CHAPTER 3

EXPERIMENT

3.1 APPARATUS

Gas chromatograph , Hewlett Packard model 5890A equipped with FID , and Hewlett Packard integrator model 3393A.

Chromatographic column , 1.8 m x 3 mm id glass column packed with 1 % SP-1240DA on Supelcoport 100/120.

Mechanical shaker , Kika - Work ,Janke & Kunket HS-500.

Ultrasonic cleaner (Branson).

Microsyringes 10.00 , 25.00 , 50.00 , 200.00 µL (Hamilton).

Vials 3 and 8 drams.

Volumetric flasks 10.00 ,100.00 , 250.00 mL.

Pipettes 1.0 , 2.0 , 5.0 , 10.0 mL.

All glasswares were cleaned with detergent, water , dil. HNO_3 (1:1) , water , rinsed with double distilled water , respectively and dried at 120 ^{o} C overnight.

3.2 CHEMICALS

3.2.1 Standard of Phenolic Compounds

Phenol , 2 - nitrophenol , 2 ,4 ,6 -

trichlorophenol and 4-chloro-3-cresol were purchased from Fluka A.G., Switzerland.

2,4-Dichlorophenol was supplied by Kanto Chemical Co. Inc., Tokyo, Japan.

4,6-Dinitro-2-cresol was obtained from Merck ,Darmstadt , Germany.

3.2.2 Organic Solvents

All organic solvents including methylene chloride , carbon disulfide , hexane , and 2-propanol were purchased from J.T. Baker Chemicals Co., Deventer, Holland and were a glass-distilled before use.

3.2.3 Reagents

Nitric acid and sulfuric acid were analytical reagent grade and were perchased from Merck , Darmstadt , Germany.

Sodium chloride and anhydrous sodium sulfate which were analytical grade were obtained from J.T. Baker Chemicals Co., Deventer, Holland. The salts were heated in an oven at 220 ^{o} C overnight and were kept in dessicator prior to use.

3.3 PREPARATION OF STANDARD SOLUTIONS

3.3.1 The Stock Standard Solutions of Phenolic Compounds in 2-Propanol.

The 1000.00 ppm stock solutions of each

phenolic compounds , i.e., phenol , 2-nitrophenol , 2,4-dichlorophenol , 2,4,6-trichlorophenol , and 4-chloro-3-cresol were prepared by weighing 0.1000 g of each standard , dissolving and diluting it to the mark with 2-propanol in 100.00 mL volumetric flask. The 1.00 ppm , 10.00 ppm of phenolic compounds solutions were prepared by transferring 2.50 mL and 0.25 mL of the 1000.00 ppm stock standard solution into 250.00 mL volumetric flasks and then diluting it to the mark with double distilled water.

3.3.2 The Stock Standard Solutions of Phenolic Compounds in Organic Solvents.

The 1000.00, 600.00, and 200.00 ppm standard solutions of each phenolic compound , i. e. , phenol , 2-nitrophenol , 2,4-dichlorophenol , 2,4,6-trichlorophenol , 4-chloro-3-cresol in each organic solvent including methylene chloride , carbon disulfide and hexane were prepared by dissolving 0.0100 , 0.0060 , and 0.0020 g of each standard and diluting it to the mark with the organic solvent in 10.00 mL volumetric flasks.

3.3.3 The Internal Standard Solutions in Organic Solvents.

The 1000.00 , 600.00 , and 200.00 ppm internal standard solutions in each organic solvent,

i.e., methylene chloride , carbon disulfide ,and hexane were prepared by weighing 0.0100 , 0.0060 , and 0.0020 g of each internal standard , dissolving and diluting it to the mark with the organic solvent in 10.00 mL volumetric flasks.

The internal standard used for the quantification of the single component solutions ,i.e., 2-nitrophenol , phenol , 2,4-dichlorophenol , 2,4,6-trichlorophenol , and 4-chloro-3-cresol was phenol, 2,4-dichlorophenol , 2,4,6-trichlorophenol , 4-chloro-3-cresol , and 4,6-dinitro-2-cresol , respectively. In addition , the internal standard used for the determination of each component in the mixture solutions was 4,6-dinitro-2-cresol.

3.4 THE VARIOUS EFFECTS ON % RECOVERY OF MICROEXTRACTION.

The investigation of various effects ,i.e., pH , and shaking time for each organic solvent including methylene chloride , carbon disulfide , and hexane for sample-to-solvent ratio 9:1 at the concentration 10.00 ppm on the percent recovery was described as follows :

3.4.1 Study on the pH

1. Prepare the 10.00 ppm standard in aqueous solutions at the pH 1, 2, 3, 4, 5, 6, 7, 8, 9.

2. Pipet 1.00 mL of the extracting organic solvent into a series of vials.

3. Transfer 9.00 mL of the prepared standard in aqueous solutions at various pHs into each vial.

4. Seal the vials with aluminium foils and close them with the caps.

5. Shaking the contents in the vials by mechanical shaker with the speed of 200 Hub/min for 30 min.

6. Allow them to stand until two phases were completely separated.

7. Remove 500.00 µL of the extract and transfer it to another vial.

8. Add 40.00 µL of the internal standard solution into the vial and then injected it into the gas chromatograph.

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

10. Plot %E against pH.

3.4.2 Study on the Shaking Time

1. Pipet 1.00 mL of the extracting organic solvent into a series of vials.

2. Pipet 9.00 mL of the 10.00 ppm standard in aqueous solution at the optimum pH as found

in section 3.4.1 into each vial.

3. Seal the vials with aluminium foils , and close them with the caps.

4. Shaking the contents in the vials by mechanical shaker for 5, 10, 15, 20, 25 and 30 min.

5. Allow them to stand until two phases were completely separated.

7. Remove 500.00 µL of the extract and transfer it into another vial.

8. Add 40.00 µl of internal standard solution into the vial and then injected it into the gas chromatograph.

9. Determine the concentration of the interested compound in the extract by means of the internal standardization method, then calculated %E of each component at various shaking times.

10. Plot %E against shaking time.

3.4.3 Study on the Salting Out

In order to enhance the percent recovery of phenolic compounds , the extraction with various solvents was carried out by salting out an aqueous standard solution with sodium chloride and anhydrous sodium sulfate at the concentration of 1.00 and 10.00 ppm levels and the results obtained from the studies were compared with non salting out for each solvent , i.e., methylene chloride, carbon disulfide , and hexane and for each sample-to-solvent ratio ,i.e., 9:1 , 5:5 ,

2:8 and the procedure was :

1. Add 2.0 g of salt , i.e., NaCl and anh. Na_2SO_4 into a series of vials.

2. Pipet the extracting organic solvent into three different vials ,i.e., vials with NaCl, anh. Na_2SO_4 , and no salt.

3. Pipet the standard in aqueous solution of pH as found in the section 3.4.1 into each vial.

4. Seal the vials with aluminium foils and close them with the caps.

5. Shaking the contents in the vials by mechanical shaker with the speed of 200 Hub/min for the time as found in section 3.4.2.

6. Allow them to stand until two phases were completely separated.

7. Remove 500.00 µL of the extract and trasfer it into another vial.

8. Add 40.00 µL of the internal standard solution into the vial and then injected it into the gas chromatograph.

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

10. Plot %E against salts used and no salt.

3.3.4 Study on the Sample-to-Solvent Ratios.

To increase the percent recovery of phenolic compounds , the sample-to-solvent ratios, including 9:1, 5:5, and 2:8 were studied for each organic solvent including methylene chloride , carbon disulfide , and hexane at two different concentrations, i.e., 1.00 and 10.00 ppm and the procedure can be described as follows :

1. Pipet 1.00, 5.00 and 8.00 mL of the extracting organic solvent into a series of vials.
2. Pipet 9.00, 5.00 and 2.00 mL of the standard in aqueous solution of pH as found in section 3.4.1 into each vial to make the sample-to-solvent ratios as specified above.
3. Seal the vials with aluminium foils, and close them with the caps.
4. Shaking the contents in the vials by mechanical shaker for the time as found in section 3.4.2.
5. Allow them to stand until two phases were completely separated.
6. Remove 500.00 µL of the extract and transfer it into another vial.
7. Add 40.00 µL of internal standard solution into the vial and then injected it into the gas chromatograph.
8. Determine the concentration of the

interested compound in the extract by using the internal standardization method , then calculated %E of each interested compound at various sample-to-solvent ratios.

9. Plot %E against sample-to-solvent ratios.

3.5 INTERNAL STANDARDIZATION METHOD (1)

The method is known as the relative or the indirect calibration. An internal standard is chosen that are similar in analytical behavior to the interested component. The internal standard is not affected by method or matrix interferences.

To determine the amount of desired component in the sample , standard solution containing known weights or concentrations of the interested component and internal standard are prepared and chromatographed. The peak area ratios obtained from the chromatograms are plotted against the weight ratios or the concentrations to obtain a graph as shown in figure 3.1 ,the plot should be linear for a particular system. From the linear curve , the response factor ,RF, of the desired component is calculated from any of the standard solutions prepared and the following equation:

$$RF = \frac{\text{weight of desired component/weight of internal standard}}{\text{peak area of desired component/peak area of internal standar}}$$

$$RF = \frac{1}{\text{slope of the linear curve}}$$

The weight of the desired component , W_c , in the unknown can then be calculated by using equation :

$$W_c = A_c \times (W_i / A_i) \times RF$$

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

A_c is the peak area of the interested component in the sample.

RF is the response factor .

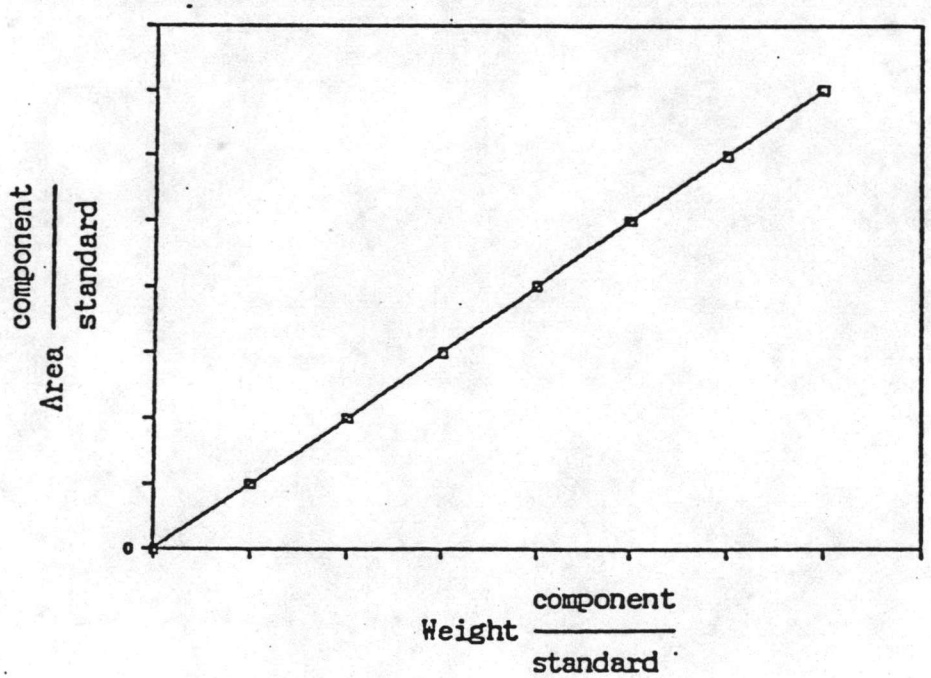


Figure 3.1 Relative calibration curve

3.6 The Calibration Curves of Each Phenolic Compound in Each Oganic Solvent.

The calibration curves of each phenolic compound , i.e. , phenol , 2-nitrophenol , 2,4-dichlorophenol , 2,4,6-trichlorophenol , and 4-chloro-3-cresol in each organic solvent including methylene chloride , carbon disulfide , and hexane were prepared from the standard solutions of phenolic compounds as mentioned below :

For studying the sample-to-solvent ratio of 9:1 , a series of standard solutions were prepared in a concentration range from 18.51-129.63 ppm by measuring 10.00-70.00 µL of 1000.00 ppm of stock standard solutions as prepared in section 3.3.2 into 500.00 µL of the extracting solvent and then 40.00 µL of 1000.00 ppm internal standard solution as prepared in section 3.3.3 was added into that content.

For studying the sample-to-solvent ratio of 5:5 , a series of standard solutions were prepared in a concentration range from 2.35-14.71 ppm by pipetting 4.00-25.00 µL of 600.00 ppm stock standard solution as prepared in section 3.3.2 into 1000.00 µL of the extracting solvent and then 20.00 µL of 600.00 ppm internal standard solution as prepared in section 3.3.3 was then added into that mixture.

For studying the sample-to-solvent ratio of

2:8, a series of standard solutions were prepared in a concentration range from 0.79-3.96 ppm by transferring 4.00-20.00 µL of 200.00 ppm stock standard solution as prepared in section 3.3.2 into 1000.00 µL of the extracting solvent and then 10.00 µL of 200.00 ppm internal standard solution as prepared in section 3.3.3 was added into that content.

The series of standard solutions in each organic solvent as prepared above were injected into a gas chromatograph , the peak area ratios obtained from the chromatograms were plotted against the concentrations of the standard phenolic compounds resulting the calibration curves of each phenolic compound in each organic solvent as can be seen in Figure 3.2-3.6.

3.7 GAS CHROMATOGRAPHIC CONDITIONS

3.7.1 Gas Chromatographic Conditions for Studying the Single Component Solution.

Standard : Phenol

Oven Temperature : 110 ^{o} C (Isothermal)

Injector and Detector Temperature: 250 ^{o}C

Detector : FID

Nitrogen Flow Rate : 30 mL/min

Air Flow Rate : 300 mL/min

Hydrogen Flow Rate : 50 mL/min

Standard : 2-Nitrophenol

Oven Temperature : 100 ^{o} C (Isothermal)

Injector and Detector Temperature: 250 ^{o}C

Detector : FID

Nitrogen Flow Rate : 30 mL/min

Air Flow Rate : 300 mL/min

Hydrogen Flow Rate : 50 mL/min

Standard : 2,4-Dichlorophenol

Oven Temperature : 125 ^{o} C (Isothermal)

Injector and Detector Temperature: 250 ^{o}C

Detector : FID

Nitrogen Flow Rate : 30 mL/min

Air Flow Rate : 300 mL/min

Hydrogen Flow Rate : 50 mL/min

Standard : 2,4,6-Trichlorophenol

Oven Temperature : 140 ^{o} C (Isothermal)

Injector and Detector Temperature: 250 ^{o}C

Detector : FID

Nitrogen Flow Rate : 30 mL/min

Air Flow Rate : 300 mL/min

Hydrogen Flow Rate : 50 mL/min

Standard : 4-Chloro-3-cresol

Oven Temperature : 150 ^{o} C (Isothermal)

Injector and Detector Temperature: 250 ^{o} C

Detector : FID

Nitrogen Flow Rate : 30 mL/min

Air Flow Rate : 300 mL/min

Hydrogen Flow Rate : 50 mL/min

3.7.2 Gas Chromatographic Conditions for Studying the Mixture Solution.

Oven Temperature : Temperature Programed, 2 min initial hold at 100 ^{o} C , then to 150 ^{o} C at rate 12 ^{o} C/min and hold until the last peak eluted.

Injector and Detector Temperature: 250 ^{o} C

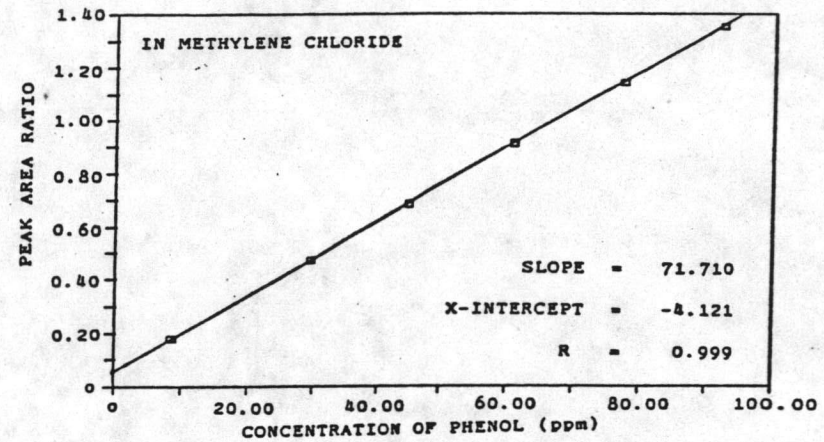
Detector : FID

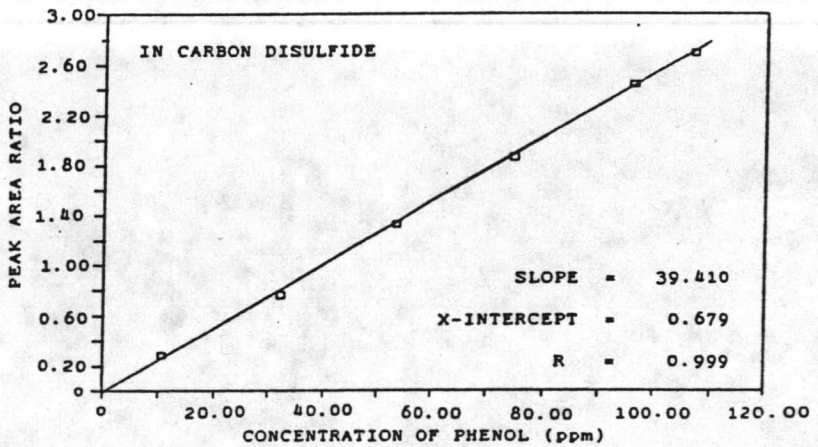
Nitrogen Flow Rate : 30 mL/min

Air Flow Rate : 300 mL/min

Hydrogen Flow Rate : 50 mL/min

The gas chromatograms of single phenolic compounds and mixture phenolic compounds in various organic solvents obtained from the chromatographic conditions as described earlier were shown in Figure 3.9 - 3.11 and Figure 3.12 - 3.14 , respectively.





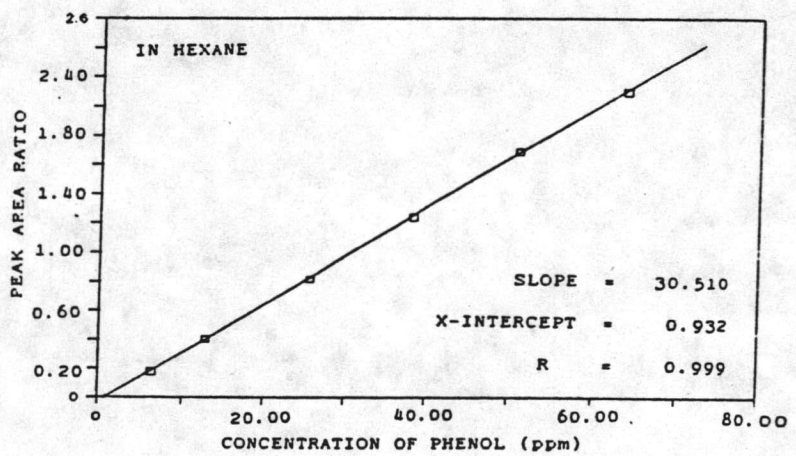


Figure 3.2 The calibration curves of phenol in the various organic solvents.

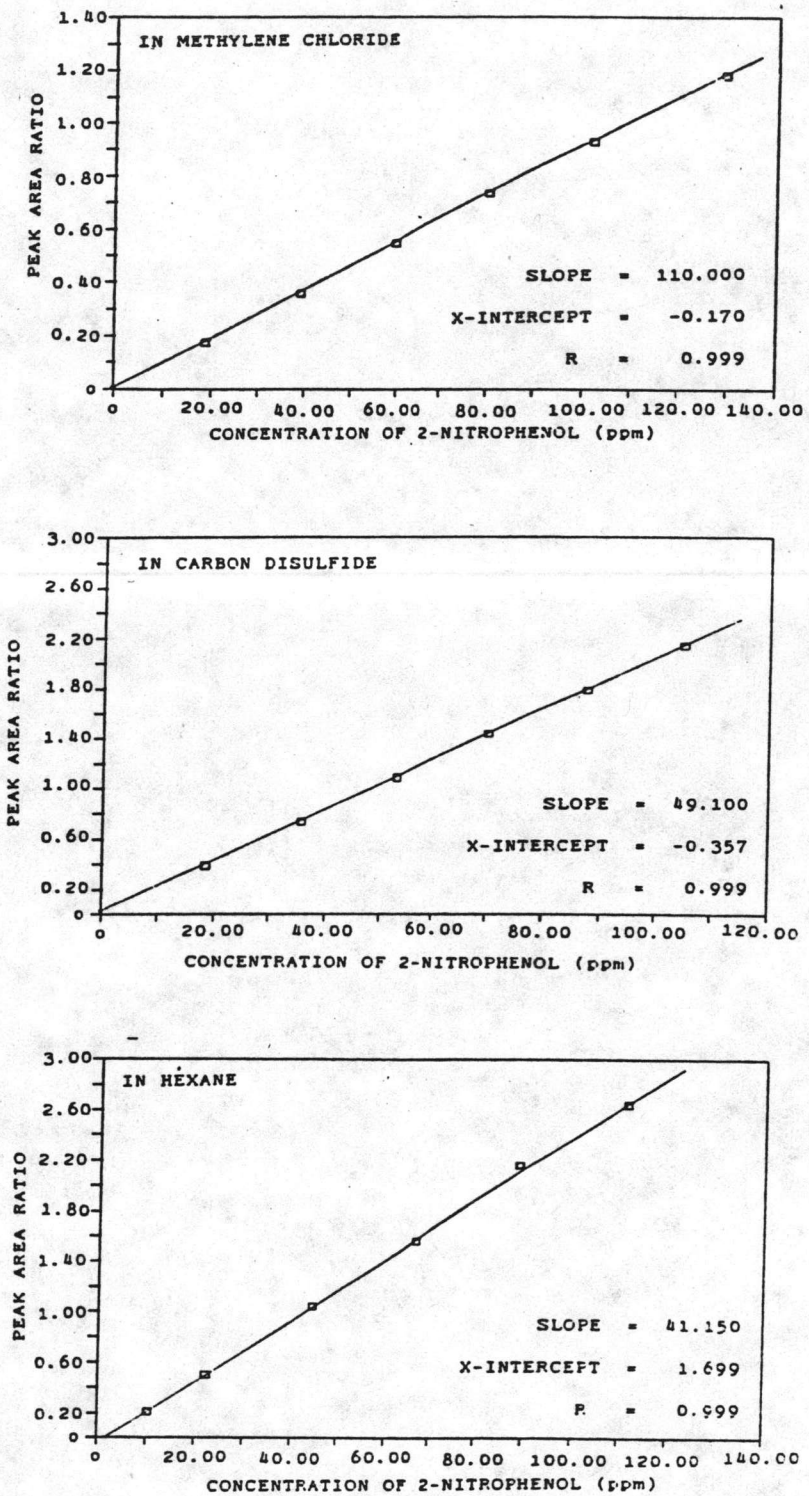
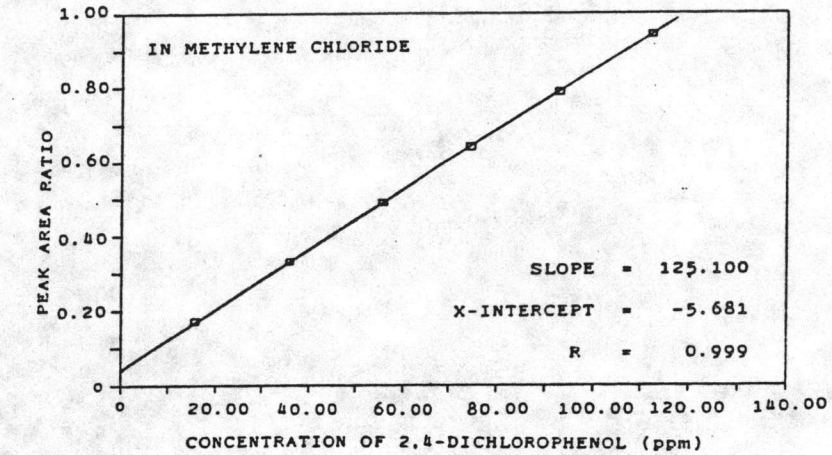
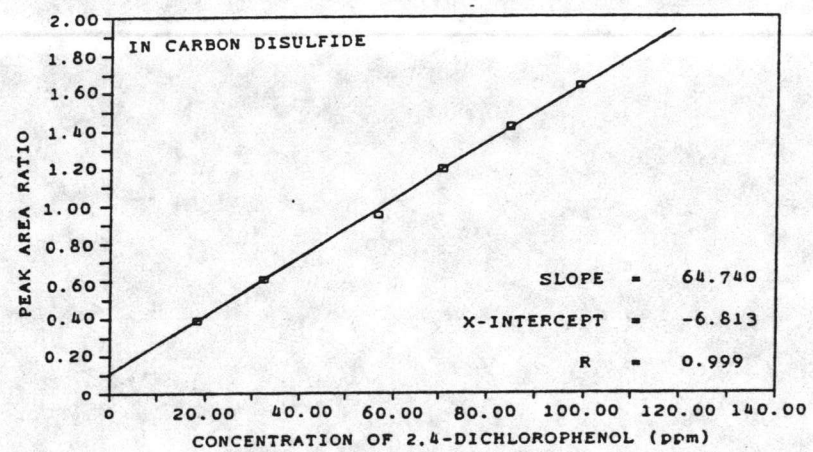


Figure 3.3 The calibration curves of 2-nitrophenol in the various organic solvents.





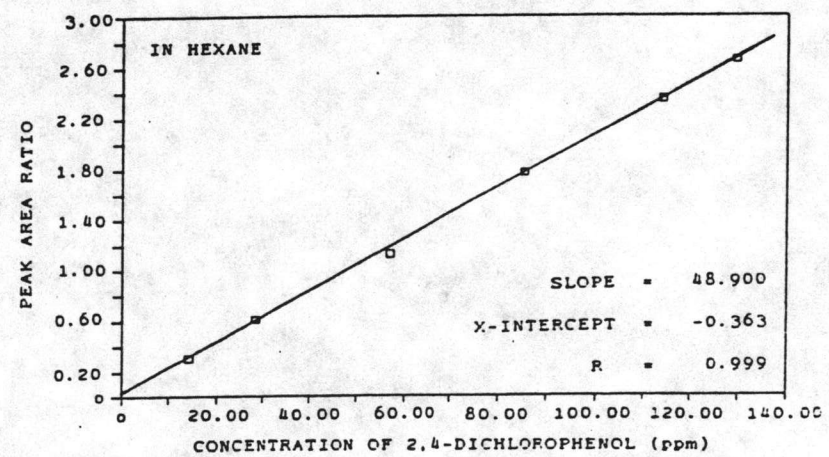


Figure 3.4 The calibration curves of 2,4-dichrolophenol in the various organic solvents.

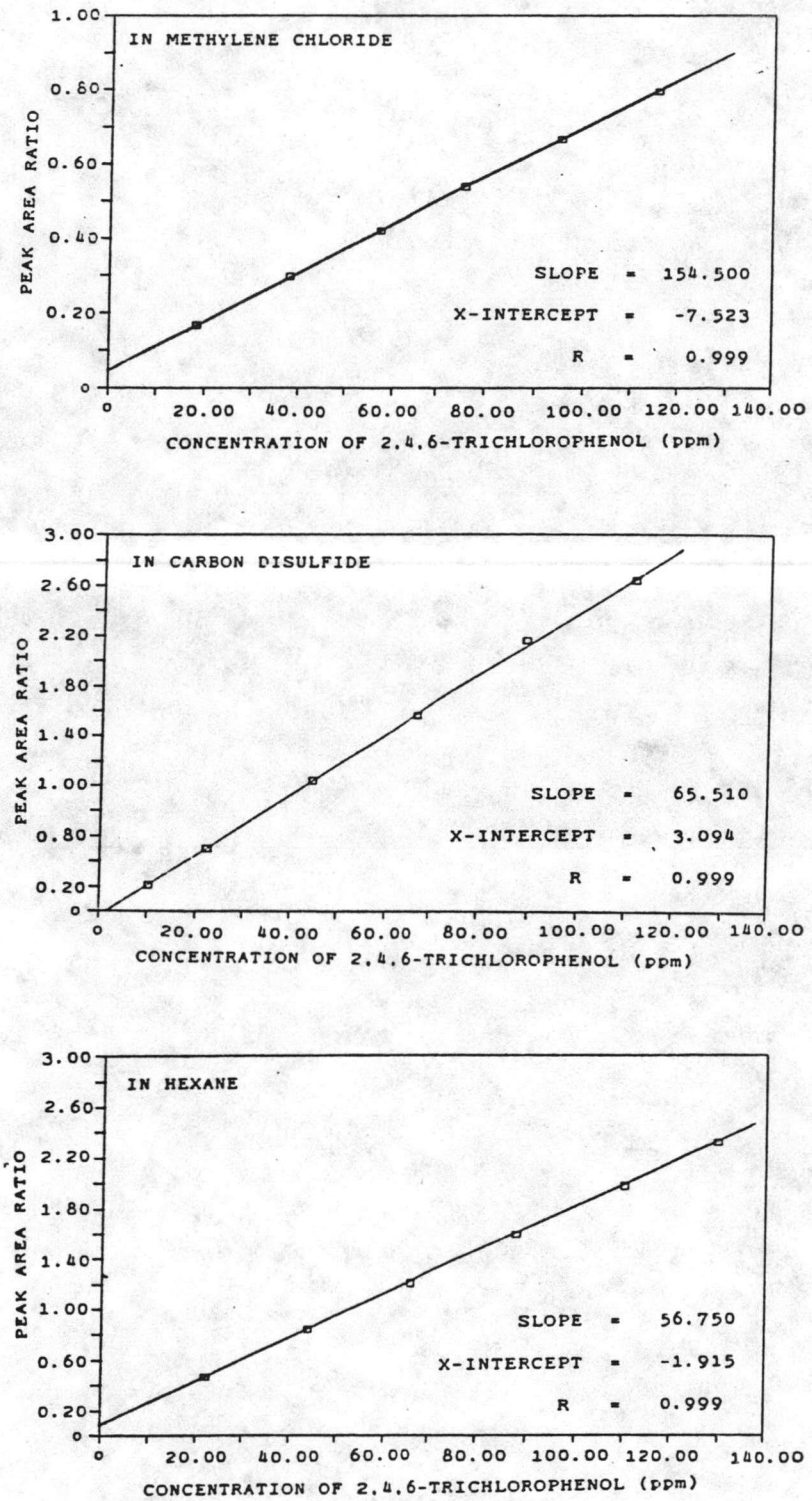
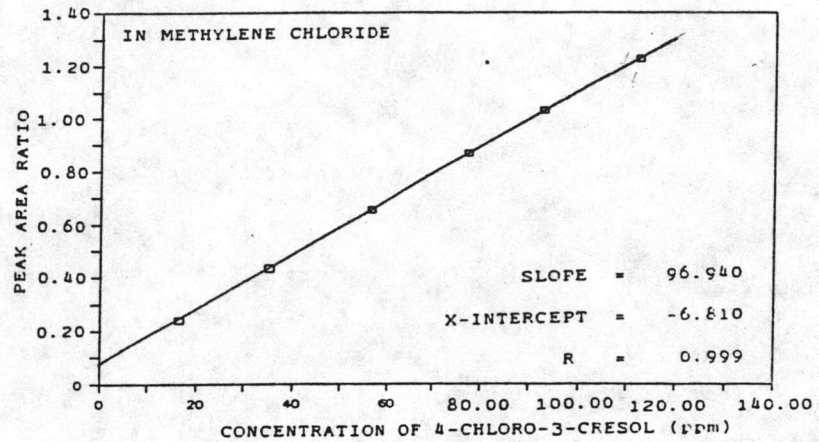
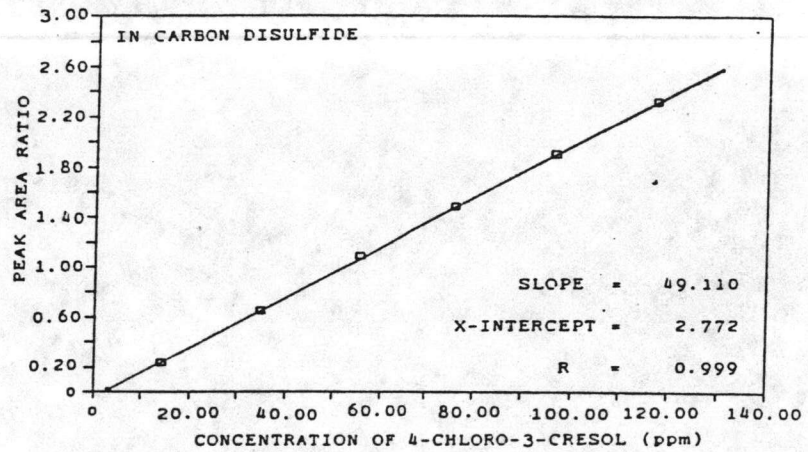


Figure 3.5 The calibration curves of 2,4,6-trichrolophenol in the various organic solvents.





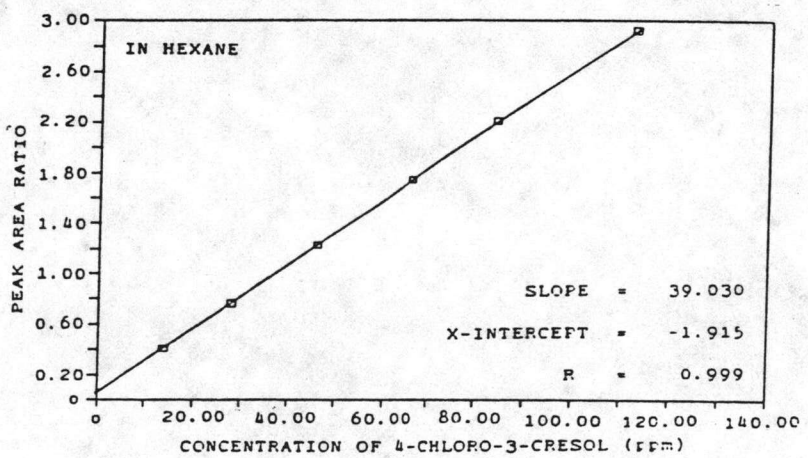


Figure 3.6 The calibration curves of 4-chrolo-3-cresol in the various organic solvents.

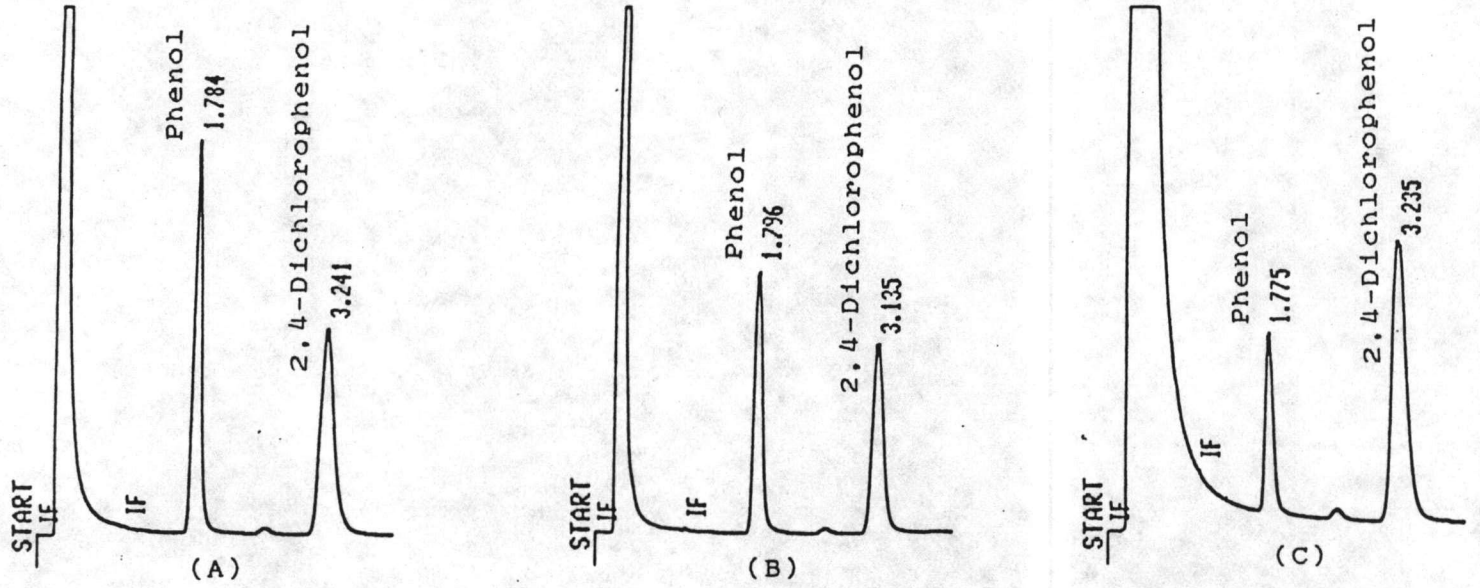


Figure 3.7 Gas chromatograms of phenol in methylene chloride (A) ,in carbon disulfide (B) , in hexane (C) ,

Chromatographic conditions : column , 1% SP - 1240DA on Supelcoport 100/120 , 110 ^{o} C : injector and FID ,250 ^{o}C : N_2 carrier gas , 30 mL/min : sample size 1.00 µL.

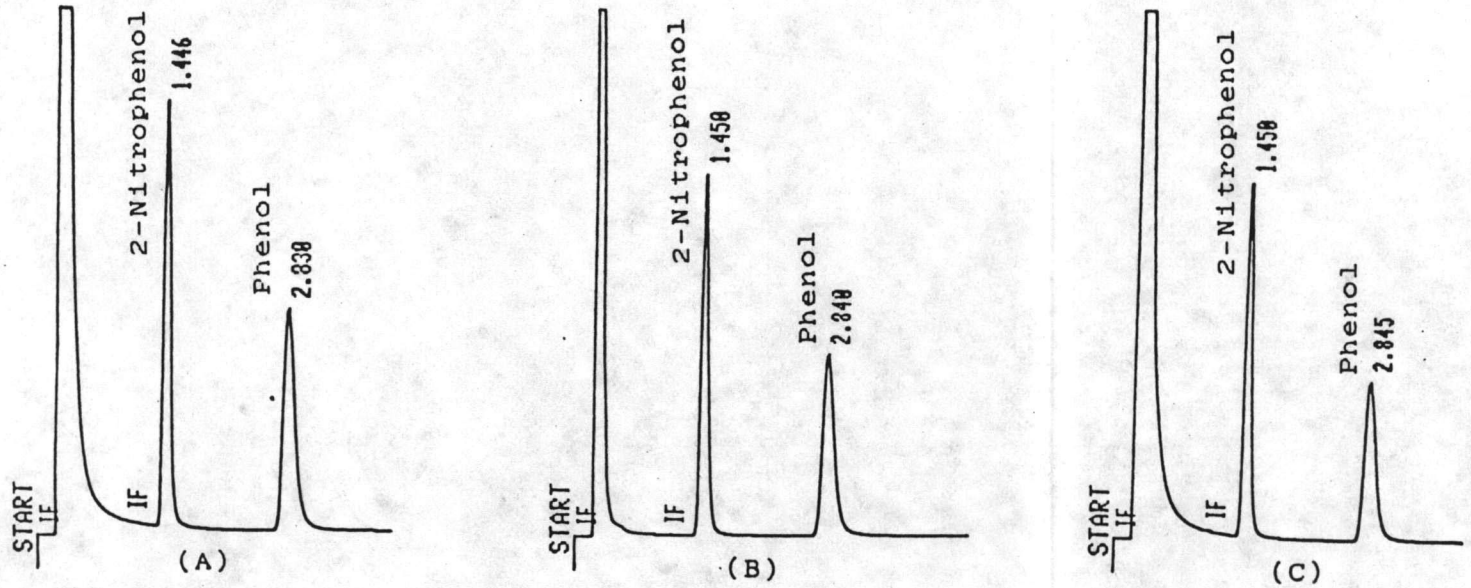


Figure 3.8 Gas chromatograms of 2-nitrophenol in methylene chloride (A) in carbon disulfide (B) , in hexane (C) , Chromatographic conditions : column , 1% SP - 1240DA on Supelcoport 100/120 , 100 ^{o} C : injector and FID ,250 ^{o}C : N_2 carrier gas , 30 mL/min : sample size 1.00 µL.

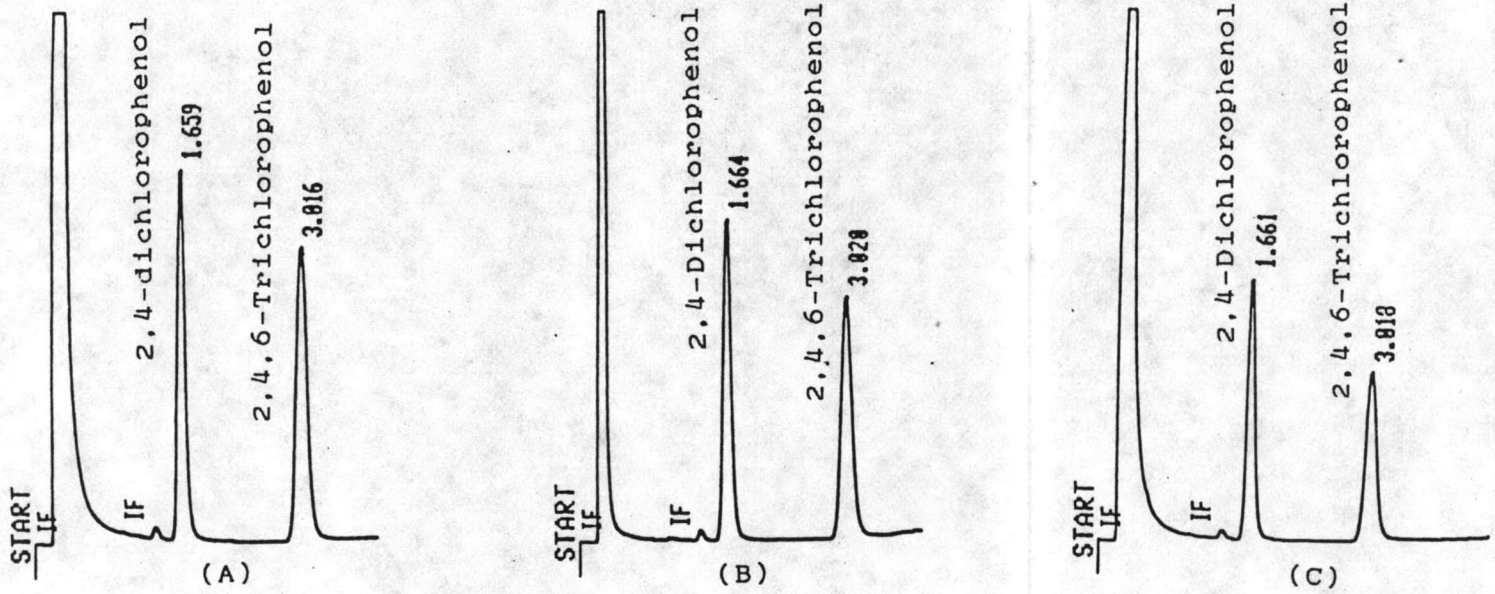


Figure 3.9 Gas chromatograms of 2,4-dichlorophenol in methylene chloride (A) in carbon disulfide (B) , in hexane (C) , Chromatographic conditions : column , 1% SP - 1240DA on Supelcoport 100/120 , 125 ^{o} C : injector and FID ,250 ^{o}C : N_2 carrier gas , 30 mL/min : sample size 1.00 uL.

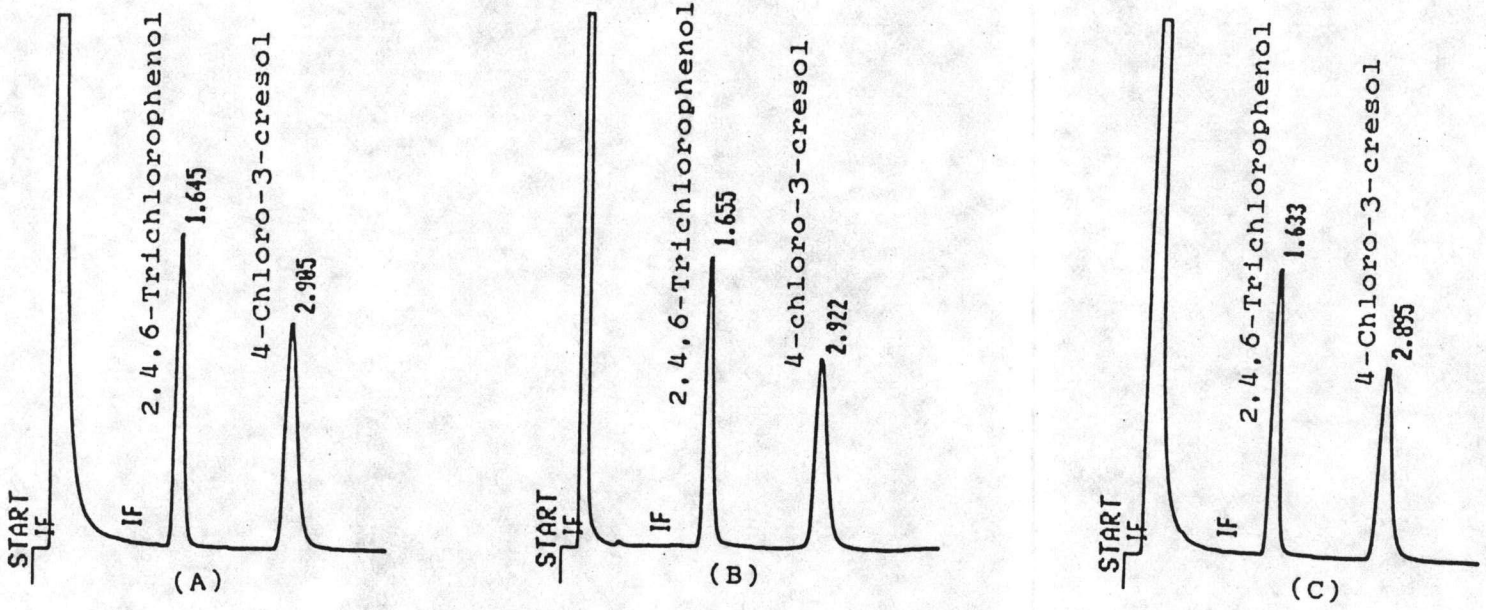


Figure 3.10 Gas chromatograms of 2,4,6-trichlorophenol in methylene chloride (A) in carbon disulfide (B) , in hexane (C) , Chromatographic conditions : column , 1% SP - 1240DA on Supelcoport 100/120 ,140 ^{o} C : injector and FID ,250 ^{o}C : N_2 carrier gas , 30 mL/min : sample size 1.00 µL.

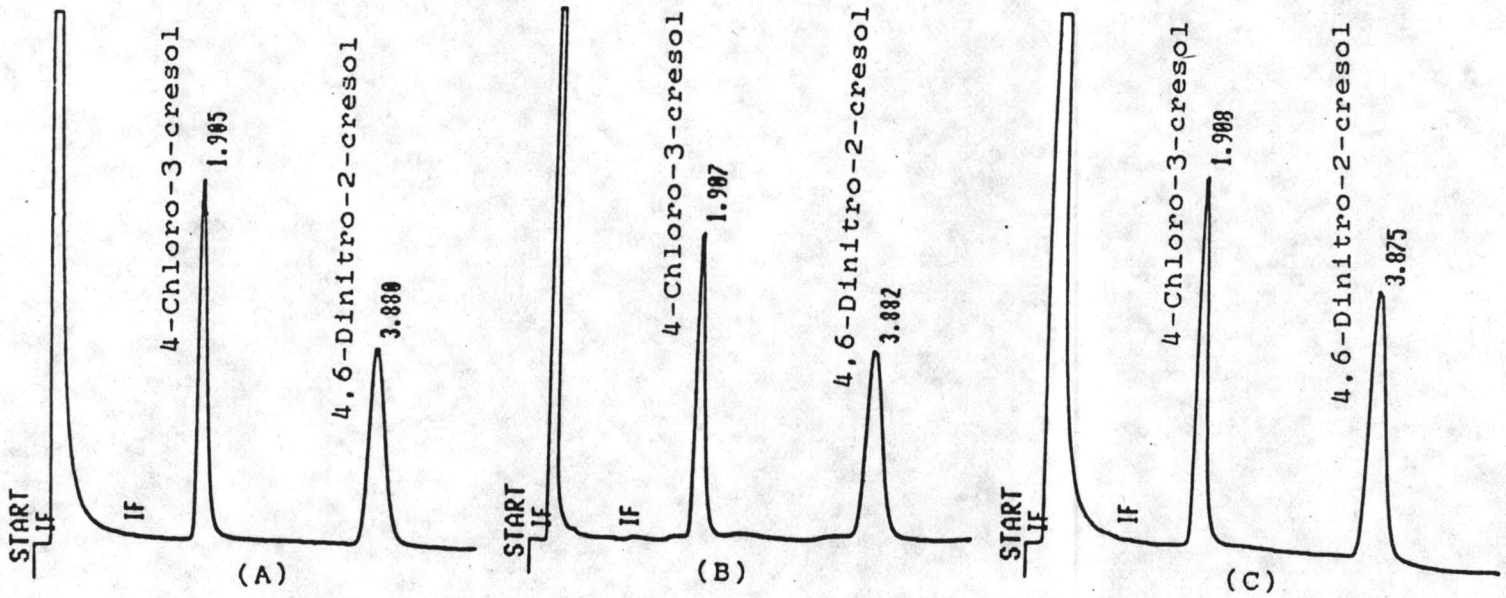


Figure 3.11 Gas chromatograms of 4-chloro-3-cresol in methylene chloride (A) in carbon disulfide (B) , in hexane (C) , Chromatographic conditions : column , 1% SP - 1240DA on Supelcoport 100/120 ,150 ^{o} C : injector and FID ,250 ^{o}C : N_2 carrier gas , 30 mL/min : sample size 1.00 µL.

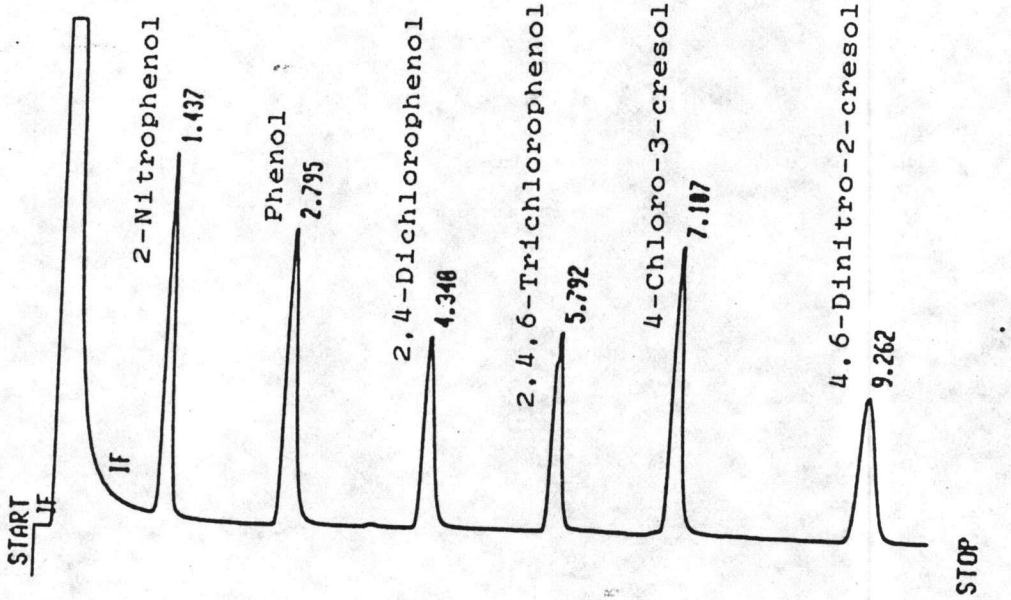


Figure 3.12 Gas chromatogram of the mixture of phenolic compounds in methylene chloride ,

Chromatographic conditions : column , 1% SP - 1240DA on Supelcoport 100/120 , 2 min initial hold at 100 ^{o} C , then to 150 ^{o} C at rate 12 ^{o} C/min and hold until the last peak eluted , injector and FID , 250 ^{o}C : N_2 carrier gas , 30 mL/min : sample size 1.00 µL.

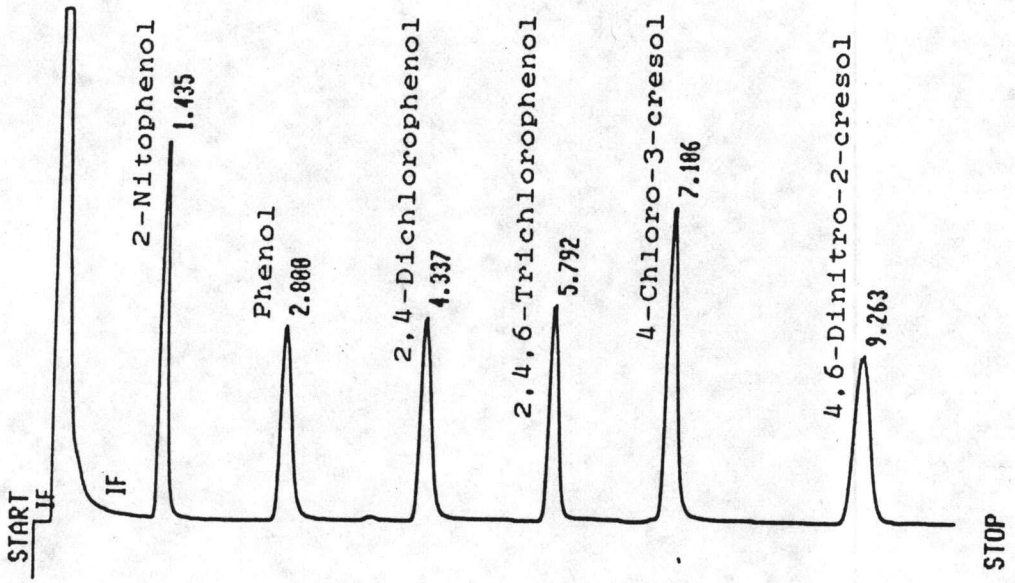


Figure 3.13 Gas chromatogram of the mixture of phenolic compounds in carbon disulfide ,

Chromatographic conditions : column , 1% SP - 1240DA on Supelcoport 100/120 , 2 min initial hold at 100 ^{o} C , then to 150 ^{o} C at rate 12 ^{o} C/min and hold until the last peak eluted , injector and FID , 250 ^{o}C : N_2 carrier gas , 30 mL/min : sample size 1.00 µL.

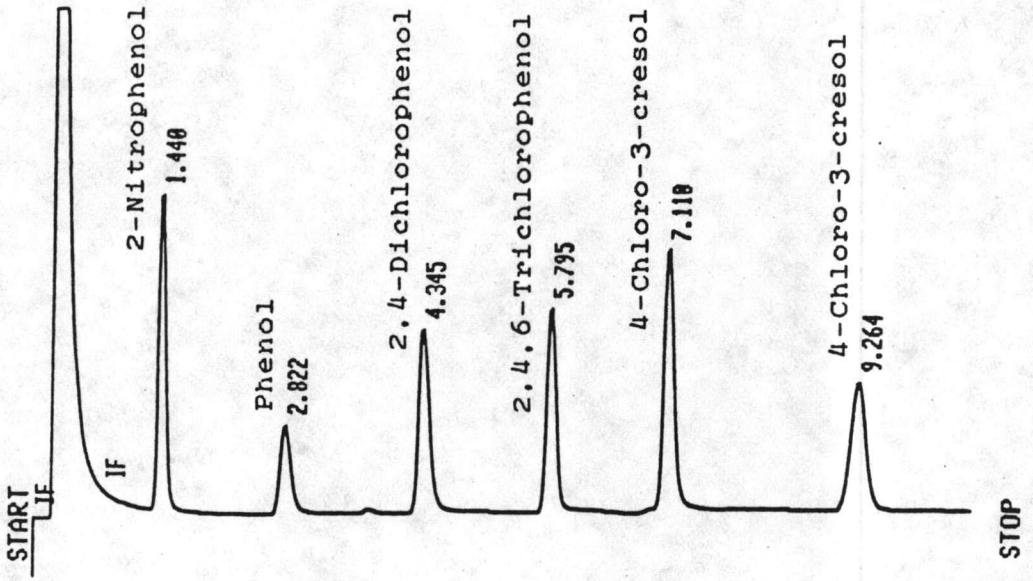


Figure 3.14 Gas chromatogram of the mixture of phenolic compounds in hexane ,

Chromatographic conditions : column , 1% SP - 1240DA on Supelcoport 100/120 , 2 min initial hold at 100 ^{o} C , then to 150 ^{o} C at rate 12 ^{o} C/min and hold until the last peak eluted , injector and FID , 250 ^{o}C : N_2 carrier gas , 30 mL/min : sample size 1.00 µL.