CHAPTER 3

EXPERIMENTAL

3.1 APPARATUS

3.1.1 Instrumentation for HPLC

A Waters model 510 pump was used with Waters model 680 gradient controller. (Waters Chromatography Division, Massachusetts, U.S.A.)

Sample injector, model Reodyne 7125 equipped with a 20 µL sample loop.(Reodyne, California, U.S.A.)

Separation of PAHs was carried out on a Lichrosphere RP-18 column (250x4.6 mm, particle size 5 µm, E. Merck, Darmstadt, F.R. Germany), protected by a C-18 guard column (Pye Unicam, U.S.A.)

Detection was accomplished via JASCO model Uvidec 100-VI UV-visible detector.(Japan Spectroscopic, Tokyu, Japan)

Chromatograms were recorded on a Waters 740 data

module.(Waters Chromatography Division, Massachusetts, U.S.A.)

3.1.2 UV-Visible Spectrophotometer

UV-Visible spectra were obtained from a Jasco model Uvidec-650 spectrophotometer.(Japan Spectroscopic, Tokyu, Japan)

3.1.3 Other Apparatuses

Microsyringe 10.00, 25.00, and 50.00 μL (Hamilton Company,Switzerland)

Ultrasonic bath (Branson) was used for degassing mobile phase. (Branson cleaning equipment company, Conn., U.S.A.)

Mettler balance No. H43 (E.Mettler, Zurich, Switzerland)

Mechanical shaker, model HS 500 (Jankle&Kunkel, Staufen i. Br., West Germany)

3.2 GLASSWARES

Pipette 1.00, 2.00, 5.00, 10.00, and 50.00 mL

Volumetric flask 25.00, 250.00, and 1000.00 mL

Vials 1, 2, 6, and 8 drams

All glasswares used were cleaned with detergent , soaked in 1:1 HNO_3 for 1 day, rinsed with double distilled water, and dried in an oven at 150^{o}C for 3 hours.

3.3 CHEMICALS

3.3.1 Standard of PAHs

Standard of PAHs (Anthracene, Fluoranthene, Fluorene, Pyrene) and internal standard (Acenaphtylene) were purchased from Chem.Service., Westchester, Pennsylvania, U.S.A.

3.3.2 Solvents

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

Acetonitrile (A.R. grade, J.T.Baker, Phillipsberg, New Jersey, U.S.A.)

Hexane (A.R. grade, J.T. Baker, Phillipsberg, New Jersey, U.S.A.)

Methylene Chloride (A.R. grade, J.T.Baker, Phillipsberg, New Jersey, U.S.A.)

Toluene (A.R. grade, J.T. Baker, Phillipsberg, New Jersey, U.S.A.)

Hexane, methylene chloride and toluene were distilled by fractional distillation in all glass distillation apparatus prior to use in the study .

3.3.3 Reagents

Nitric acid (A.R. grade, Merck, Darmstadt, Germany)

Sodium chloride (A.R. grade, J.T. Baker, Phillipsberg, New Jersey, U.S.A.)

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

The salts i.e., sodium chloride and anhydrous sodium sulfate were dried in electric furnace at 300 ^{o}C for 6 hours and were kept in dessicator before used.

Distilled water used in this study was obtained from all glass distillation apparatus.

3.4 PREPARATION OF STANDARD SOLUTIONS

3.4.1 The Single Component Standard Solution of PAHs in Methanol

The 1000.00 ppm single component standard stock solution of each PAH, i.e., anthracene, fluoranthene, fluorene, pyrene and internal standard in methanol was prepared by accurate weighing 0.0100 g of each standard, dissolving, and diluting it to the mark with methanol in 10.00 mL volumetric flask and mix thoroughly.

3.4.2 The Standard Mixture of PAHs in Methanol

A 1000.00 ppm standard mixture of each PAH, i.e., anthracene, fluoranthene, fluorene and pyrene in methanol was prepared by accurate weighing 0.0100 g of each standard, dissolving, and diluting it to the mark with methanol in 10.00 mL volumetric flask and mix thoroughly.

3.4.3 The Standard Solution of Internal Standard

The 1000.00 ppm standard stock solution of internal standard in each extracting solvent i.e., toluene, hexane, and methylene chloride were prepared by accurate weighing 0.0100 g of acenaphtylene and then dissolved and diluted it with the solvent to the mark in 10.00 mL volumetric flask and mixed thoroughly.

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

3.4.4. The 1.00 ppm Single Component Aqueous Standard Solution of PAHs

The 1.00 ppm single component aqueous standard solution of each PAH i.e., anthracene, fluoranthene, fluorene and pyrene was prepared by transfering 250.00 µL and 1.00 mL of the 1000.00 ppm single component standard solution of each PAH from 3.4.1 into 250.00 and 1000.00 mL volumetric flasks respectively and was then diluted them to the marks with distilled water and mixed thoroughly.

3.4.5 The 50.00 ppb Single Component Aqueous Standard Solution of PAHs

The 50.00 ppb single component aqueous standard solution of each PAH was prepared by transfering 12.50 and 50.00 µL of the 1000.00 ppm single component standard solution of each PAH from3.4.1 into 250.00 and 1000.00 mL volumetric flasks respectively and was then diluted them to the marks with distilled water and mixed thoroughly.

3.4.6 The 1.00 ppm Aqueous Standard Mixture of PAHs

The 1.00 ppm aqueous standard mixture of PAHs i.e., anthracene, fluoranthene, fluorene and pyrene was prepared by transfering 250.00 µL and 1.00 mL of the 1000.00 ppm standard mixture of each PAH from 3.4.2 into 250.00 and 1000.00 mL volumetric flasks respectively and was then diluted them to the marks with distilled water and mixed thoroughly.

3.4.7 The 50.00 ppb Aqueous Standard Mixture of PAHs

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ophotometer

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(Japan Spectroscopic, Tokyu,

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model HS 500 (Jankle&Kunkel,

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Spectra for each PAH

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3.5.2 The Effect of Shaking Time

The shaking time of each PAH in each extracting solvent at different sample to solvent ratios i.e., 10:1, and 50:1 was studied and the procedure was :

1. Pipet 1.00 mL of the extracting solvent which was prepared from 3.4.3 and 10.00 ,50.00 mL (for 10:1, 50:1 sample to solvent ratios extraction, respectively) of the 1.00 ppm single component aqueous standard solution of each PAH from 3.4.4 into a series of vials, sequentially.

2. Seal each vial with aluminum foil, black-rubber septum, and closed it tightly with the cap.

3. Shake the contents in the vials with mechanical shaker for 2, 5, 10,..., 30 min with a speed of 200 Hub/min and then allow them to stand until the two phases were completely separated.

4. Remove 0.3 mL of the extract from the vial by pasteur pipette and transfer it to another vial.

5. Blow the extract gently with nitrogen gas until it was nearly dried.

6. The remaining extract was finally dissolved in

0.2 mL of acetonitrile and then 30.00 μL of the solution was injected into HPLC.

7. Determine the concentration of interested component by means of the internal standard method at the wavelength of 254.0 nm.

8. Calculate the percentage of recovery (% E) of each PAH at various shaking time and plot the graph of % E vs. shaking time. The equilibration time of each sample to solvent ratio can be determined from the graphs and these time would be used as the equilibration time in the next study.

3.5.3 Microextraction

The investigation of sample to solvent ratios i.e., 10:1 and 50:1 and the effect of adding salts i.e., sodium chloride and anhydrous sodium sulfate on % recovery of each PAH were studied. Therefore, each extraction consisted of three systems, the condition of three systems were described below.

1. Non salting out (no salt)

2. 2.00 and 5.00 g of sodium chloride were used for 10:1 and 50:1 sample to solvent ratio microextraction, respectively

3. 2.00 and 5.00 g of anhydrous sodium sulfate were used for 10:1 and 50:1 sample to solvent ratios microextraction, respectively.

In each system, two concentrations of standard solutions of PAH, i.e., 1.00 ppm and 50.00 ppb of the single component aqueous standard solution and aqueous standard mixture were studied. The procedure for the study of microextraction was :

1. Add the solid salt, pipet 1.00 mL of the extracting solvent which was prepared from 3.4.3 and pipet 10.00 and 50.00 mL of the aqueous standard solution of each PAH ,for 10:1 and 50:1 sample to solvent ratios extraction respectively ,from 3.4.4, 3.4.5, 3.4.6 and 3.4.7 into a series of vials, sequentially.

2. Seal each vial with aluminum foil, black-rubber septum, and closed it tightly with the cap.

3. Shake the contents in the vials with mechanical shaker with a speed of 200 Hub/min for the equilibration

time as found in 3.5.2 and then allow it to stand until the two phases were completely separated.

4. Remove 0.3 mL of the extract from the vial by pasteur pipette and transfer it to another vial.

5. Blow the extract gently with nitrogen gas until it was nearly dried.

6. The remaining extract was finally dissolved in 0.2 mL of acetonitrile and then 30.00 µL of the solution was injected into HPLC.

7. Determine the concentration of interested component by means of the internal standard method at the wavelength of 254.0 nm. For single component aqueous standard solution, the detection would also be performed at the optimum wavelength of each PAH as found in 3.5.1.

8. Calculate the percentage of recovery (% E) of each PAH.

3.6 HPLC OPERATING CONDITION

3.6.1 The Study of Single Component Solution

ANALYTICAL COLUMN : Lichrosphere RP-18 column

(250x4.6 mm., particle size 5 μm.) protected by a guard column.

MOBILE PHASE : 75 % actonitrile in water (isocratic)

FLOW RATE : 1.00 mL/min

PRESSURE : 1200 psi

TEMPERATURE : room temperature

INJECTOR : 20 μL sample loop

DETECTOR : UV detector , at 254.0 nm and at optimum wavelength of each PAH (260.4, 254.0, 286.4 and 272.4 nm for fluorene, anthracene, fluoranthene and pyrene, respectively)

3.6.2 The Study of Mixture

ANALYTICAL COLUMN : Lichrosphere RP-18 column (250x4.6 mm., particle size 5 μm.) protected by a guard column.

MOBILE PHASE : 75 % actonitrile in water (isocratic)

FLOW RATE : 1.00 mL/min

PRESSURE : 1200 psi

TEMPERATURE : room temperature

INJECTOR : 20 μL sample loop

DETECTOR : UV detector, at 254.0 nm

3.7 Internal Standardization Method

The internal standardization method is a relative calibration method which is used for determining the weight of the interested component in the unknown sample. The preparation of several standard solutions containing known weight of interested component and an internal standard is performed and chromatographed. A plot of weight ratio as abscissa and peak area (or peak height) ratio as ordinate is obtained to be linear for a particular system. The slope of this plot means the response factor, F, from the following equation :

$$\text{Slope} = \frac{\text{peak area ratio}}{\text{weight ratio}} = F$$

$$= \frac{A_c/A_i}{W_i/W_c}$$

where A_c is the peak area of the interested

component.

W_c is the weight of the interested component.

A_i is the peak area of internal standard.

W_i is the weight of internal standard.

After preparing the calibration curve of each PAH as can be seen from Figures 3.1-3.4, a known weight of the internal standard is added into the known weight of sample in order to determine the amount of the interested component in the sample. This mixture is then chromatographed and the peak area ratio of component c to i is measured. The weight of interested component in the unknown can be calculated by using the following equation :

$$W_c = \frac{A_c x W_i x F}{A_i}$$

and the percent of interested component in unknown is,

$$\% C = \frac{W_c x 100}{\text{sample weight}}$$

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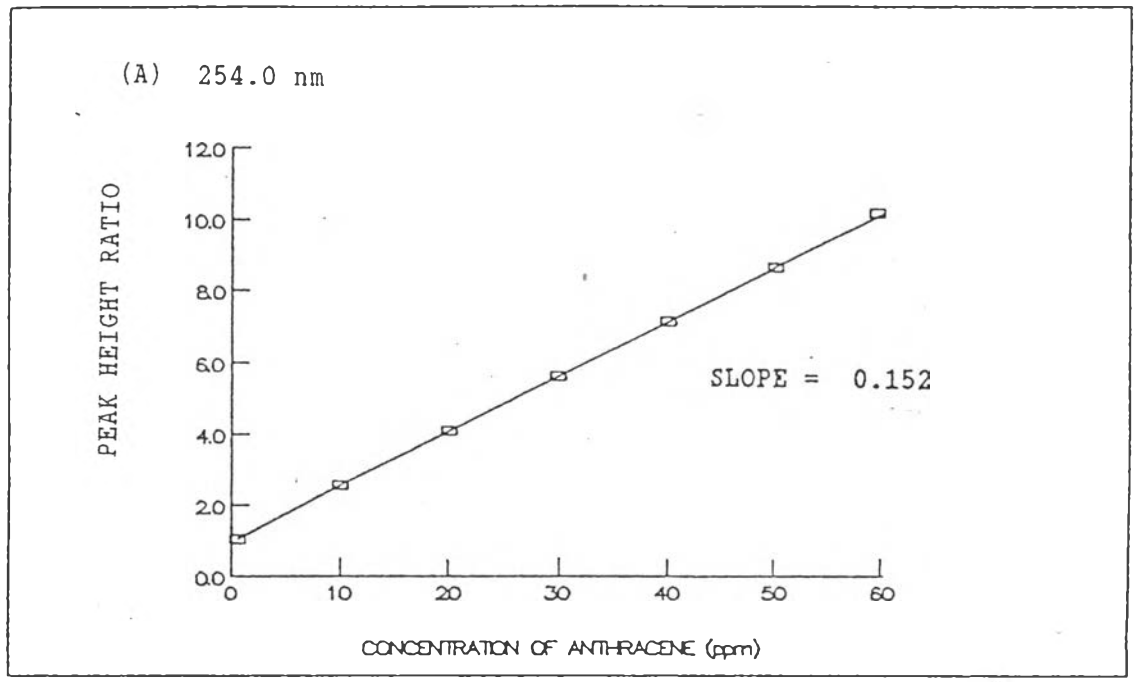


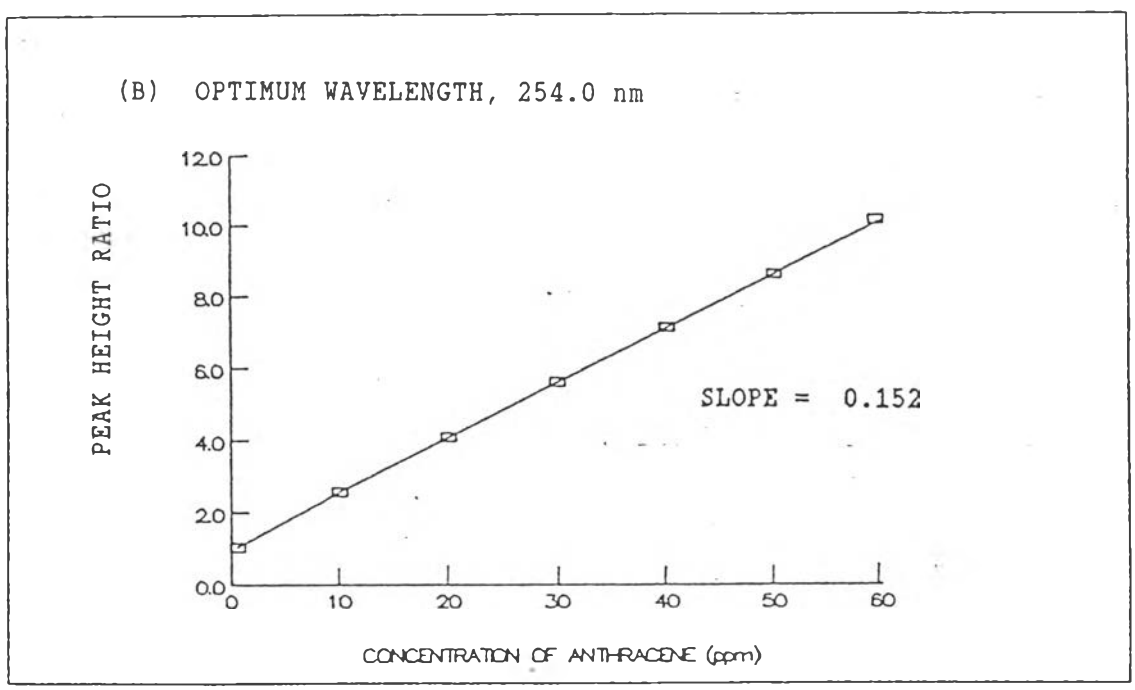
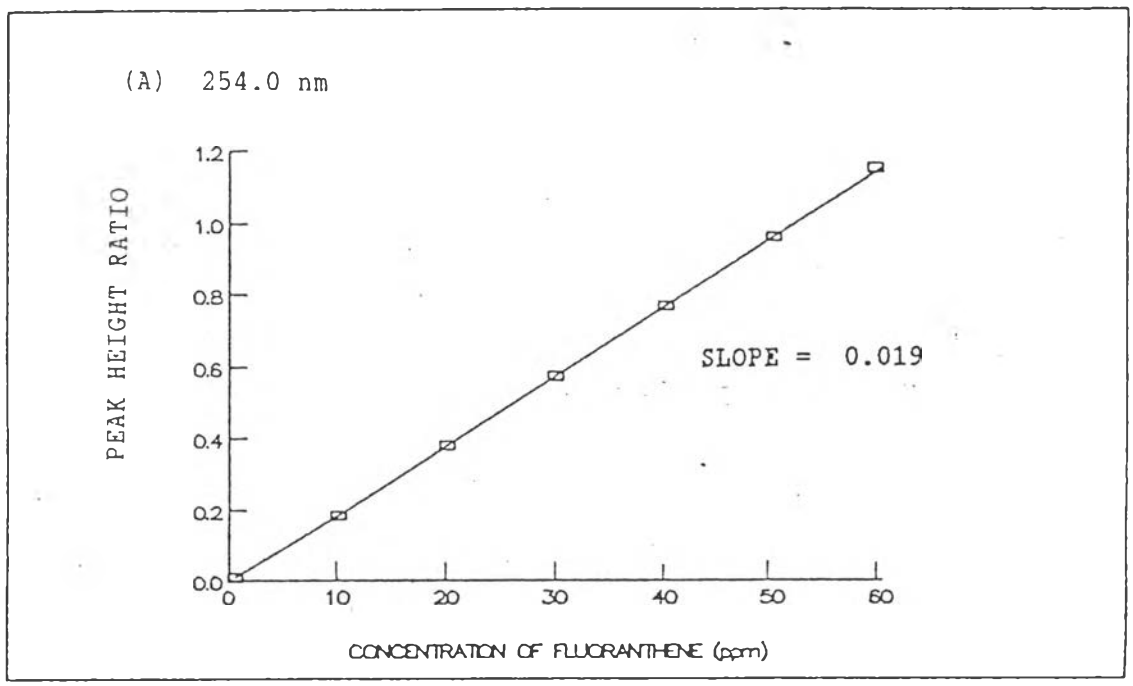


Figure 3.2 The calibration curve of anthracene in methanol



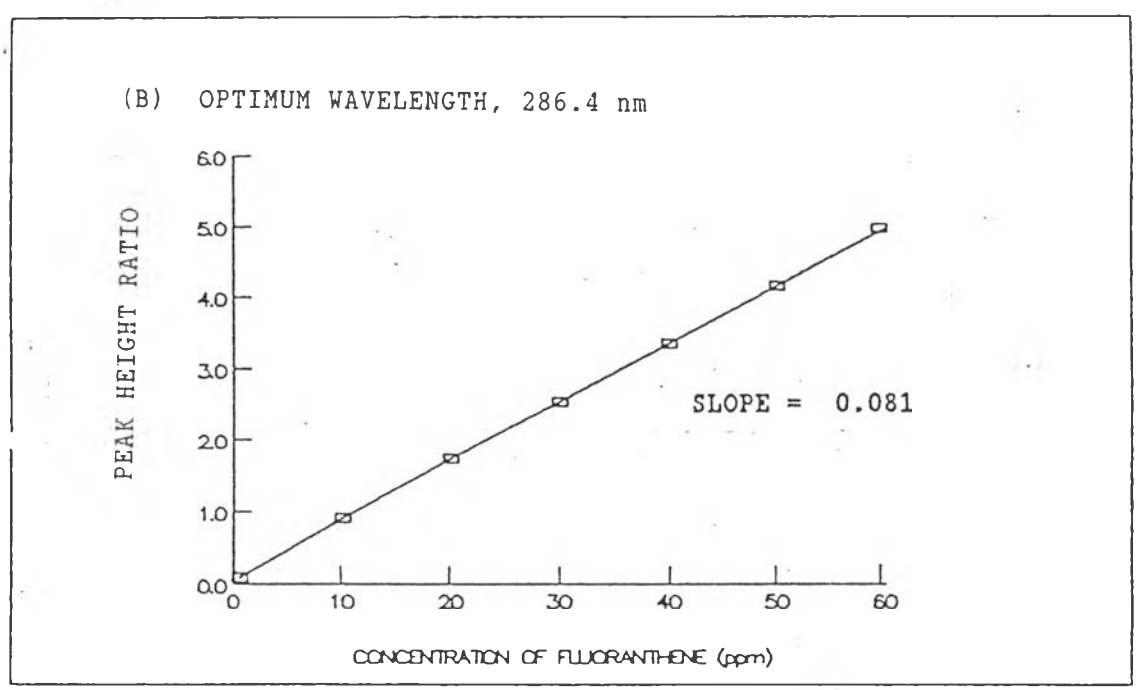


Figure 3.3 The calibration curve of fluoranthene in methanol

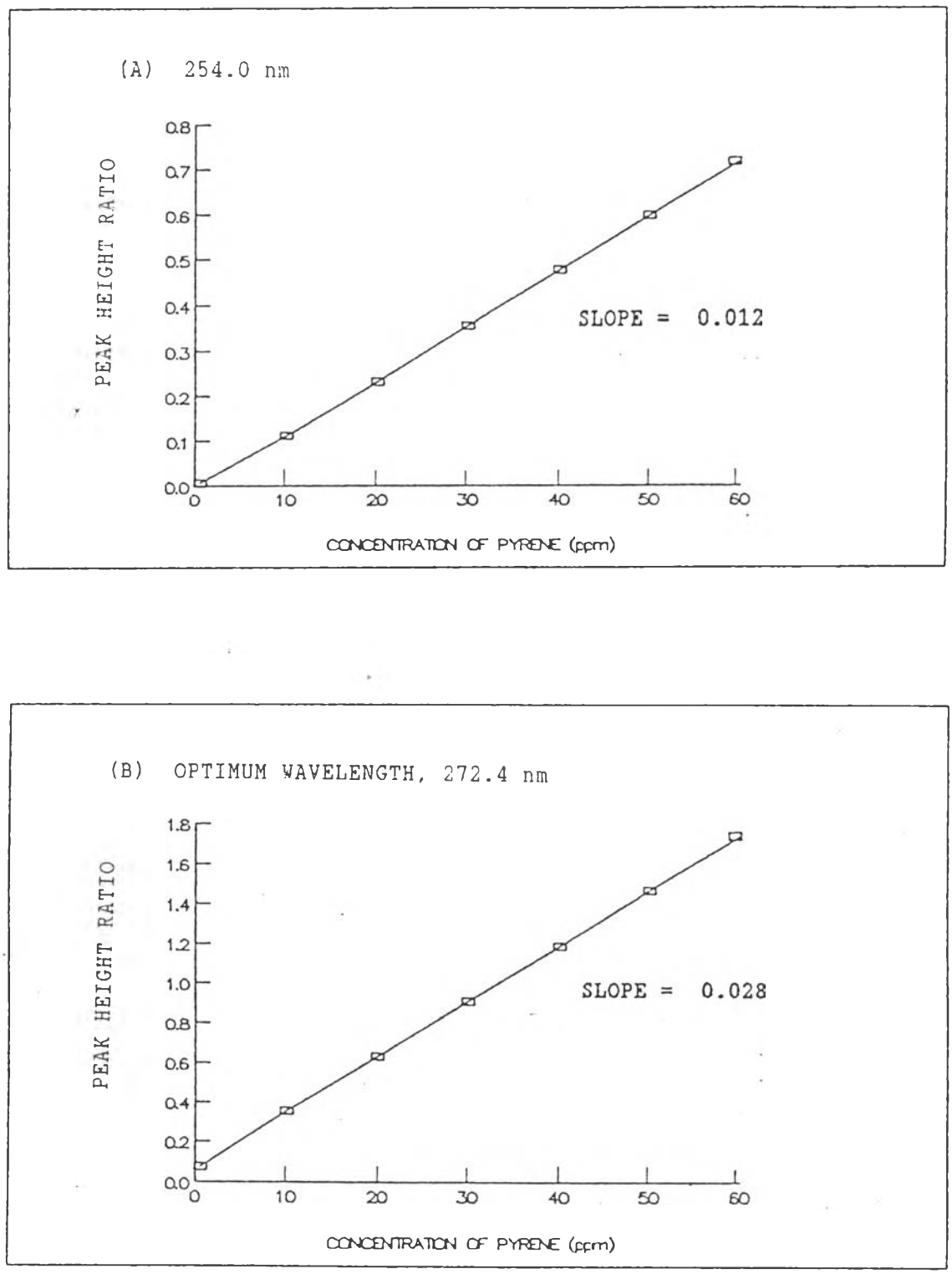


Figure 3.4 The calibration curve of pyrene in methanol

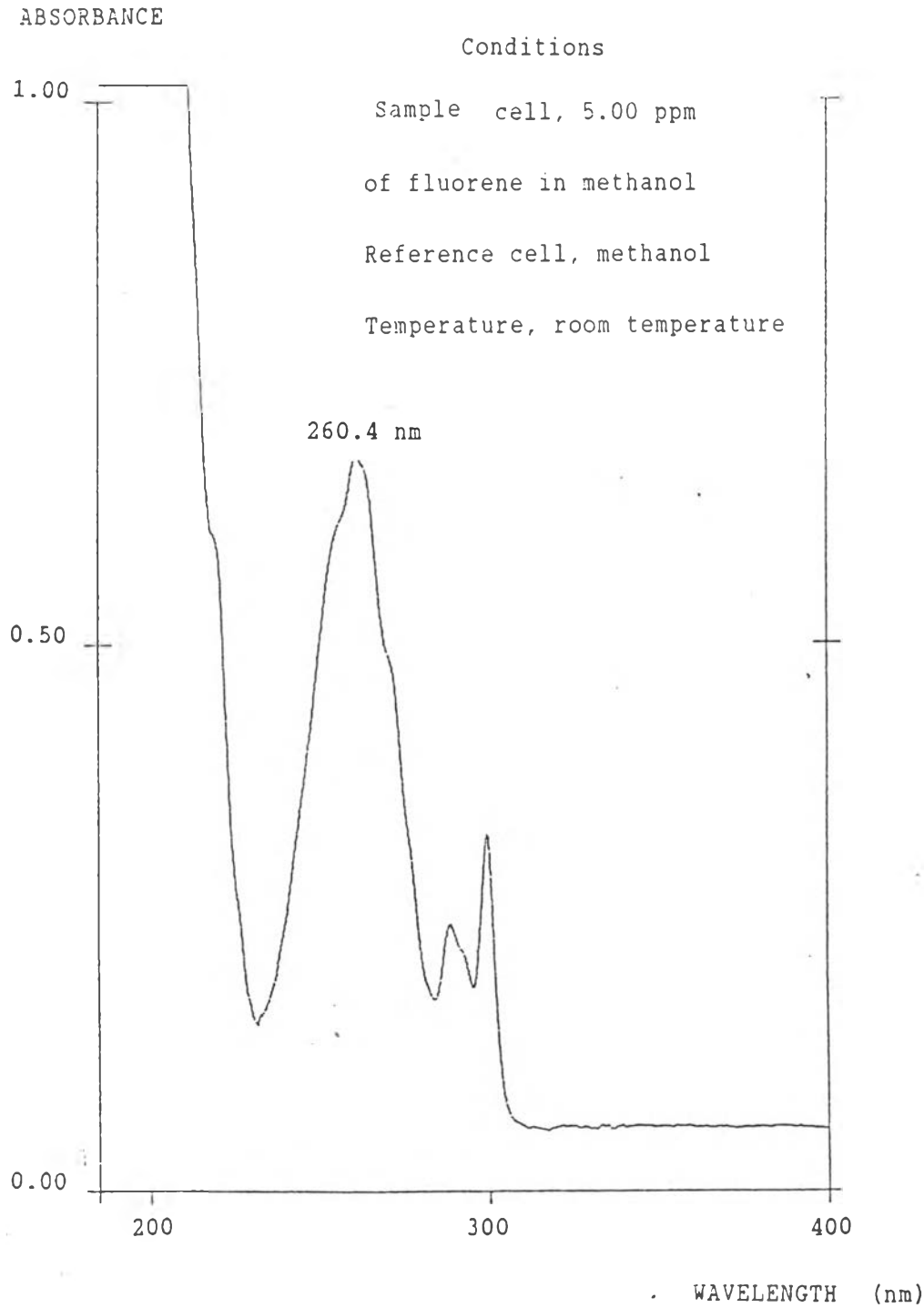


Figure 3.5 UV absorption spectra of fluorene in methanol

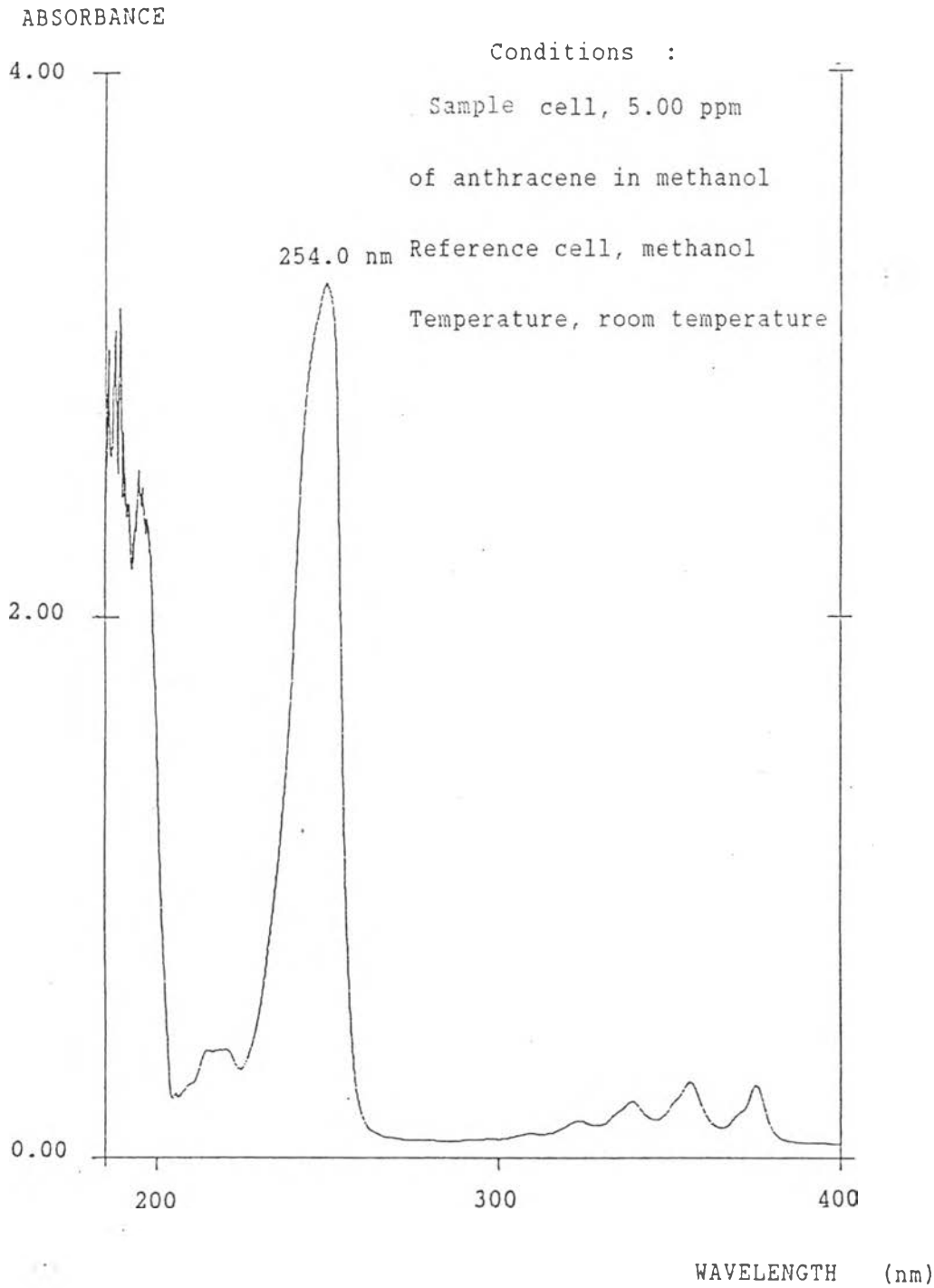


Figure 3.6 UV absorption spectra of anthracene in methanol

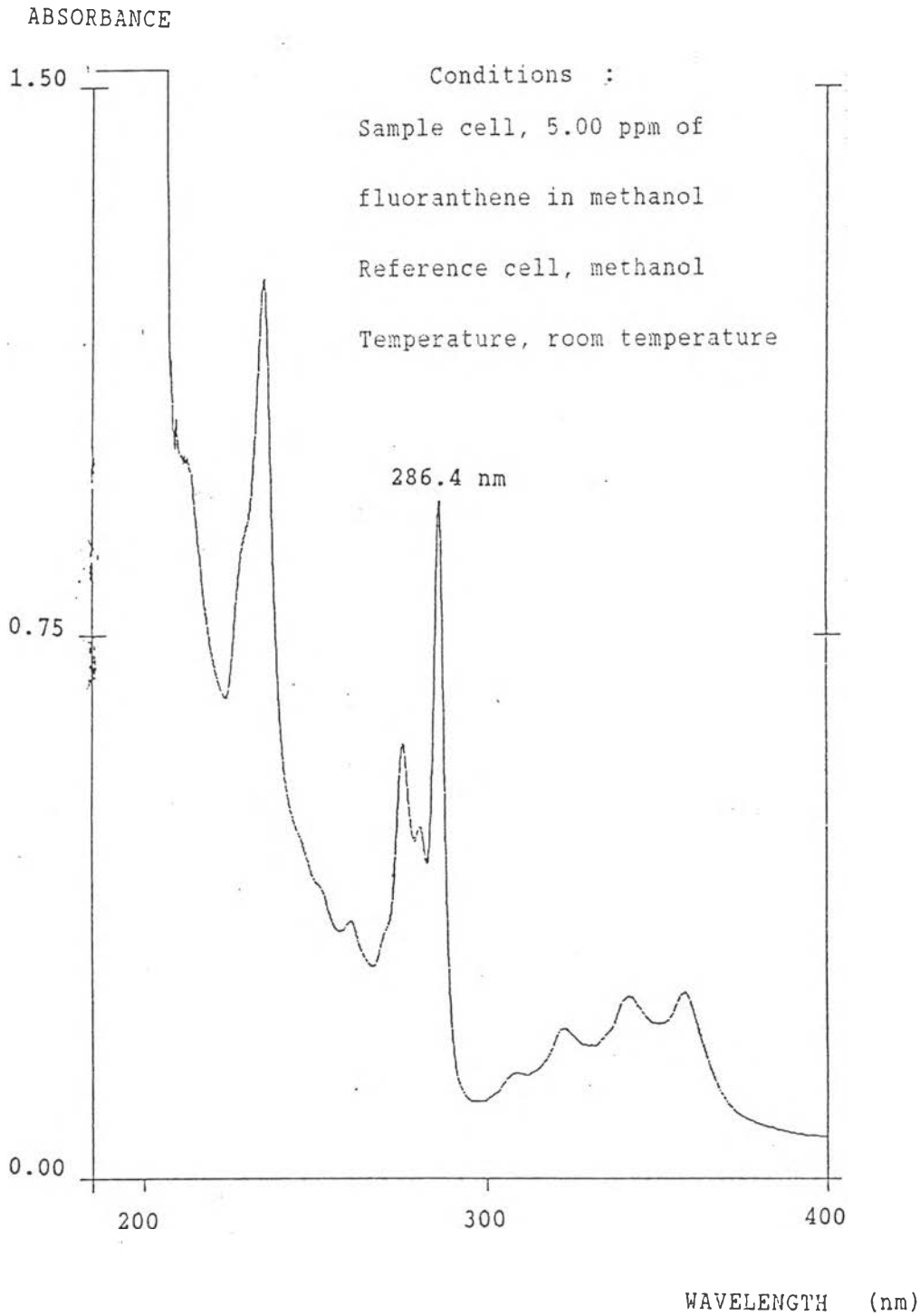


Figure 3.7 UV absorption spectra of fluoranthene in methanol

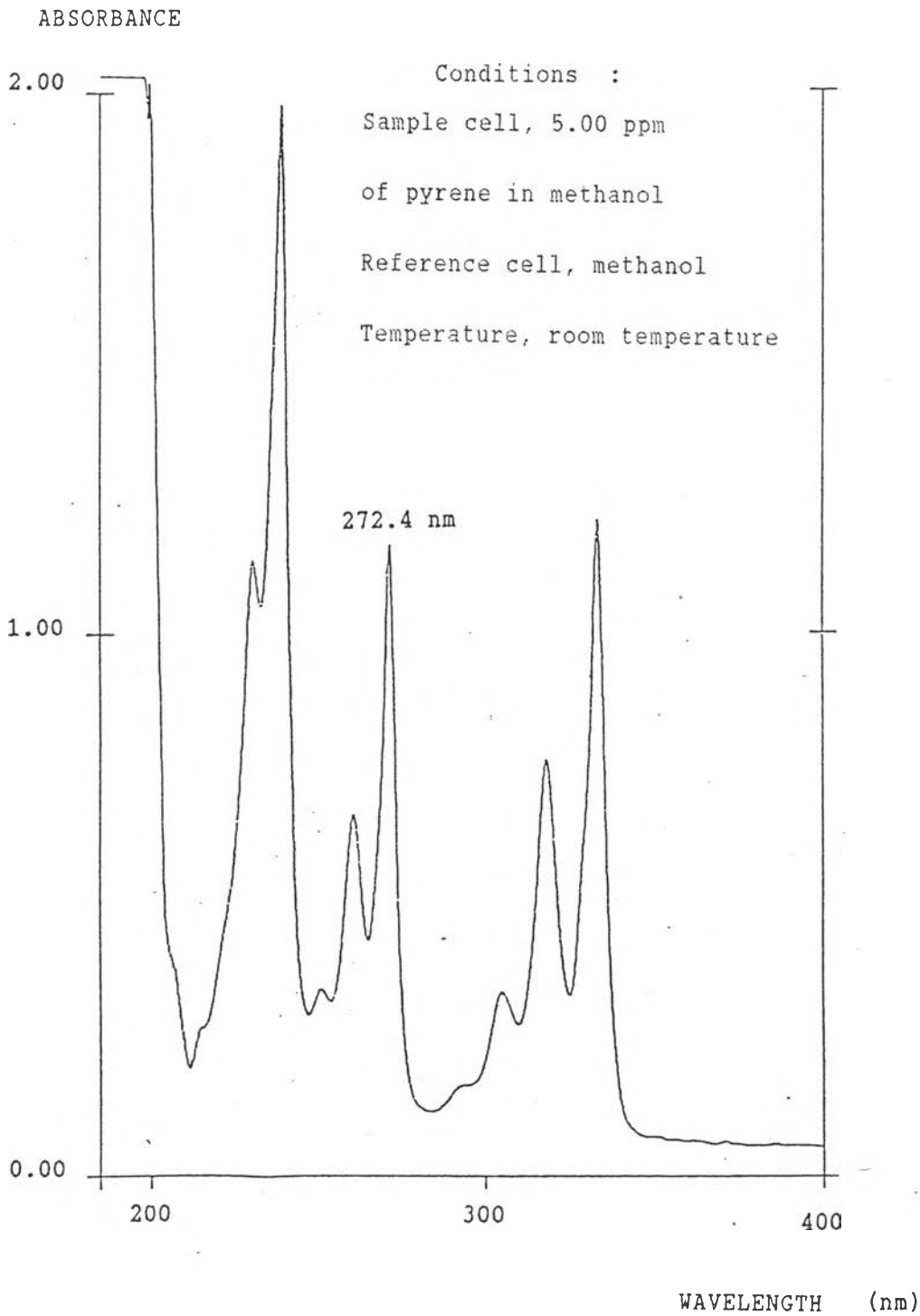


Figure 3.8 UV absorption spectra of pyrene in methanol

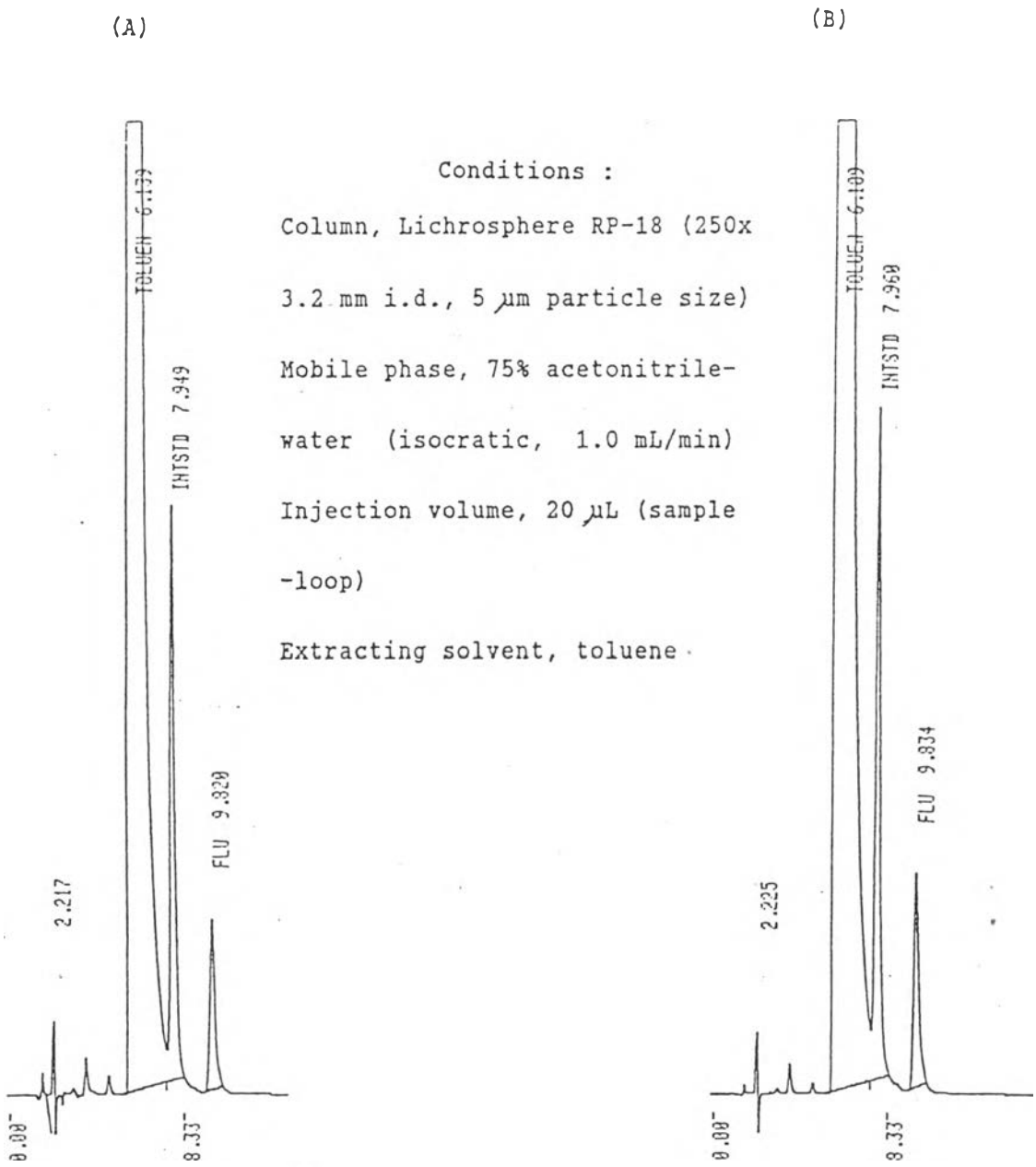


Figure 3.9 Liquid chromatograms of microextractions of fluorene with UV absorbance detection at 254.0 nm (A) and at its optimum wavelength, 260.4 nm (B)

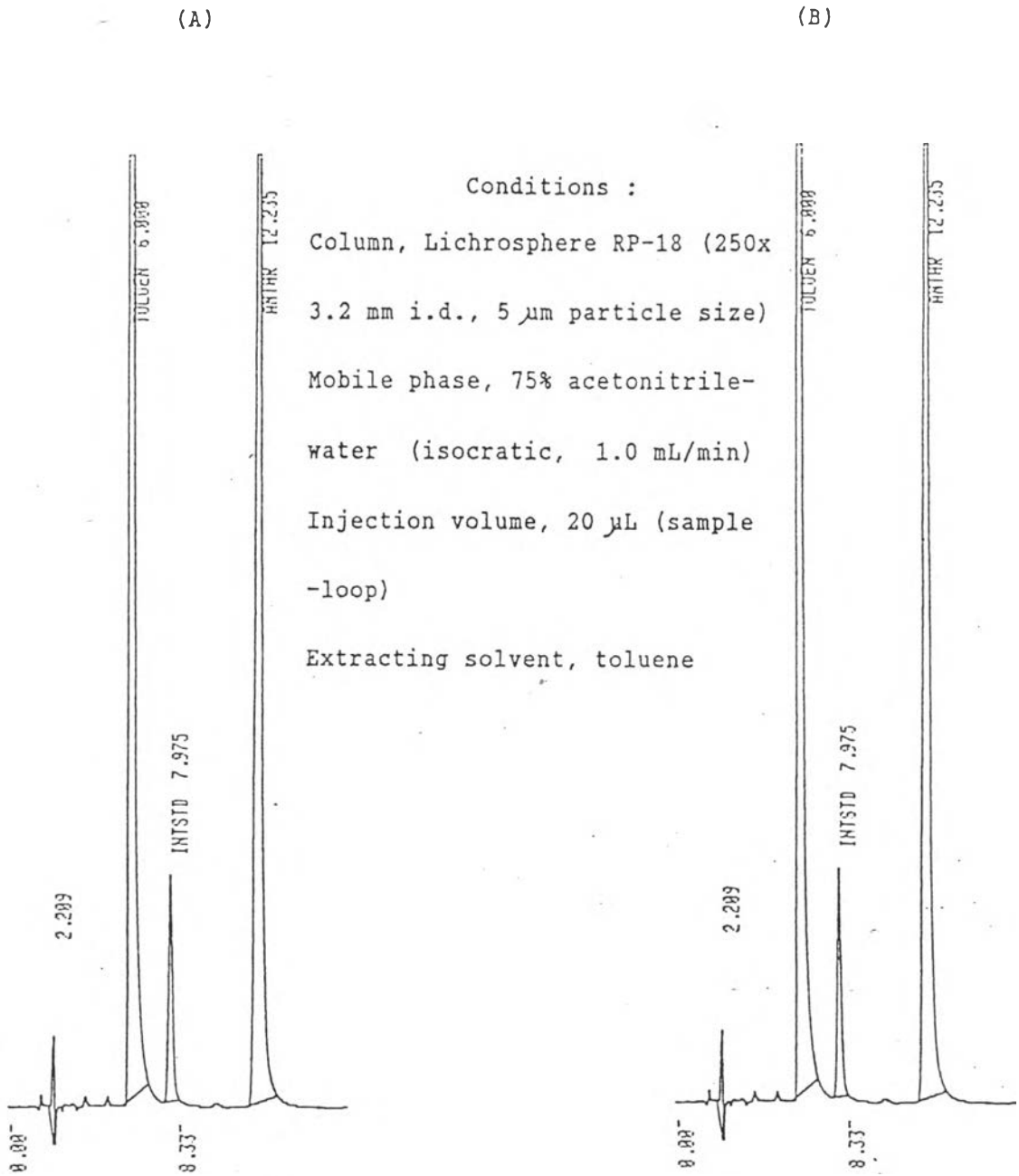
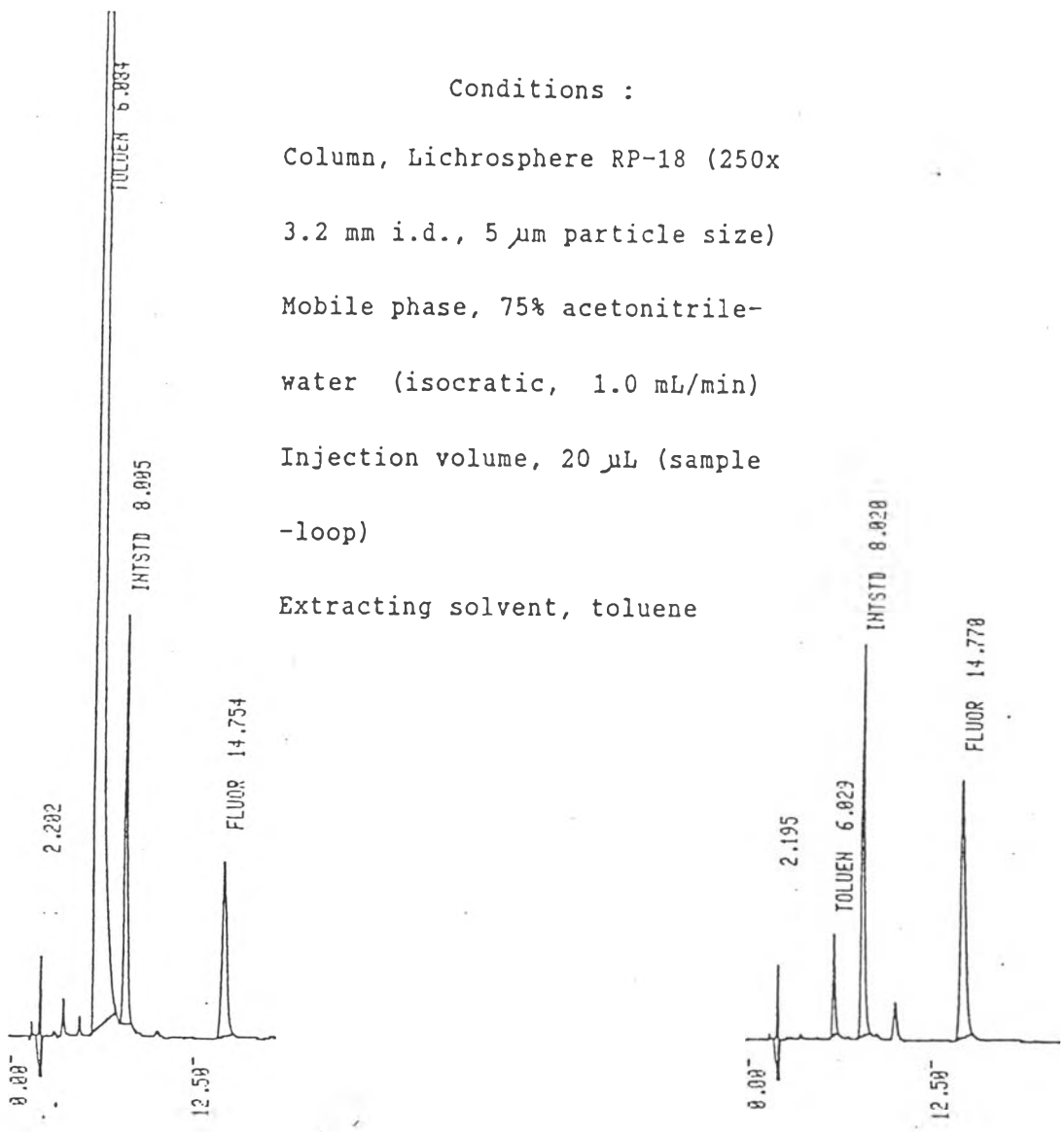


Figure 3.10 Liquid chromatograms of microextractions of anthracene with UV absorbance detection at 254.0 nm (A) and at its optimum wavelength, 254.0 nm (B)

(A)

(B)



Conditions :

Column, Lichrosphere RP-18 (250x 3.2 mm i.d., 5 μm particle size)

Mobile phase, 75% acetonitrile-water (isocratic, 1.0 mL/min)

Injection volume, 20 μL (sample -loop)

Extracting solvent, toluene

Figure 3.11 Liquid chromatograms of microextractions of fluoranthene with UV absorbance detection at 254.0 nm (A) and at its optimum wavelength, 286.4 nm (B)

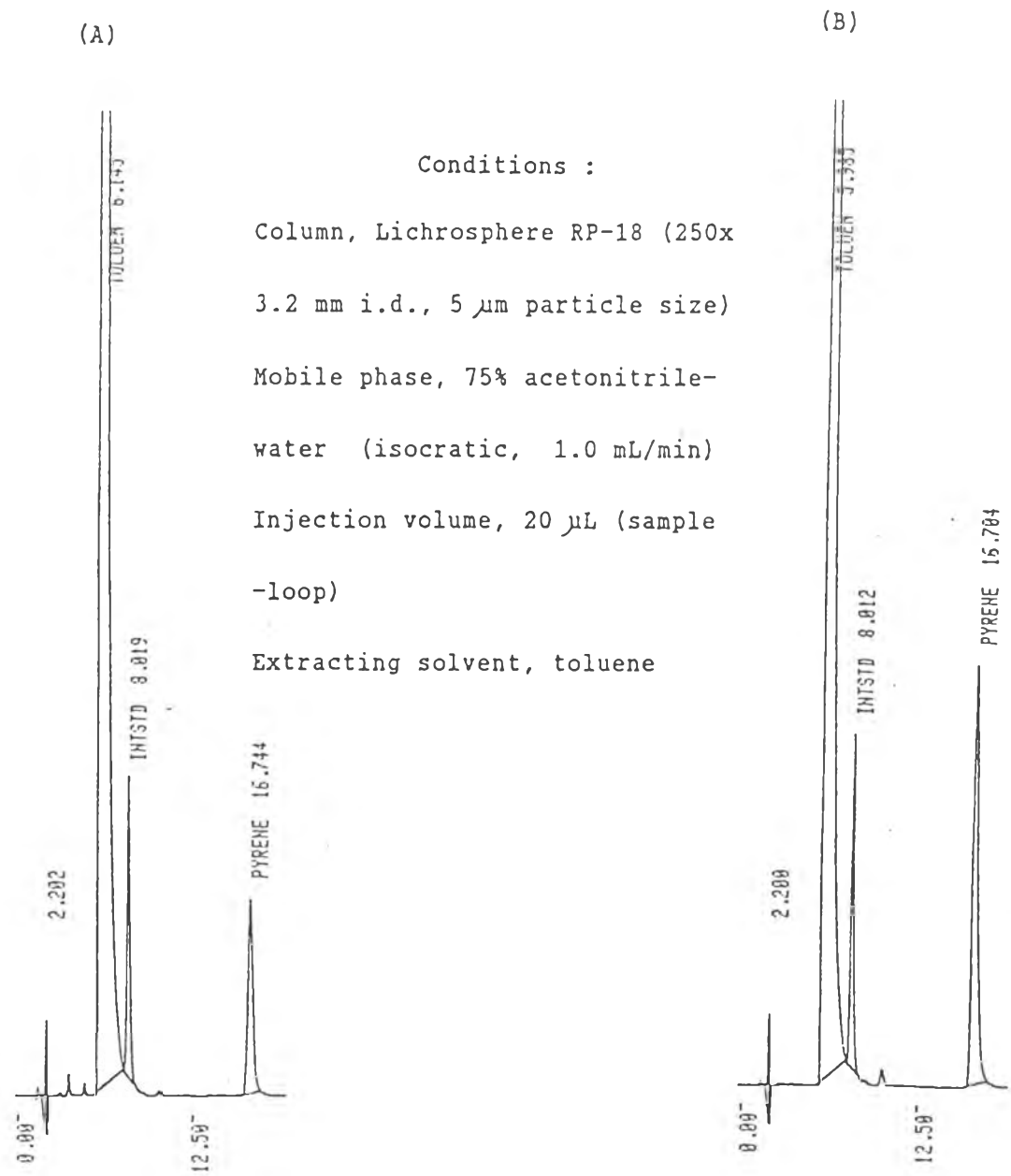
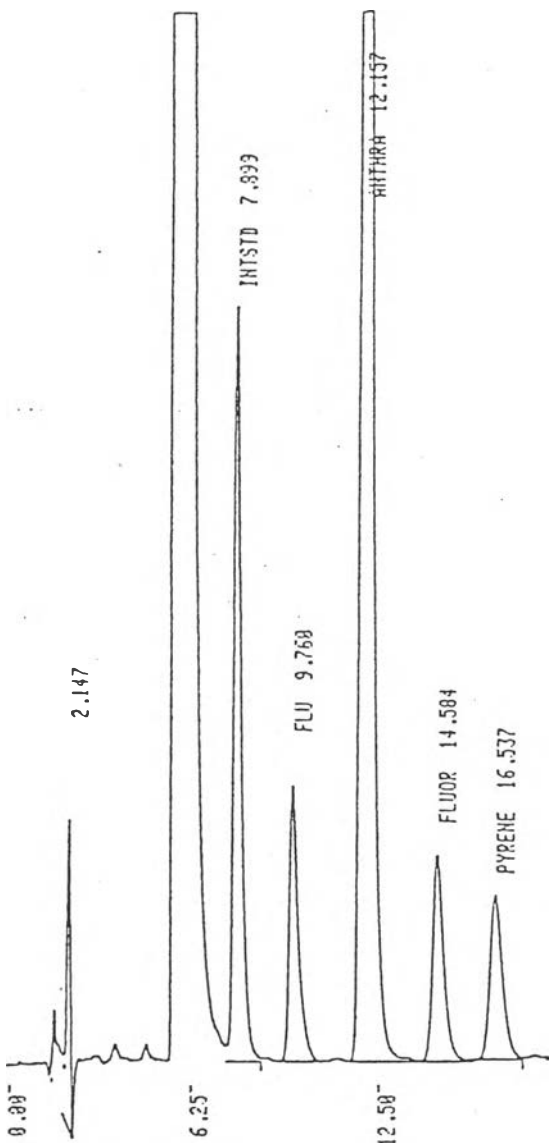


Figure 3.12 Liquid chromatograms of microextractions of pyrene with UV absorbance detection at 254.0 nm (A) and at its optimum wavelength, 272.4 nm (B)



Conditions :

Column, Lichrosphere RP-18 (250x 3.2 mm i.d., 5 μm particle size)

Mobile phase, 75% acetonitrile-water (isocratic, 1.0 mL/min)

Injection volume, 20 μL (sample -loop)

Extracting solvent, toluene

Figure 3.13 Liquid chromatogram of microextractions of a standard mixture of PAHs with UV absorbance detection at 254.0 nm