CHAPTER 3

EXPERIMENT

3.1 APPARATUSES

Gas Chromatograph equipped with Flame Ionization Detector (FID), Shimadzu, model GC-9A and integrator is Chromatopac, Shimadzu, model CR-3A.

Mechanical Shaker, model HS 500 (Jankel & Kunkel)

A Mettler Balance, No.H43

Ultrasonic Cleanser (Branson)

Microsyringe 1.00, 10.00, and 50.00 µL (Hamilton)

Pipette 1.00, 2.00, 5.00 and 10.00 mL

Vials 3 and 8 drams

Volumetric Flask 25.00, 50.00 and 100.00 mL

All glasswares were cleaned with detergent, dil. HNO_3, water, rinsed with double distilled water, respectively. The vials were cleaned in the same way and were dried at 150 ^{o}C for 3 hours.

3.2 CHEMICALS

3.2.1 Standard of PAHs

Acenaphthene (CHEM SERVICE, West Chester, Pennsylvania, U.S.A.)

Fluoranthene (CHEM SERVICE, West Chester, Pennsylvania, U.S.A.)

Fluorene (CHEM SERVICE, West Chester, Pennsylvania, U.S.A.)

Phenanthrene (CHEM SERVICE, West Chester, Pennsylvania, U.S.A.)

Pyrene (CHEM SERVICE, West Chester, Pennsylvania, U.S.A.)

Di tert-butyl naphthalene was synthesized in laboratory and was checked for the purity by using GC (97%).

3.2.2 Organic Solvents

Absolute methanol (AnalaR grade, J.T.Baker, Phillipsberg, New Jersey, U.S.A.)

Carbon disulfide (AnalaR grade, Merck, Darmstadt, Germany)

Cyclohexane (AnalaR grade, J.T. Baker, Phillipsberg, New Jersey, U.S.A.)

Methylene chloride (AnalaR grade, J.T.Baker, Phillipsberg, New Jersey, U.S.A.)

All solvents were distilled in glass prior to use in the extraction.

3.2.3 Reagents

Nitric acid (AnalaR grade, Merck, Darmstadt, Germany)

Sodium chloride (AnalaR grade, Merck, Darmstadt, Germany)

Anhydrous sodium sulfate (AnalaR grade, J.T. Baker, Phillipsberg, New Jersey, U.S.A.)

The salts were dried in electric furnace at 300 °C for 6 hours and were kept in dessicator before used.

3.3 PREPARATION OF STANDARD SOLUTIONS

3.3.1 The Standard Solutions of PAHs

The 1000.00 ppm stock solutions of each PAH, e.g., acenaphthene, fluorene, phenanthrene, fluoranthene and pyrene in each solvent, i.e., cyclohexane, methylene chloride and carbon disulfide were prepared by accurate weighing 0.0250 g of each standard, dissolving and diluting it with the solvent to the mark in 25.00 mL volumetric flask.

3.3.2 The Standard Solutions of PAHs in Methanol

The 1000.00 ppm stock solutions of each PAH, e.g., acenaphthene, fluorene, phenanthrene, fluoranthene and pyrene in methanol were prepared by accurate weighing 0.0250 g of each standard, dissolving and diluting it with methanol to the mark in 25.00 mL volumetric flask. The 1.00 ppm and 5.00 ppm aqueous solutions of each standard were prepared by further dilution of 1000.00 ppm standard stock solution with double distilled water in 100.00 mL volumetric flask.

3.3.3 The Standard Solutions of Internal Standard

The 1000.00 ppm stock solutions of internal standard in each solvent, i.e., carbon disulfide, cyclohexane, and methylene chloride were prepared by accurate weighing 0.0250 g of di tert-

butyl naphthalene, dissolving and diluting it with the solvent to the mark in 25.00 mL volumetric flask.

This internal standard solution was added to extracting solvent prior to the extraction.

3.4 STUDY VARIOUS EFFECTS ON % RECOVERY OF MICROEXTRACTION

The shaking time and salts content were studied in each solvent, i.e., carbon disulfide, cyclohexane and methylene chloride in each sample to solvent ratio, i.e., 9:1, 5:5 and 2:8 as described in the following :

3.4.1 Study of the Shaking Time

1. Pipet the extracting solvent into a series of vials.
2. Pipet the 5.00 ppm standard aqueous solution into each vial.
3. Seal a vial with aluminum foil, butyl-rubber septum and tight them with open-top cap.
4. Shake the contents in a vial by mechanical shaker with the speed of 200 Hub/min for 2, 4,..., min and allow it to stand until two phases were completely separated.
5. Remove the extract from the vial by microsyringe and then injected it into gas chromatograph.
6. Determine the concentration of interested component in the extract by using the internal standardization method, then calculated % E of each compound at various shaking times.
7. Plot % E against shaking times.

3.4.2 Determination of the Optimum Amounts of Sodium Chloride Used in the Extraction.

1. Add 0, 0.5, 1.0, 1.5, 2.0,..., g of sodium chloride into a series of vials.

2. Pipet the extracting solvent into each vial.

3. Pipet the 5.00 ppm standard aqueous solution into each vial.

4. Seal a vial with aluminum foil, butyl-rubber septum and tight them with open-top cap.

5. Shake the contents in a vial by mechanical shaker with the speed of 200 Hub/min for the time as found in section 3.4.1. and allow it to stand until two phases were completely separated.

6. Remove the extract from the vial by microsyringe and then injected it into gas chromatograph.

7. Determine the concentration of interested component in the extract by using the internal standardization method, then calculated % E of each compound.

8. Plot % E against the amounts of sodium chloride used.

3.4.3 Determination of the Optimum Amounts of Sodium Sulfate Used in the Extraction.

1. Add 0, 0.5, 1.0, 1.5, 2.0,..., g of sodium sulfate into a series of vials.

2. Pipet the extracting solvent into each vial.

3. Pipet the 5.00 ppm standard aqueous solution into each vial.

4. Seal a vial with aluminum foil, butyl-rubber septum and tight them with open-top cap.

5. Shake the contents in a vial by mechanical shaker with the speed of 200 Hub/min for the time as found in section 3.4.1. and allow it to stand until two phases were completely separated.

6. Remove the extract from the vial by microsyringe and then injected it into gas chromatograph.

7. Determine the concentration of interested compound in the extract by using the internal standardization method, then calculated % E of each compound.

8. Plot % E against the amounts of sodium sulfate used.

3.4.4 Microextraction Procedures

The investigation of the shaking time, the amounts of sodium chloride and sodium sulfate used as in the section 3.4.1 3.4.2 and 3.4.3 were evaluated. Therefore, each extraction consisted of there systems. The three systems were :

1. non salting out (no salt)

2. the optimum amount of sodium chloride as found in section 3.4.2.

3. the optimum amount of sodium sulfate as found in section 3.4.3.

In each system, two concentrations of standard solution, i.e., 1.00 ppm and 5.00 ppm of the single component standard solutions and the standard mixture solutions were studied. The solid salt, extracting solvent and standard aqueous solution were

added into a vial sequentially for each extraction. The contents in vial were shaken by mechanical shaker and were analyzed by gas chromatograph.

3.5 INTERNAL STANDARDIZATION METHOD

This method is also known as the relative or the indirect calibration. Several standard solutions containing known weights of interested component and a chosen standard (internal standard) are prepared and chromatographed. The peak area ratios obtained from the chromatograms are plotted against the weight ratios to obtain a graph, the plot should be linear for a particular system. Once the linearity is established for a given sample system and type only one standard mix need be used to define the slope of that plot.

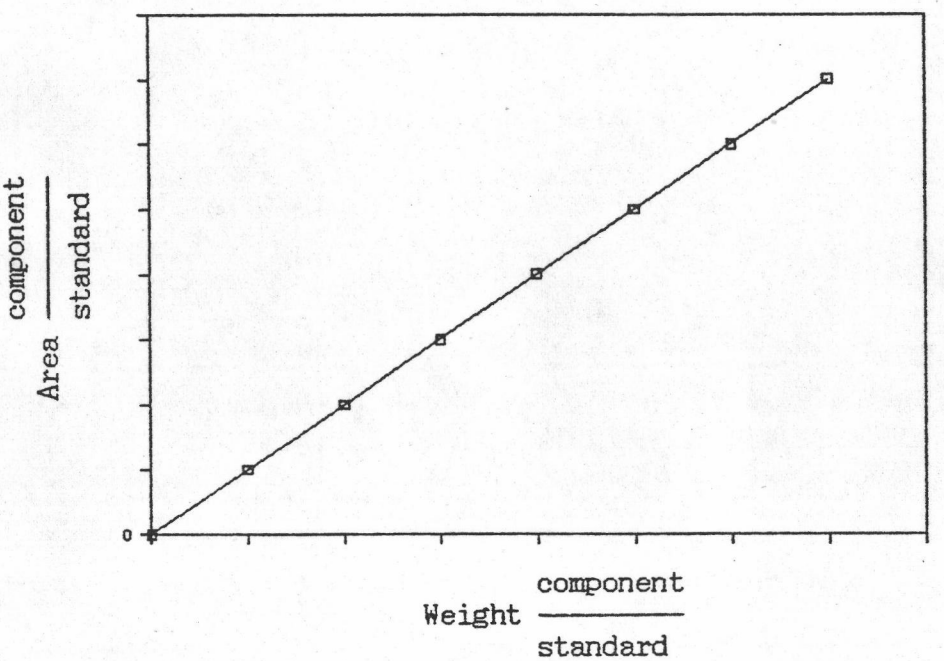


Figure 3.1 Relative calibration curve

Therefore the standard is actually used to determine the response factor, F, from the following equation :

$$\text{slope} = \frac{\text{peak area ratio}}{\text{weight ratio}} = F \qquad (28)$$

$$= \frac{A_c}{A_i} \times \frac{W_i}{W_c} \qquad (29)$$

Where A_c and W_c are the peak area and weight of the interested component.

and A_i and W_i are the peak area and weight of the internal standard.

To determine the amount of the interested component in the sample, a known weight of the internal standard is added into the sample. The mixture is then chromatographed and the peak area ratio of components are measured. The weight of interested component in unknown could be calculated by using the following equation :

$$W_c = A_c \times \frac{W_i}{A_i} \times F \qquad (30)$$

The calibration curves of each PAH, i.e., acenaphthene, fluorene, phenanthrene, fluoranthene and pyrene in each solvent, e.g., carbon disulfide, cyclohexane and methylene chloride were obtained from the standard solutions of PAHs as decribed below :

For studying the sample to solvent ratio of 9:1, a series of standard solutions were prepared in concentration range from 5.00 ppm to 50.00 ppm by taking 50.00-500.00 µL of 1000.00 ppm stock solution of standard into 10.00 mL volumetric flask and diluting it to the mark with the extracting solvent.

For studying the sample to solvent ratio of 5:5, a series of standard solutions were prepared in concentration range from 1.00 ppm to 10.00 ppm by taking 10.00-100.00 µL of 1000.00 ppm stock solution of standard into 10.00 mL volumetric flask and diluting it to the mark with the extracting solvent.

For studying the sample to solvent ratio of 2:8, a series of standard solutions were prepared in concentration range from 0.10 ppm to 2.00 ppm by taking 1.00-20.00 µL of 1000.00 ppm stock solution of standard into 10.00 mL volumetric flask and diluting it to the mark with the extracting solvent.

The series of standard solutions in each solvent were injected into a gas chromatograph, the peak height ratios obtained from the chromatograms were plotted against the concentrations of standard PAHs.

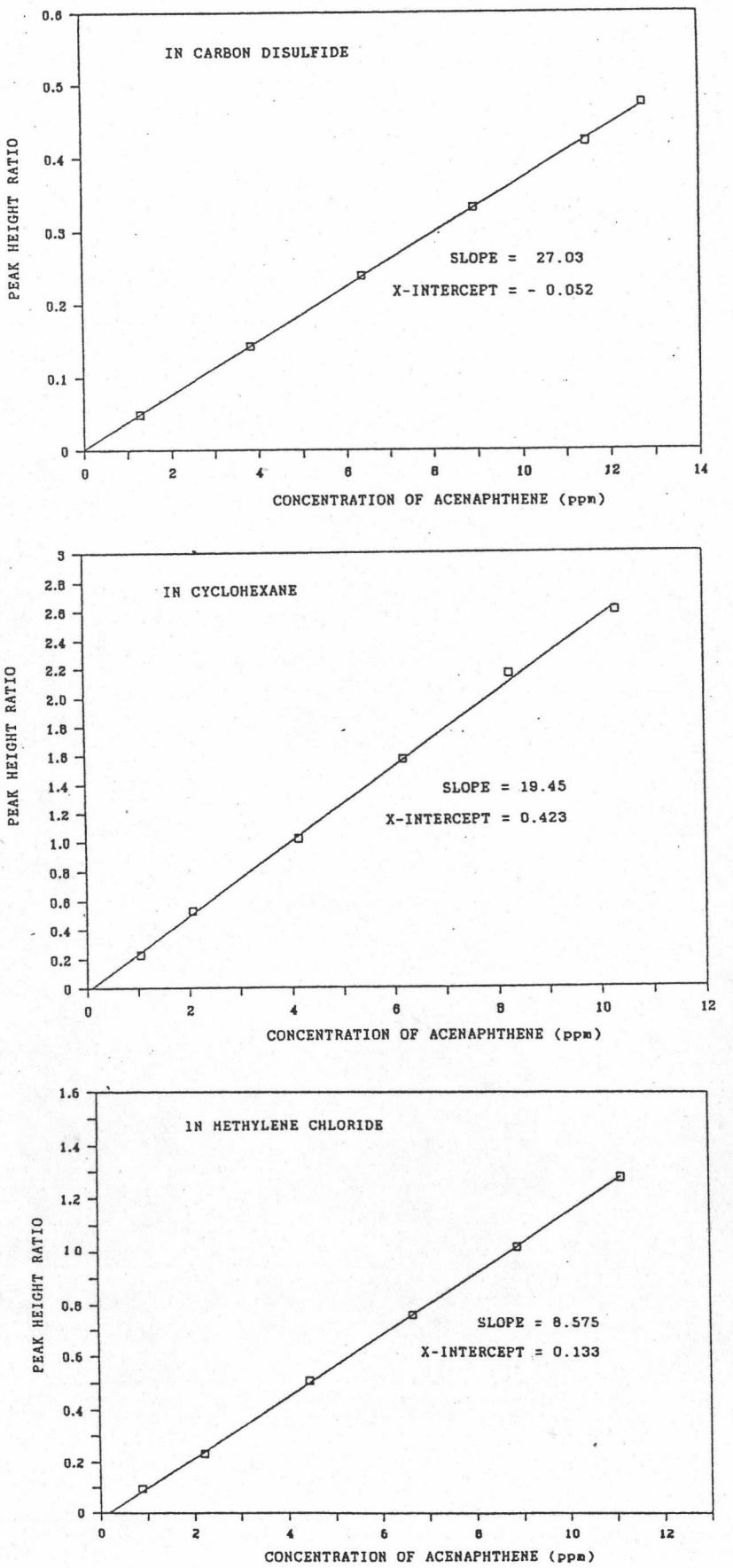


Figure 3.2 The calibration curve of acenaphthene in various solvents

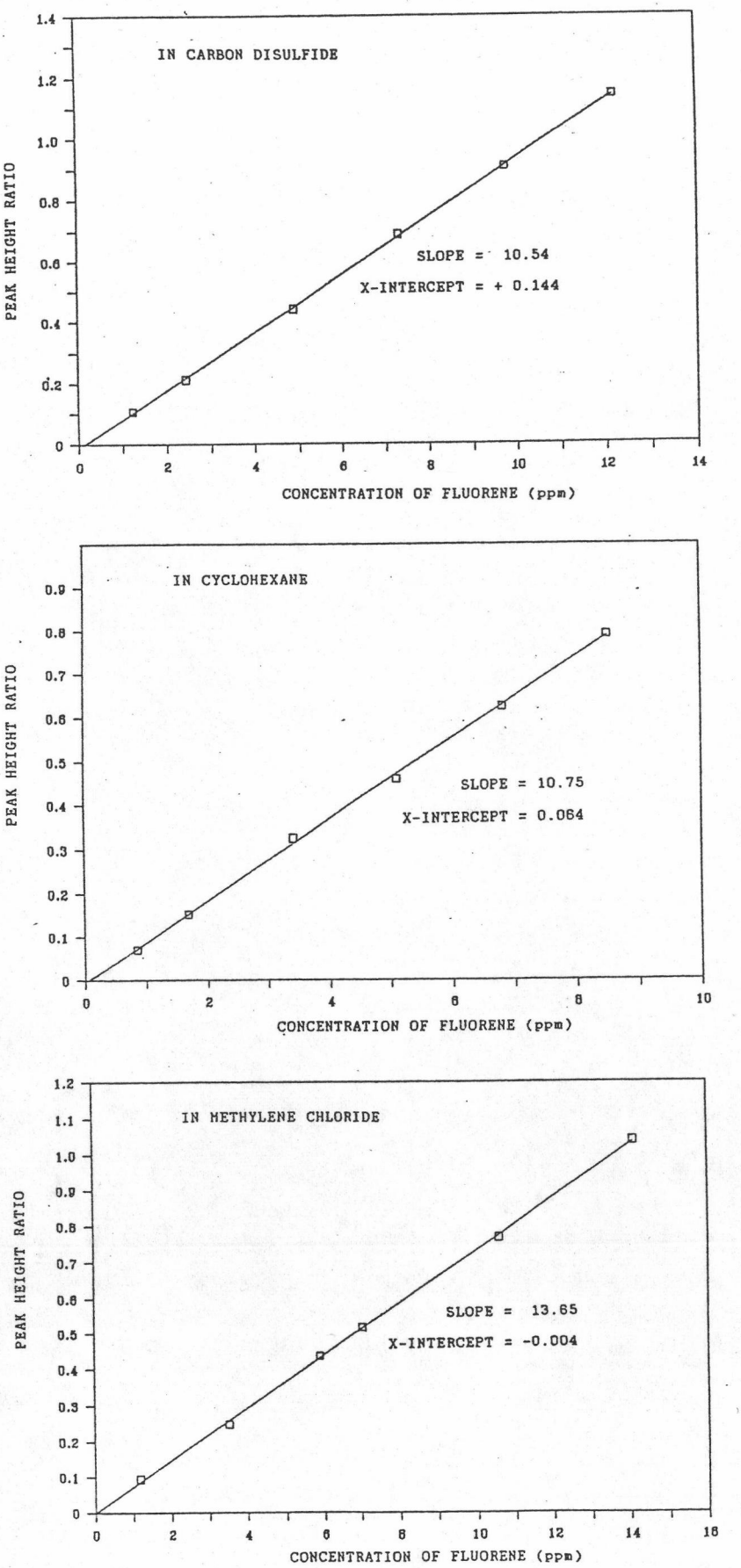
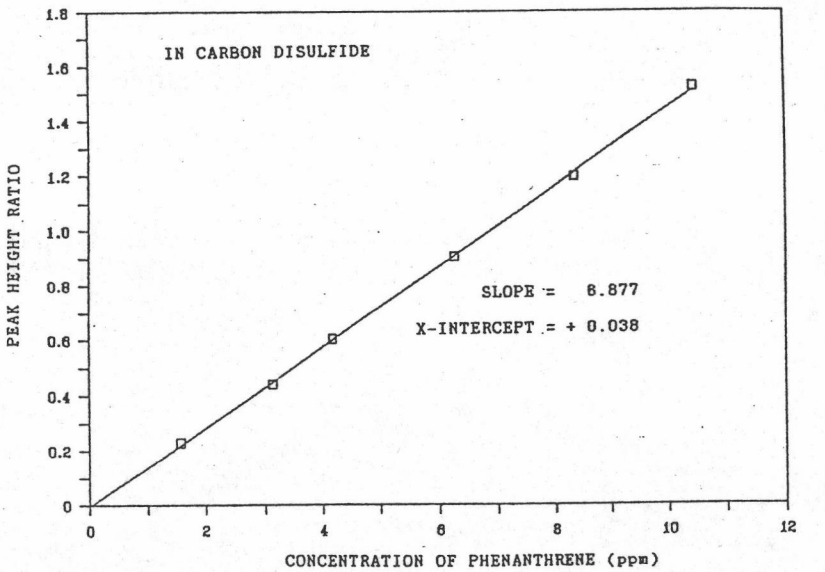
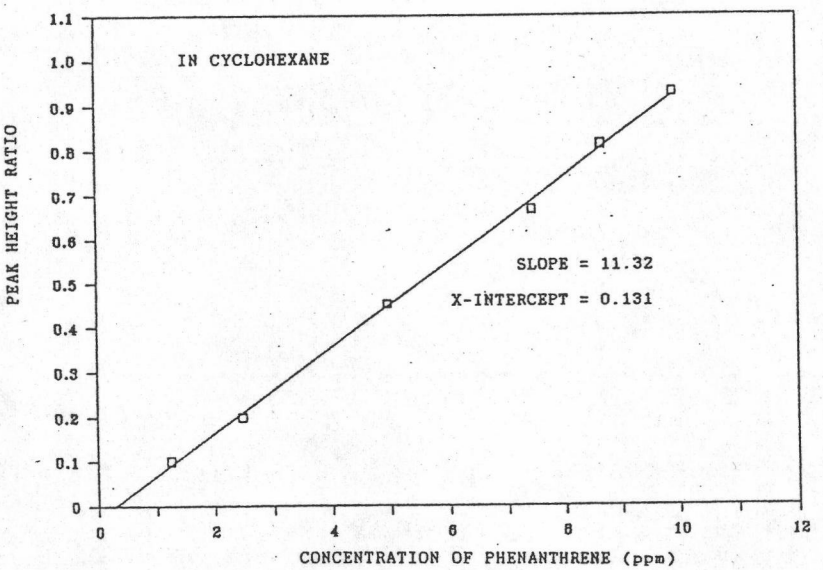


Figure 3.3 The calibration curve of fluorene in various solvents





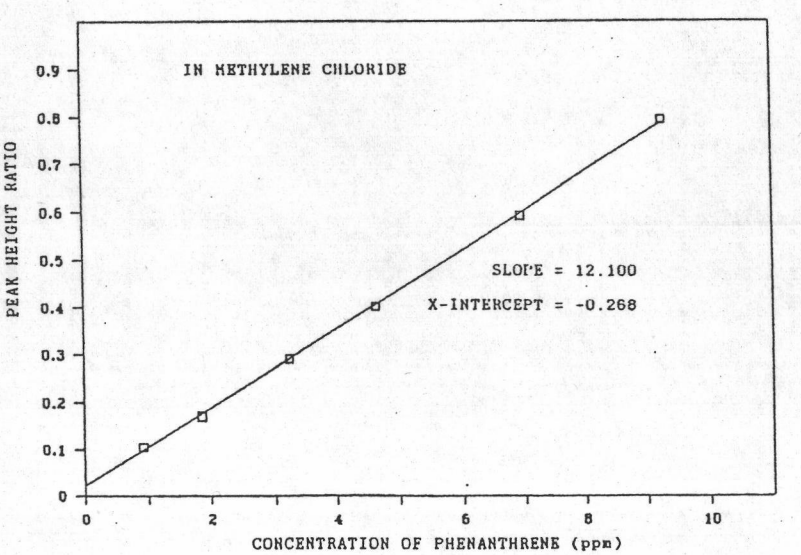


Figure 3.4 The calibration curve of phenanthrene in various solvents

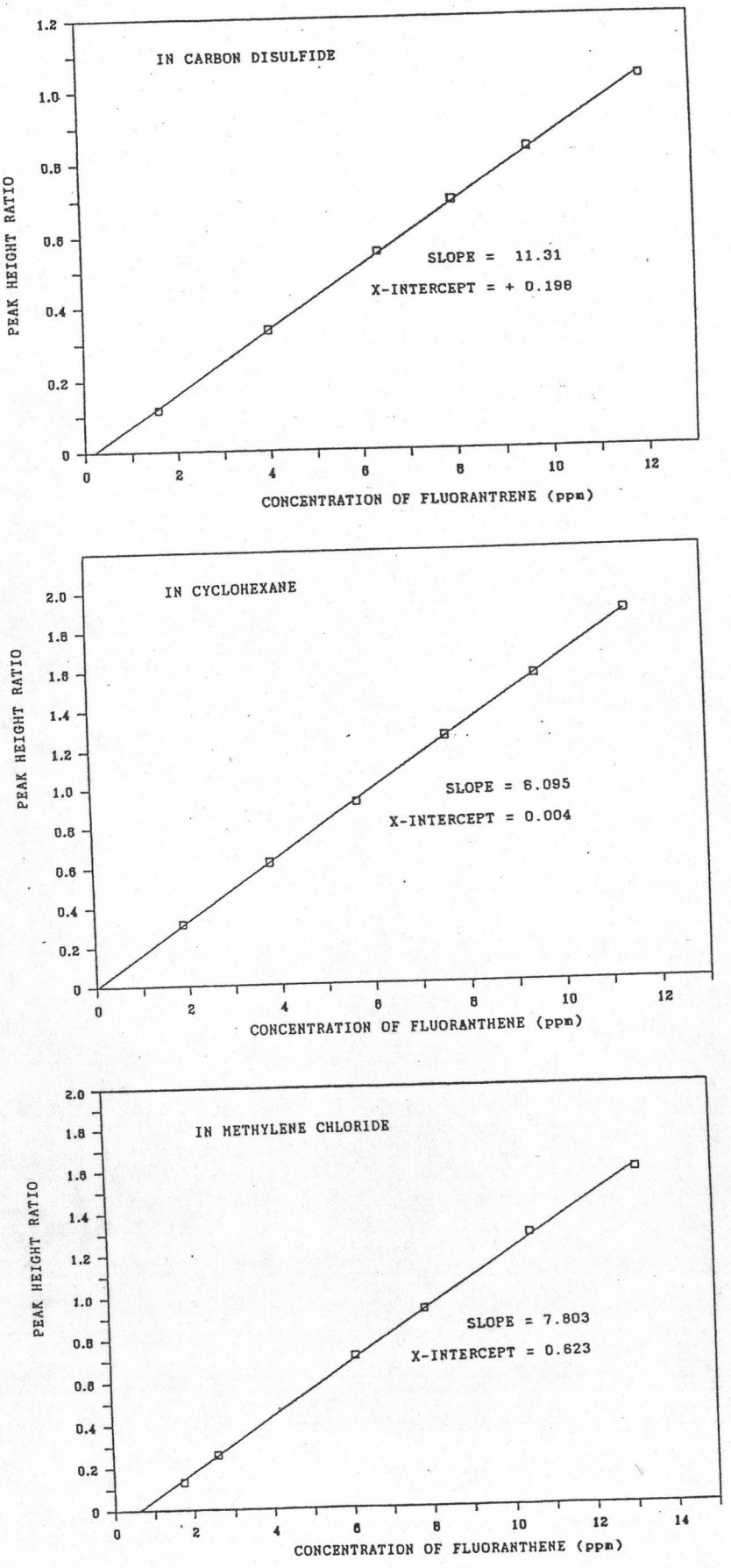
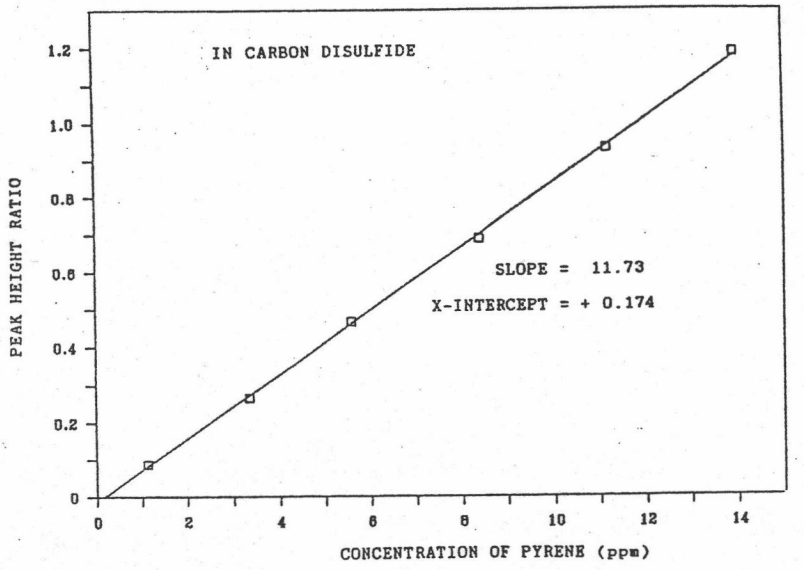
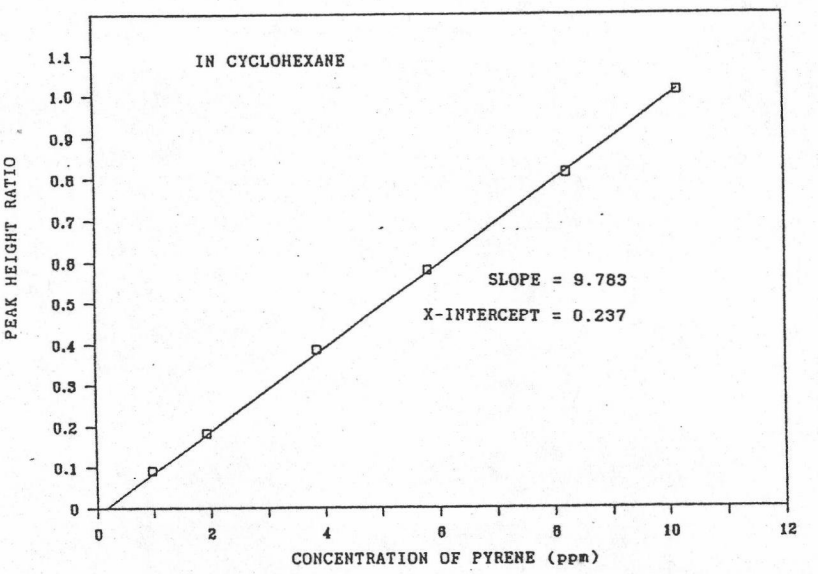


Figure 3.5 The calibration curve of fluoranthene in various solvents





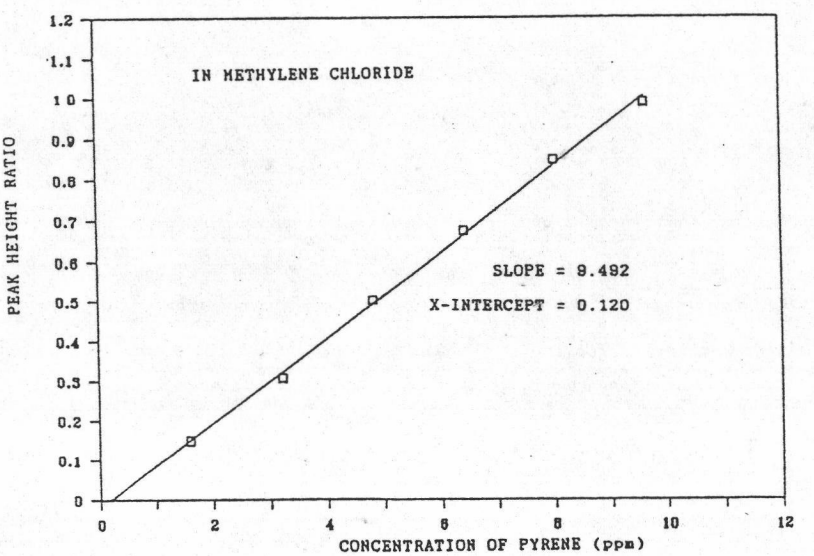


Figure 3.6 The calibration curve of pyrene in various solvents

3.6 GAS CHROMATOGRAPHIC CONDITIONS

3.6.1 Gas Chromatographic Conditions for Studying the Single Component Solutions

STANDARD: Acenaphthene

ANALYTICAL COLUMN: 3/8" O.D.x 2 m stainless steel column packed with 5% OV-17 on Chromosorb WAW-DMCS, 60/80 mesh

OVEN TEMPERATURE: 170 °C (isothermal)

INJECTOR AND DETECTOR TEMPERATURES: 350 °C

NITROGEN FLOW RATE: 30 mL/min

DETECTOR: FID

Air flow rate 300 mL/min

Hydrogen flow rate 50 mL/min

STANDARD: Fluorene

ANALYTICAL COLUMN: 3/8" O.D.x 2 m stainless steel column packed with 5 % OV-17 on Chromosorb WAW-DMCS, 60/80 mesh

OVEN TEMPERATURE: 185 °C (isothermal)

INJECTOR AND DETECTOR TEMPERATURES: 350 °C

NITROGEN FLOW RATE: 30 mL/min

DETECTOR: FID

Air flow rate 300 mL/min

Hydrogen flow rate 50 mL/min

STANDARD: Phenanthrene

ANALYTICAL COLUMN: 3/8" O.D.x 2 m stainless steel column packed with 5 % OV-17 on Chromosorb WAW-DMCS, 60/80 mesh

OVEN TEMPERATURE: 190 °C (isothermal)

INJECTOR AND DETECTOR TEMPERATURES: 350 °C

NITROGEN FLOW RATE: 30 mL/min

DETECTOR: FID

Air flow rate 300 mL/min

Hydrogen flow rate 50 mL/min

STANDARD: Fluoranthene

ANALYTICAL COLUMN: 3/8" O.D.x 2 m stainless steel column packed with 5 % OV-17 on Chromosorb WAW-DMCS, 60/80 mesh

OVEN TEMPERATURE: 200 °C (isothermal)

INJECTOR AND DETECTOR TEMPERATURES: 350 °C

NITROGEN FLOW RATE: 40 mL/min

DETECTOR: FID

Air flow rate 300 mL/min

Hydrogen flow rate 50 mL/min

STANDARD: Pyrene

ANALYTICAL COLUMN: 3/8" O.D.x 2 m stainless steel column packed with 5 % OV-17 on Chromosorb WAW-DMCS, 60/80 mesh

OVEN TEMPERATURE: 200 °C (isothermal)

INJECTOR AND DETECTOR TEMPERATURES: 350 °C

NITROGEN FLOW RATE: 40 mL/min

DETECTOR: FID

Air flow rate 300 mL/min

Hydrogen flow rate 50 mL/min

3.6.2 Gas Chromatographic Condition for Studying the Mixture Solution

ANALYTICAL COLUMN: 3/8" O.D. x 2 m stainless steel column packed with 5 % OV-17 on Chromosorb WAW-DMCS, 60/80 mesh

OVEN TEMPERATURE: Temperature programmed;

1 min initial hold at temperature 185°C, then to 240 °C at 4 °C/min and hold until the last peak eluted

INJECTOR AND DETECTOR TEMPERATURES: 350 °C

NITROGEN FLOW RATE: 30 mL/min

DETECTOR: FID

Air flow rate 300 mL/min

Hydrogen flow rate 50 mL/min

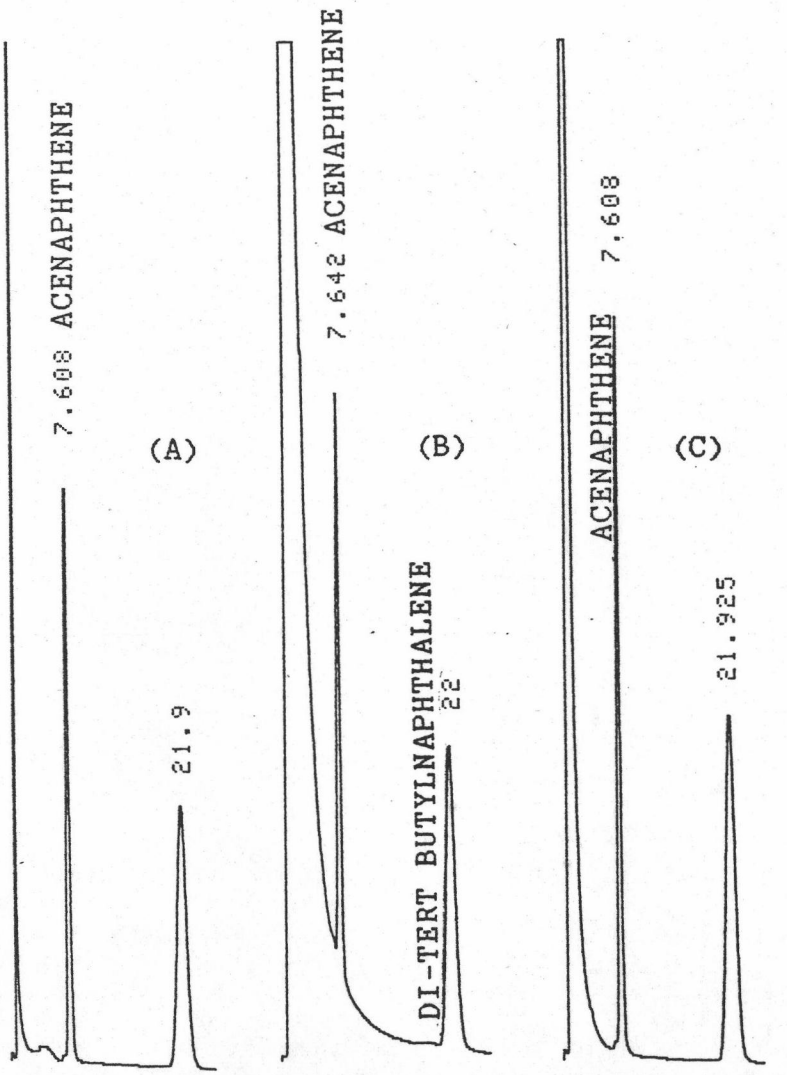


Figure 3.7 Gas chromatograms of acenaphthene in CS_2 (A), in cyclohexane (B) and in CH_2Cl_2 (C)
Conditions: column, 170 °C; injector and FID, 350 °C; N_2 carrier gas, 30 mL/min; sample size, 1 uL; detector range, x10°; attenuation, 5; chart speed, 1 mm/min

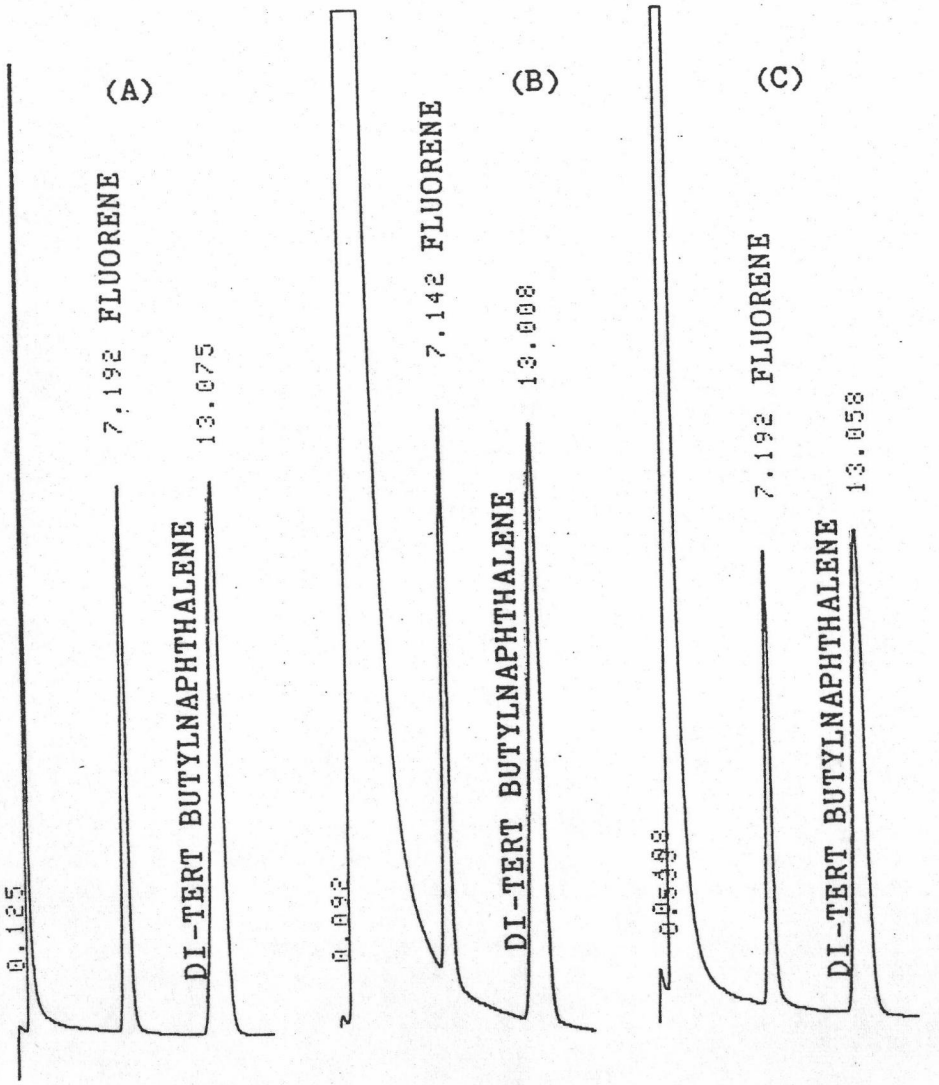


Figure 3.8 Gas chromatograms of fluorene in CS_2 (A), in cyclohexane (B) and in CH_2Cl_2 (C)
Conditions: column, 185 °C; injector and FID, 350 °C; N_2 carrier gas, 30 mL/min; sample size, 1 uL; detector range, x10^0; attenuation, 5; chart speed, 1 mm/min

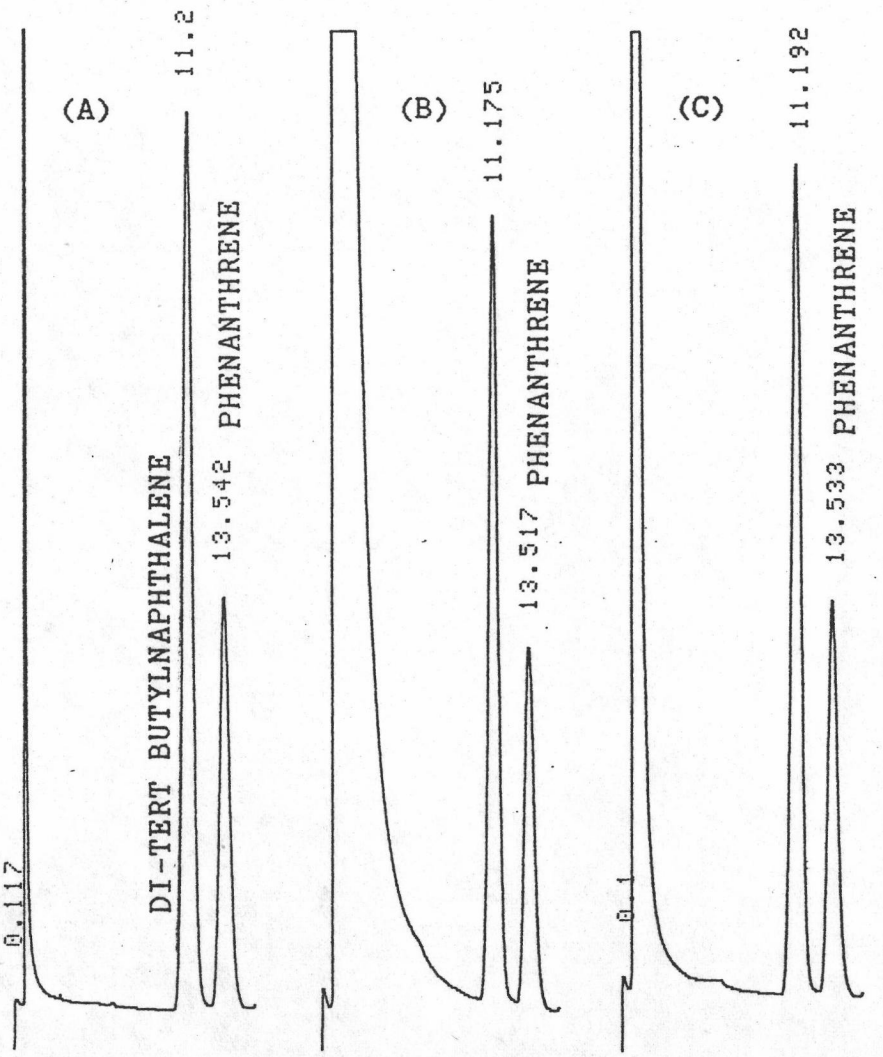


Figure 3.9 Gas chromatograms of phenanthrene in CS_2 (A), in cyclohexane (B) and in CH_2Cl_2 (C)
Conditions: column, 190 °C; injector and FID, 350 °C; N_2 carrier gas, 30 mL/min; sample size, 1 uL; detector range, x10°; attenuation, 5; chart speed, 1 mm/min

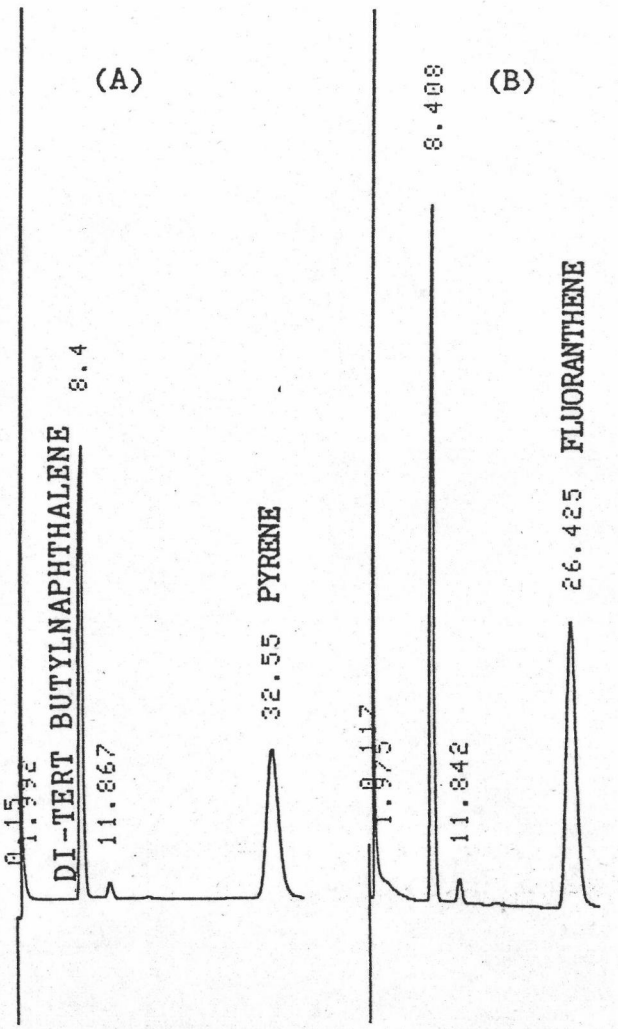


Figure 3.10 Gas chromatograms of pyrene in CS_2 (A) and fluoranthene in CS_2 (B)
Conditions: column, 200 °C; injector and FID, 350 °C; N_2 carrier gas, 30 mL/min; sample size, 1 uL; detector range, x10°; attenuation, 5; chart speed, 1 mm/min

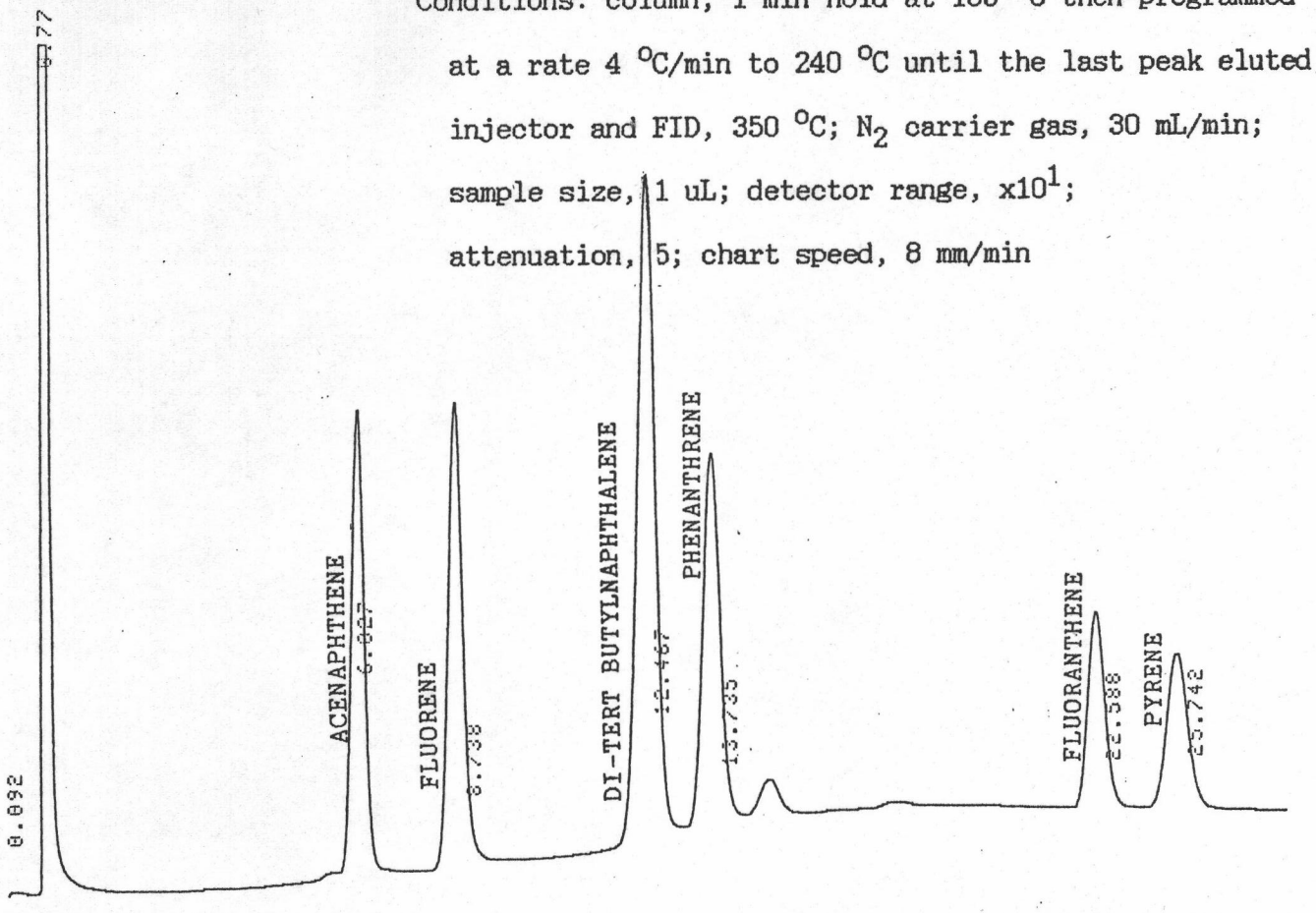


Figure 3.11 Gas chromatograms of PAHs mixture in CS_2

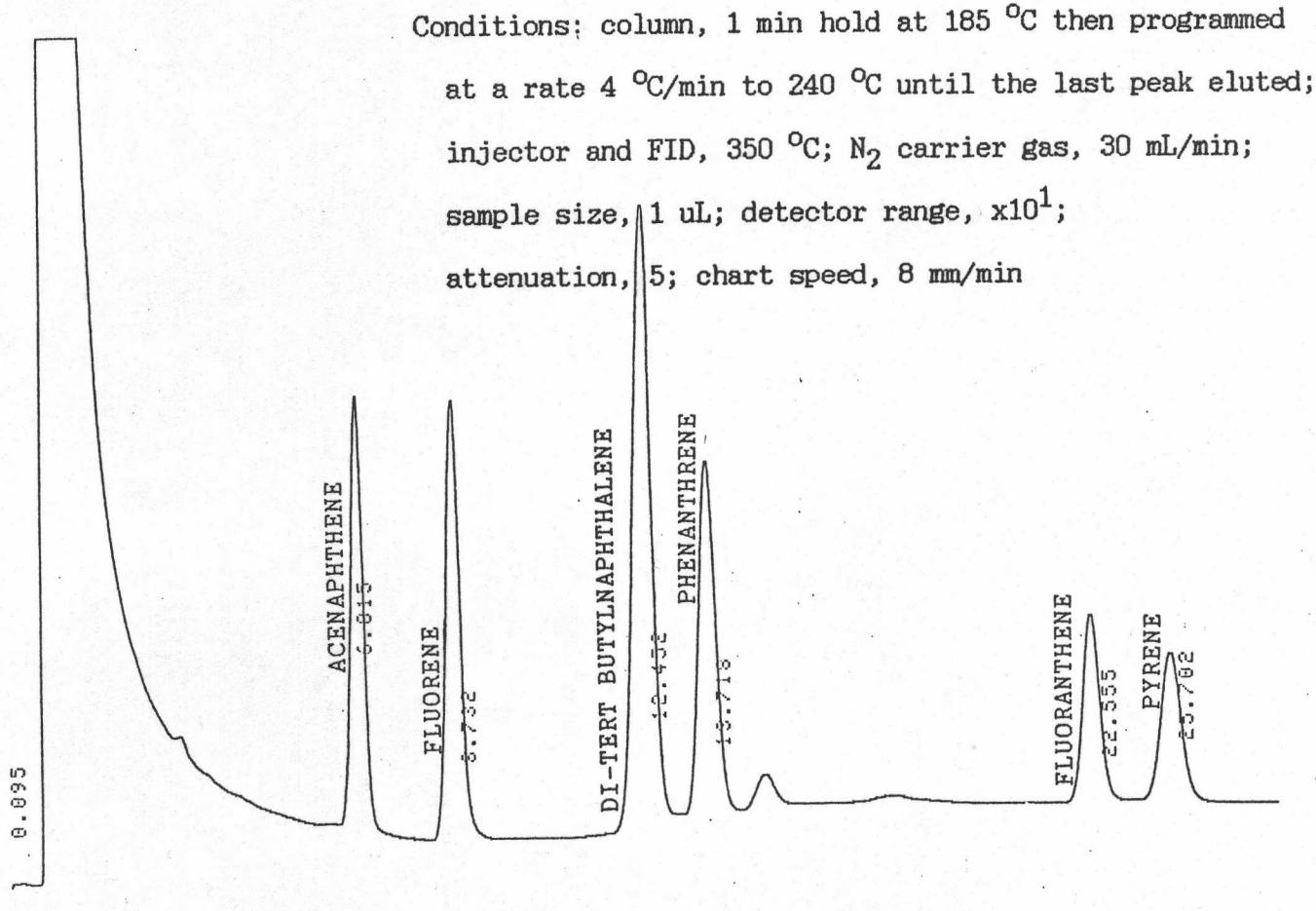


Figure 3.12 Gas chromatograms of PAHs mixture in cyclohexane

Conditions: column, 1 min hold at 185 °C then programmed at a rate 4 °C/min to 240 °C until the last peak eluted; injector and FID, 350 °C; N_2 carrier gas, 30 mL/min; sample size, 1 uL; detector range, $x10^1$; attenuation, 5; chart speed, 8 mm/min

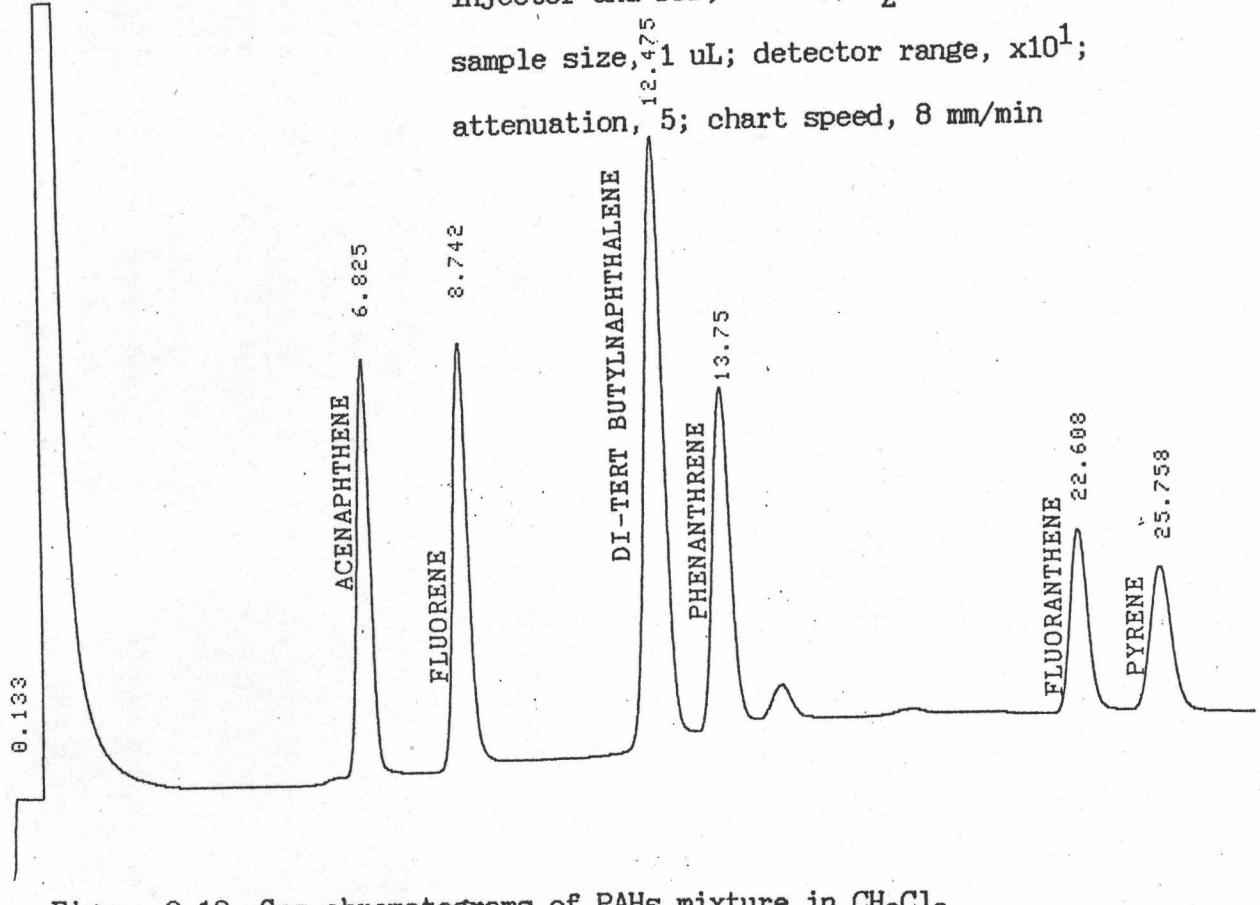


Figure 3.13 Gas chromatograms of PAHs mixture in CH_2Cl_2