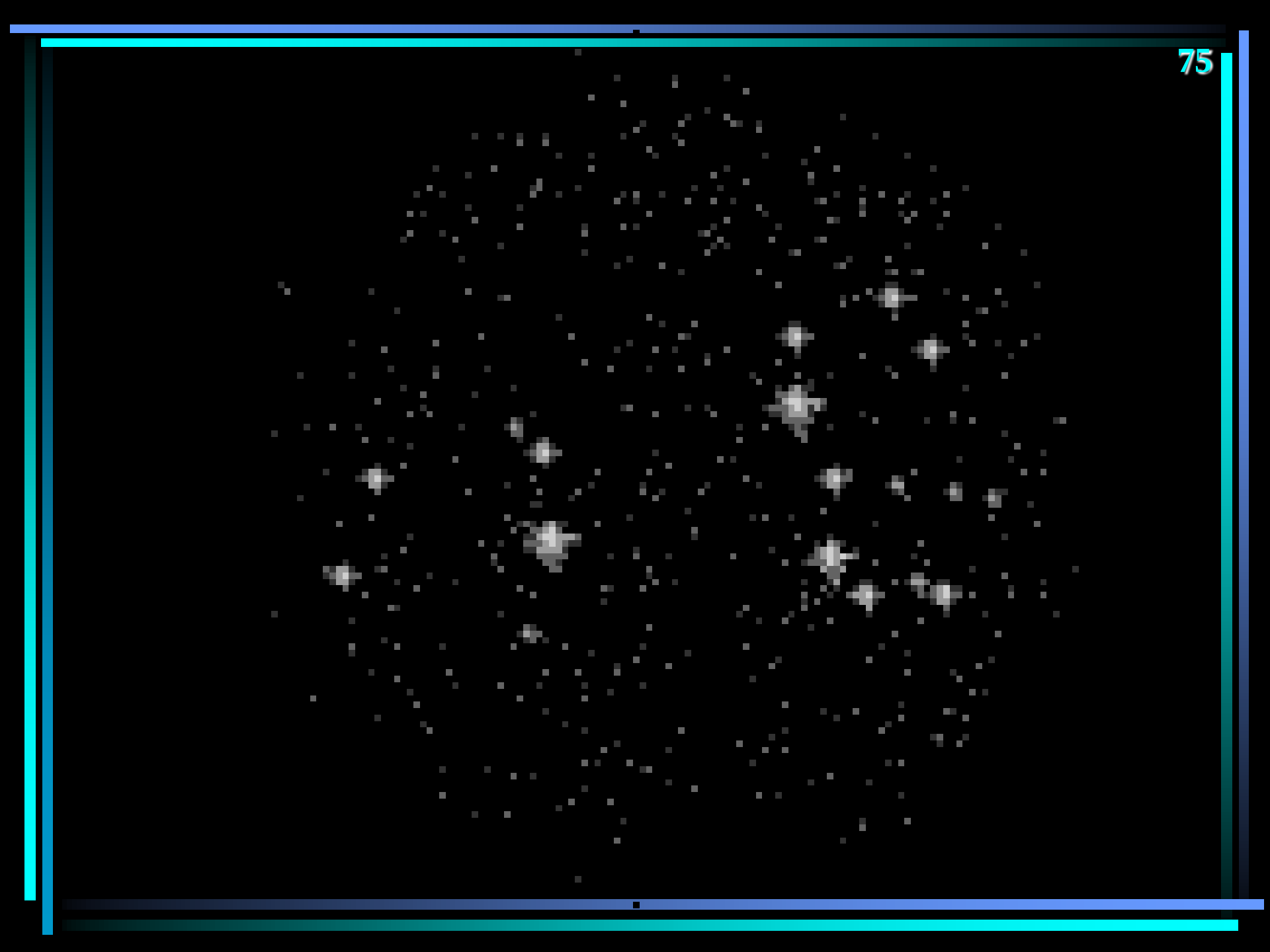
The microwave technique was a good alternative to extract PAHs in soil and sediment samples.

The optimum condition of microwave were 20 ml of cyclohexane:acetone (3:2) at 140% of BP of acetone for 15 minutes. When compared to the certified value, the total %recovery obtained by PMAE was 96.55 %.

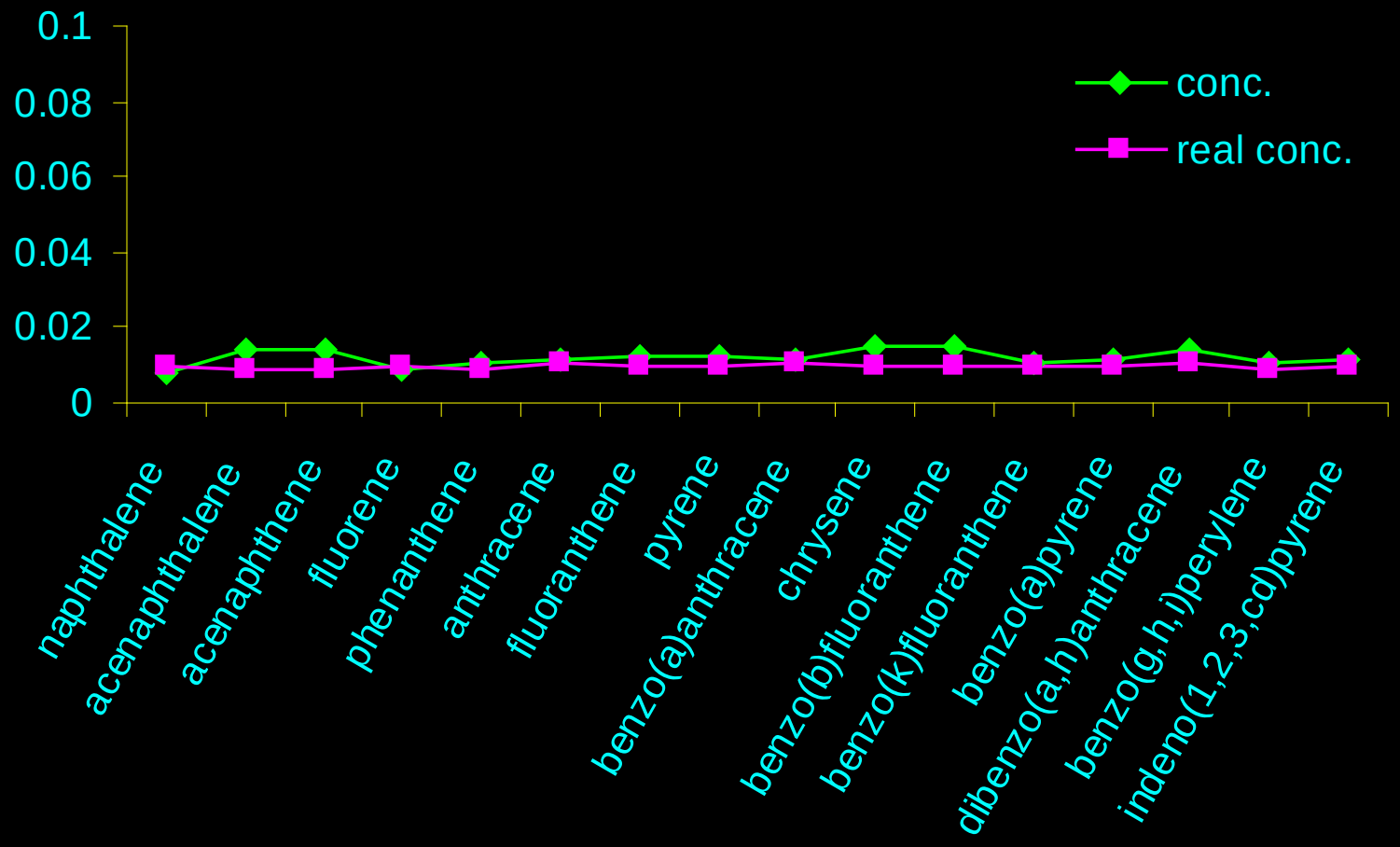
The main advantage of PMAE were the reduction of the volume of extraction solvent, the reduction of decomposition of extracted PAHs, the reduction in extraction time.



THANK YOU

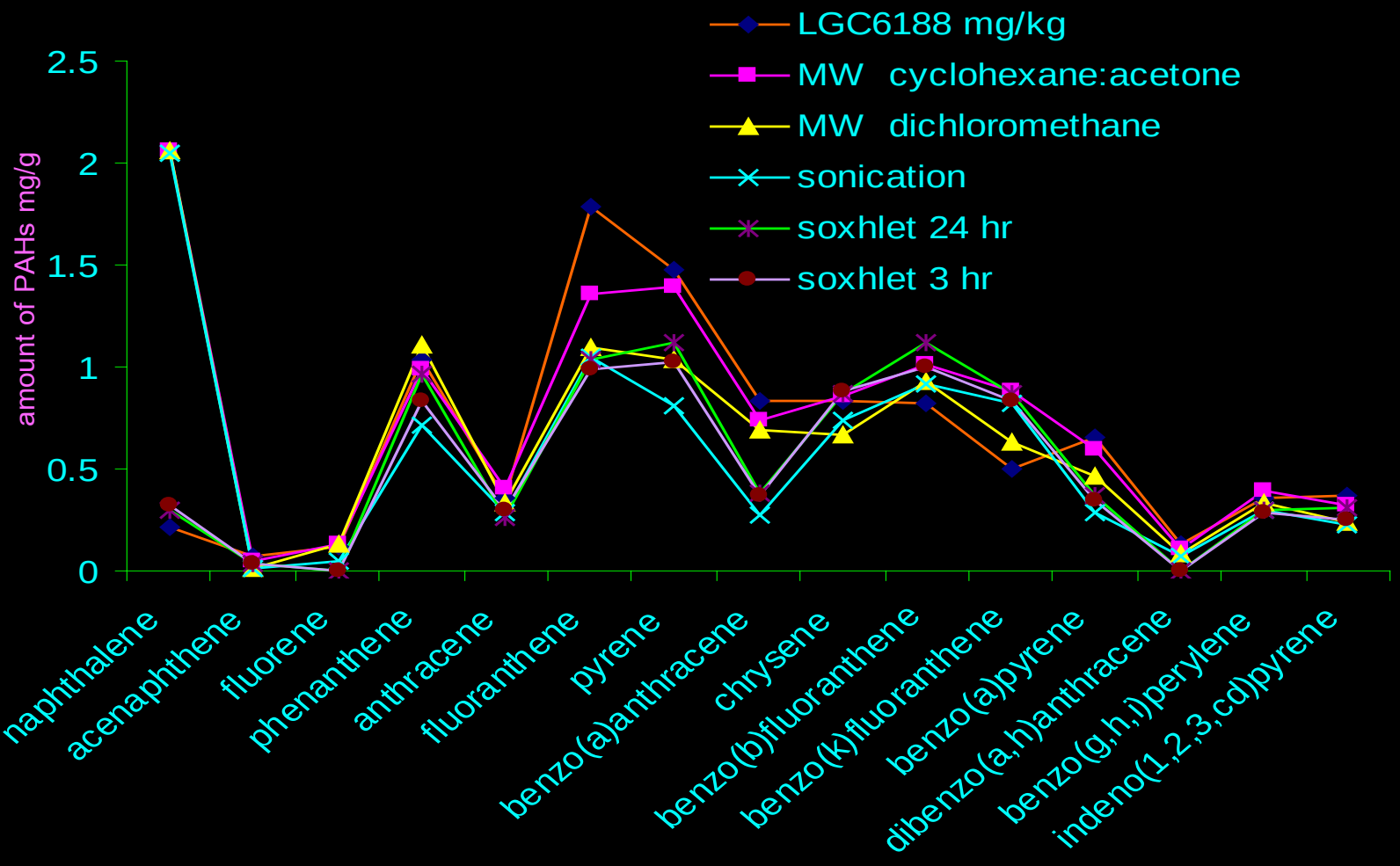


Test method for HPLC



Results

Amount of CRM extracts (mg/g) prepared by different procedures (n=3)



% Recovery of CRM extracts prepared by different procedures (n=3)