CHAPTER III

EXPERIMENTAL

3.1 Apparatus

A Hewlett Packard HP 5890 gas chromatograph equipped with Flame Ionization Detector (FID) , Electron Capture Detector (ECD) and Hewlett Packard HP 3393 A integrator.

A Griffin No. 7 constant temperature water bath (Griffin & George Ltd., GT. Britain).

A Pressure-Lok series A2 gas-tight syringe 2.00 milliliters (Scientific Glass Engineering, SGE). (see appendix A)

Microsyringes 5.00, 10.00, 50.00 and 100.00 μL (Hamilton Company, Switzerland).

Pipettes 1.00 , 2.00, 5.00, 10.00 and 25.00 mL.

Vials 5.00 and 10.00 mL.

Volumetric flasks 5.00, 10.00, 25.00, 50.00, 100.00, 500.00 and 1000.00 mL.

60 mL serum vials. (see appendix A)

Black rubber septa, aluminum foils, aluminum caps. (see appendix A)

Manual Hand Operated Crimper (Supelco.Inc., Bellefonte, PA, USA.). (see appendix A)

All glasswares, including vials and serum vials were cleaned with detergent, diluted HNO_3 (1:1), water, and rinsed with double

distilled water respectively and dried in an oven at 150 °C at least 3 hours.

The procedure for calibrating the volume of all serum vials is described in appendix B.

3.2 CHEMICALS

3.2.1 The Standard of Volatile Chlorinated Hydrocarbons.

Methylene chloride, Chloroform, Carbon tetrachloride, 1,1,1-Trichloroethane and Trichloroethylene were purchased from Chem Service, Inc., West Chester, PA., USA.

The purities of the standard chemicals were shown in Table 3.1.

Table 3.1 The purities of the standard chlorinated hydrocarbons used in the headspace study.

Name of compound	% purity of standard compounds	Density at 20 °C (g/mL)
Methylene chloride	99.80	1.316
Chloroform	99.30	1.470
Carbon tetrachloride	99.00	1.594
1,1,1-Trichloroethane	99.00	1.336
Trichloroethylene	99.00	1.460
2-Bromo-1-chloropropane	98.50	1.537

3.2.2 Organic Solvents.

Absolute methanol, hexane and heptane were purchased from J. T. Baker Chemical Company, Deventer, Holland and carbon disulfide was purchased from E. Merck, Darmstdt,Germany. All sovents were analytical reagent grade (AR Grade) and they were purified by fractional distillation and the distillate was checked for the purity by gas chromatograph prior to use in the study.

3.2.3 Salts.

Sodium chloride (AR Grade) and Calcium carbonate (AR Grade) were obtained from E. Merck, Darmstadt, Germany and anhydrous sodium sulfate (AR Grade) was obtained from J.T. Baker Chemical Company, Deventer, Holland. They were heated in an oven at 210 ^{o}C for 6 hours and were kept in desiccator before used.

3.2.4 Double distilled Water.

The double distilled water used in this study was distilled by the Yamato distillator model WA-52R (Yamato Scientific Co; Ltd. Tokyo, Japan) and boiled at 100 ^{o}C for 1 hours in 1000 mL breaker. It was checked for purity by the headspace technique prior to use in this study.

3.3 Preparation of the Standard Solutions

3.3.1 The Standard Solution for the Headspace Study Using GC with FID as a Detector.

3.3.1.1 The Single Component Standard Solutions of Methylene chloride, Chloroform, Carbon tetrachloride, 1,1,1-Trichloro ethane and Trichloroethylene in Methanol.

A single component standard solutions of each chlorinated hydrocarbon i.e., methylene chloride, chloroform, carbon tetrachloride,1,1,1-trichloroethane and trichloroethylene in methanol were prepared by measuring each chlorinated hydrocarbon by micro syringe and diluting it with methanol to the mark in 25.00 mL volumetric flasks. The measuring volume of each standard chlorinated hydrocarbon was calculated from the density as shown in Table 3.1 and its concentration were shown in Table 3.2.

The single component standard solution of 579.04 ppm methylene chloride, 564.48 ppm chloroform, 599.34 ppm carbon tetrachloride, 598.53 ppm 1,1,1-trichloroethane and 584.00 ppm trichloroethylene in methanol were prepared by pipetting 1.00 mL of each single component standard solution in methanol as shown in Table 3.2 into 5.00 mL volumetric flasks and diluting to the mark with methanol.

Table 3.2 The measuring volume of standard chlorinated hydrocarbons and the concentration of the single component standard solution in methanol.

Compounds	the volume of standard (μL)	the concentration of the single standard solution (ppm)
Methylene chloride	55.00	2895.20
Chloroform	48.00	2822.40
Carbon tetrachloride	47.00	2996.70
1,1,1-trichloroethane	56.00	2992.64
Trichloroethylene	50.00	2920.00

3.3.1.2 The Standard Mixture Solution of Methylene chloride, Chloroform, Carbon tetrachloride, 1,1,1-trichloroethane and Trichloroethylene in Methanol.

A first standard mixture solution containing 3000.28, 2940.00, 2996.72, 2992.64 and 2978.40 ppm of methylene chloride, chloroform, carbon tetrachloride, 1,1,1-trichloroethane and trichloroethylene , respectively , in methanol was prepared by measuring 57.00 μL of methylene chloride, 50.00 μL of chloroform, 47.00 μL of carbon tetrachloride, 56.00 μL of 1,1,1-trichloroethane and 51.00 μL of trichloroethylene into a 25.00 mL volumetric flask containing about 10 mL of methanol and diluting to the mark with methanol. A 5.00 mL of the first standard mixture solution was pipetted into a 25.00 mL volumetric flask and was then diluted with

methanol to the mark. The second standard mixture solution was composed of 600.06 ppm of methylene chloride, 588.00 ppm of chloroform, 599.34 ppm of carbon tetrachloride, 598.53 ppm of 1,1,1-trichloroethane and 595.68 ppm of trichloroethylene.

3.3.1.3 The Single Component Standard Solution of Methylene chloride, Chloroform, Carbon tetrachloride, 1,1,1-Trichloro ethane and Trichloroethylene in water.

The single component standard solutions of each chlorinated hydrocarbon including methylene chloride,chloroform, carbon tetrachloride, 1,1,1-trichloroethane and trichloroethylene in water were prepared by pipetting each single component standard solution in methanol into 1000.00 mL volumetric flasks and diluting it with water to the mark. The volume of the standard solution in methanol used in the preparation of the standard in water and its concentration were shown in Tables 3.3 and 3.4.

Table 3.3 The concentration of the single component standard solution in water.

Compounds	Concentration of standard in methanol (ppm)	Volume of standard (μL)	The concentration of standard in water (ppb)
Methylene chloride	2895.20	340.00	984.40
Chloroform	2822.40	350.00	984.40
Carbon tetrachloride	2996.70	330.00	988.60
1,1,1-Trichloroethane	2992.64	330.00	987.60
Trichloroethylene	2920.00	340.00	992.50

Table 3.4 The concentration of the single component standard solution in water.

Compounds	Concentration of standard in methanol (ppm)	Volume of standard (μL)	The concentration of standard in water (ppb)
Methylene chloride	579.04	340.00	196.87
Chloroform	564.48	360.00	203.21
Carbon tetrachloride	599.34	330.00	197.78
1,1,1-Trichloroethane	598.53	330.00	197.51
Trichloroethylene	584.00	340.00	198.56

3.3.2 The Standard Solutions for the Headspace Study Using GC with ECD as a Detector.

3.3.2.1 The Single Component Standard Solutions of Methylene chloride, Chloroform, Carbon tetrachloride, 1,1,1-Trichloro ethane and Trichloroethylene and in Methanol.

A single component standard stock solutions of each chlorinated hydrocarbon i.e., methylene chloride, chloroform, carbon tetrachloride, 1,1,1-trichloroethane and trichloroethylene in methanol were prepared by measuring the standard chlorinated hydrocarbons by microsyringe and diluting it with methanol to the mark in 25.00 mL volumetric flasks. The volume of each chlorinated

hydrocarbon was calculated from the density as shown in Table 3.1 and its concentration were shown in Table 3.5

Table 3.5 The concentration of the single component standard stock solutions in methanol.

Compounds	Volume of standard (μL)	The concentration of the standard stock solution in methanol (ppm)
Methylene chloride	19.00	1000.16
Chloroform	17.00	999.60
Carbon tetrachloride	16.00	1020.16
1,1,1-trichloroethane	19.00	1015.30
Trichloroethylene	17.00	992.80

The first single component standard solution of 150.02 ppm methylene chloride, 149.94 ppm chloroform, 153.02 ppm carbon tetrachloride, 152.30 ppm 1,1,1-trichloroethane and 148.92 ppm trichloroethylene in methanol was prepared by pipetting 0.75 mL of each standard stock solution as shown in Table 3.5 into 5.00 mL volumetric flask and was diluted to the mark with methanol.

The second single component standard solution of 15.00 ppm methylene chloride, 14.99 ppm chloroform, 15.30 ppm carbon tetrachloride, 15.23 ppm 1,1,1-trichloroethane and 14.89 ppm trichloroethylene in methanol was prepared by pipetting 0.50 mL of

the first single component standard solution into 5.00 mL volumetric flask and was diluted to the mark with methanol.

3.3.2.2 The Standard Mixture Solution of Methylene chloride, Chloroform, Carbon tetrachloride, 1,1,1-trichloroethane and Trichloroethylene in Methanol.

A first standard mixture solution containing 150.02, 149.94, 153.02, 152.30 and 148.92 ppm of methylene chloride, chloroform, carbon tetrachloride, 1,1,1-trichloroethane and trichloro ethylene, respectively in methanol was prepared by pipetting 0.75 mL of each standard stock solution as shown in Table 3.5 into a 5.00 mL volumetric flask and diluting to the mark with methanol. A 0.50 mL of the first standard mixture solution was transferred into a 5.00 mL volumetric flask and was then diluted with methanol to the mark. The second standard mixture solution contained 15.00 ppm of methylene chloride, 14.99 ppm of chloroform, 15.30 ppm of carbon tetrachloride, 15.23 ppm of 1,1,1-trichloroethane and 14.89 ppm of trichloroethylene.

3.3.2.3 The Internal Standard Solution of 2-Bromo-1-Chloropropane in Methanol.

A 983.68 ppm standard solution of 2-bromo-1-chloropropane in methanol was prepared by transferring 16.00 μL of 2-bromo-1-chloropropane by 50.00 μL microsyringe into 25.00 mL volumetric flask which was filled with 10 mL methanol and diluting to

the mark.

A 118.04 ppm standard solution of 2-bromo-1-chloropropane in methanol was prepared by transferring 1.20 mL of 983.68 ppm of 2-bromo-1-chloropropane into 10.00 mL volumetric flask, diluting it with methanol to the mark and mixing thoroughly.

3.3.3 The Standard Solutions for the calibration curve Using GC with FID as a Detector.

3.3.3.1 The Single Component Standard Solutions of Chloroform, Carbon tetrachloride, 1,1,1-Trichloroethane and Trichloro ethylene in Carbon disulfide.

The single component standard stock solutions of 999.60 ppm chloroform, 1020.16 ppm carbon tetrachloride, 1015.36 ppm 1,1,1-trichloroethane and 992.80 ppm trichloroethylene in carbon disulfide were prepared by transferring 17.00 μL of chloroform, 16.00 μL of carbon tetrachloride, 19.00 μL of 1,1,1-trichloroethane and 17.00 μL of trichloroethylene into each 25.00 mL volumetric flask containing carbondisulfide about 10 mL, diluting each flask to the mark and mixing thoroughly.

3.3.3.2 The Single Component Standard Solution of Methylene chloride in Heptane.

The 1000.16 ppm single component standard stock solution of methylene chloride was prepared by transferring

19.00 μL of methylene chloride into 25.00 mL volumetric flask with 10 mL heptane and diluting it to the mark.

3.3.4 The Standard Solutions for the calibration curve Using GC with ECD as a Detector.

3.3.4.1 The Single Component Standard Solutions of Methylene chloride, Chloroform, Carbon tetrachloride, 1,1,1-Trichloro ethane and Trichloroethylene in Hexane.

The single component standard stock solutions of 1000.16 ppm methylene chloride, 999.60 ppm chloroform, 1020.16 ppm carbon tetrachloride, 1015.36 ppm 1,1,1-trichloroethane and 992.80 ppm trichloroethylene in hexane were prepared by transferring 19.00 μL of methylene chloride, 17.00 μL of chloroform, 16.00 μL of carbon tetrachloride, 19.00 μL of 1,1,1-trichloroethane and 17.00 μL of trichloroethylene into each 25.00 mL volumetric flask containing hexane about 10 mL, diluting each flask to the mark and mixing thoroughly.

3.4 Gas Chromatographic Conditions

Table 3.6 The gas chromatographic conditions used FID as a detector for the study of single component solution.

GC Parameter	GC Condition
Analytical Column	5 m x 0.53 mm ID., HP-1 (Methyl silicone) Capillary column.
Temperature Program	36°C (1 min) to 100°C (10 min) at 5 °C/min.
Splitless time	0.60 min.
Split Ratio	25 : 1
Flow Rate of Carrier Gas (He)	1.50 mL/min.
H_2	40.00 mL/min.
Air	380.00 mL/min.
N_2	50.00 mL/min.
Detector	Flame Ionization Detector (FID)
Detector Temperature	250 °C
Inlet Temperature	150 °C

Table 3.7 The gas chromatographic conditions used FID as a detector for the study of mixture solution.

GC Parameter	GC Condition
Analytical Column	25 m x 0.32 mm ID., HP-5 (5% Phenyl Methyl silicone) Capillary column.
Temperature Program	36°C (1 min) to 100°C (10 min) at 5°C/min.
Splitless time	0.60 min.
Split Ratio	25 : 1
Flow Rate of Carrier Gas (He)	1.50 mL/min.
H_2	40.00 mL/min.
Air	380.00 mL/min.
N_2	50.00 mL/min.
Detector	Flame Ionization Detector (FID)
Detector Temperature	250 °C
Inlet Temperature	150 °C

Table 3.8 The gas chromatographic conditions used ECD as a detector for the study of single and mixture component solution.

GC Parameter	GC Condition
Analytical Column	25 m x 0.32 m ID., HP-5 (5% Phenyl Methyl silicone) Capillary column
Temperature Program	36°C (1 min) to 100°C (10 min) at 5°C/min.
Splitless time	0.60 min.
Split Ratio	25 : 1
Flow Rate of Carrier Gas (He)	1.50 mL/min.
N_2	40.00 mL/min.
Detector	Electron Capture Detector (ECD)
Detector Temperature	300 °C
Inlet Temperature	150 °C

3.5 The Study of the Various Parameters on the Sensitivity of Headspace Technique.

The various parameters which have the effect on the sensitivity of the headspace technique including the equilibration time, temperature, phase ratio, injection volume and salting out were studied in order to be able to determine the optimum headspace analysis condition. Both of the concentrations of each standard solution were studied and all procedure for the studies were triplicated analyses. The procedures were described as follows :

3.5.1 Equilibration Time

The procedure for the study of the effect of time i.e., 10, 20, 30, 40, 50, 60, 80, 100 and 120 min. on the equilibrium system of headspace technique was shown in Figure 3.1 and described as the follow :

1. Pipet 30.00 mL of water into a series of 60 mL serum vials.

2. Inject 10.00 μL of the single standard solution in methanol into the series of 60 mL serum vials.

3. Close each vial with aluminum foil, black rubber septum and aluminum cap sequentially, and then tightly crimp it with Manual Hand Operated Crimper.

4. Shake the sealed serum vials for about 1 min and place in a constant temperature water bath which the temperature is set at 50.0 ^{o}C for 0, 10, 20, 30, 40, 50, 60, 80, 100 and 120 minutes.

5. When it reaches the time as set in 4., withdraw 1.00 mL of vapor phase from sample vial with a 2.00 mL Pressure-Lok series A2 gas tight syringe and then inject into gas chromatograph under GC condition as described in Table 3.6.

6. Plot peak area of each studied compound (A) against time (min).

The optimum equilibration time of all interested compounds found in this section would be used in the next study.

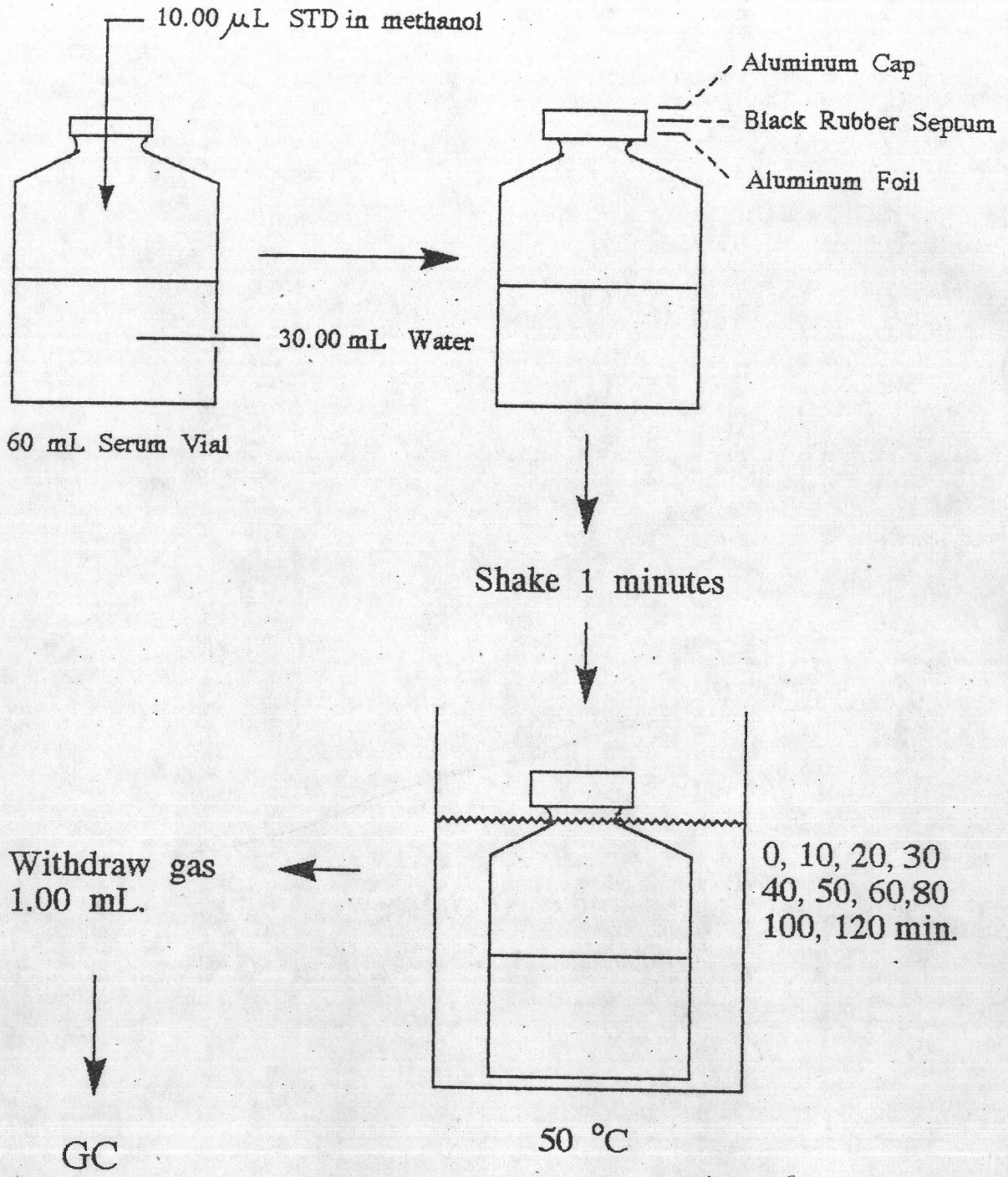

Figure 3.1 The procedure for the study of the effect of time.

3.5.2 Temperature

The procedure for the study of effect of the temperature i.e., 50 °, 60 °, 70 ° and 80 °C on the sensitivity of headspace technique was shown in Figure 3.2 and described as the follow :

1. Pipet 30.00 mL of water and inject 10.00 μL of the single component standard solution in methanol into a series of 60 mL serum vials.

2. Close each vial with aluminum foil, black rubber septum and aluminum cap sequentially, and then tightly crimp it with Manual Hand Operated Crimper.

3. Shake vigorously the sealed serum vials for about 1 min and place them in a constant temperature water bath which is set at verious temperature, i.e., 50.0 °, 60.0 °, 70.0 ° and 80.0 °C and leave them to stand in the water bath until they reach an equilibration time as found in the section 3.5.1.

4. Withdraw 1.00 mL of vapor phase from sample vial with a 2.00 mL Pressure-Lok series A2 gas tight syringe and then inject into gas chromatograph under GC condition as described in Table 3.6.

5. Calculated the concentration of interested volatile component in vapor phase (C_g) by means of external standardization method, then determine the distribution coefficient of each interested component (K).

6. Calculate the sensitivity (S) of interested volatile component from equation (2.24)

7. Plot the sensitivity (S) and the distribution coefficient

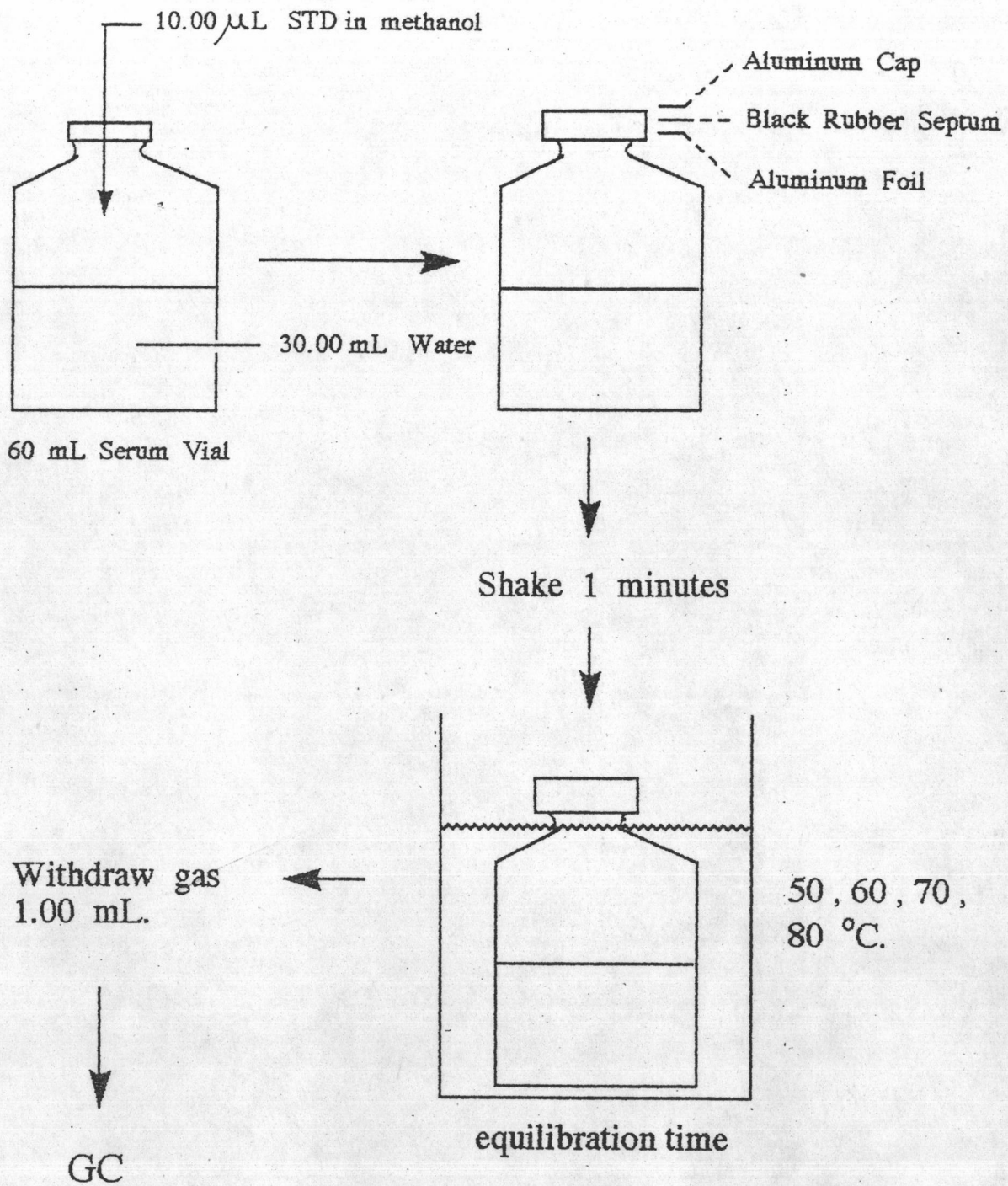

Figure 3.2 The procedure for the study of the effect of temperature.

(K) against the temperature (^{o}C)

The optimum temperature of each chlorinated hydrocarbon found in this section would be used in the next study.

3.5.3 Liquid to Gas Phase Volume Ratio

The liquid to gas phase volume ratios, i.e., 10:50, 20:40, 30:30, 40:20 and 50:10 of each chlorinated hydrocarbon were studied in order to be able to determine the optimum phase ratio. The single component standard solution in water in the Section 3.3.1.3 were studied in this procedure which was shown in Figure 3.3 and described as follows :

1. Pipet 10.00, 20.00, 30.00, 40.00 and 50.00 mL of the single component standard in aqueous solution into a series of 60 mL serum vials.

2. Close each vial with aluminum foil, black rubber septum and aluminum cap sequentially, and then tightly crimp it with Manual Hand Operated Crimper.

3. Shake vigorously the sealed serum vials for about 1 min and place them in a constant temperature water bath which the temperature is set at the optimum temperature as found in section 3.5.2.

4. Withdraw 1.00 mL of vapor phase from sample vial with a 2.00 mL Pressure-Lok series A2 gas tight syringe and then inject into gas chromatograph under GC condition as described in Table 3.6 when it reaches the equilibration time.

5. Calculated the concentration of the interested compounds

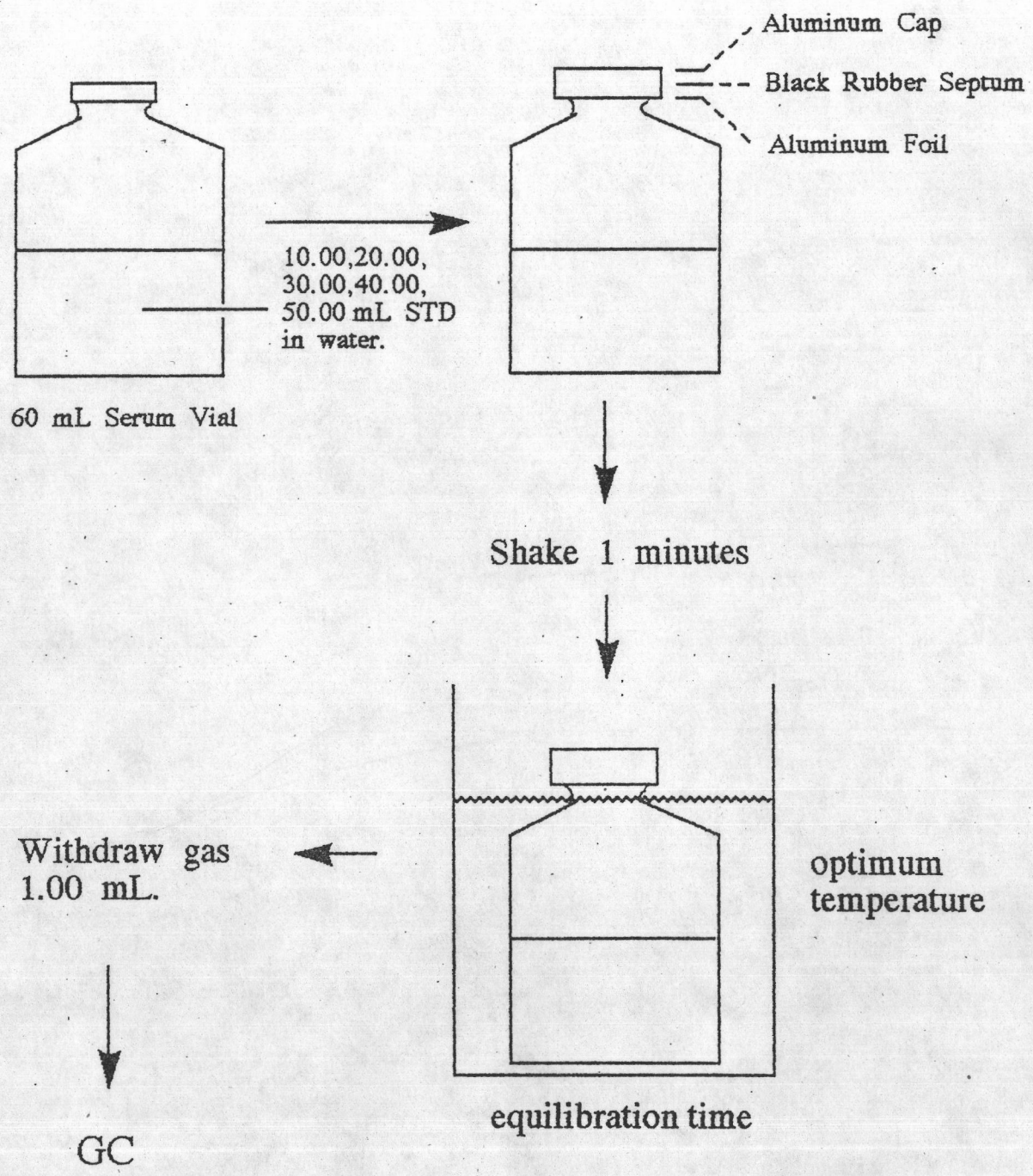

Figure 3.3 The procedure for the study of the effect of liquid to gas phase volume ratio.

in vapor phase (C_g) by means of external standardization method then determine the distribution coefficient of each interested compound.

6. Calculate the sensitivity(S) of each interested compound from equation (2.24)

7. Plot the sensitivity (S) and the distribution coefficient (K) against the phase ratio (V_l/V_g).

The optimum liquid to gas phase volume ratio of each chlorinated hydrocarbon found in this study would be used in the study of salting out effect, minimum detectable level and sample analysis.

3.5.4 Injection Volume

The injection volumes of headspace gas for each interested compound i.e., 0.50, 1.00, 1.50 and 2.00 mL were studied in order to be able to determine the optimum injection volume. The procedure for the study of the injection volume was shown in Figure 3.4 and described as follows :

1. Pipet 30.00 mL of water and inject 10.00 μL of the single component standard solution in methanol into a series of 60 mL serum vials.

2. Close each vial with aluminum foil, black rubber septum and aluminum cap sequentially, and then tightly crimp it with Manual Hand Operated Crimper.

3. Shake vigorously the sealed serum vials for about 1 min and place them in a constant temperature water bath which the

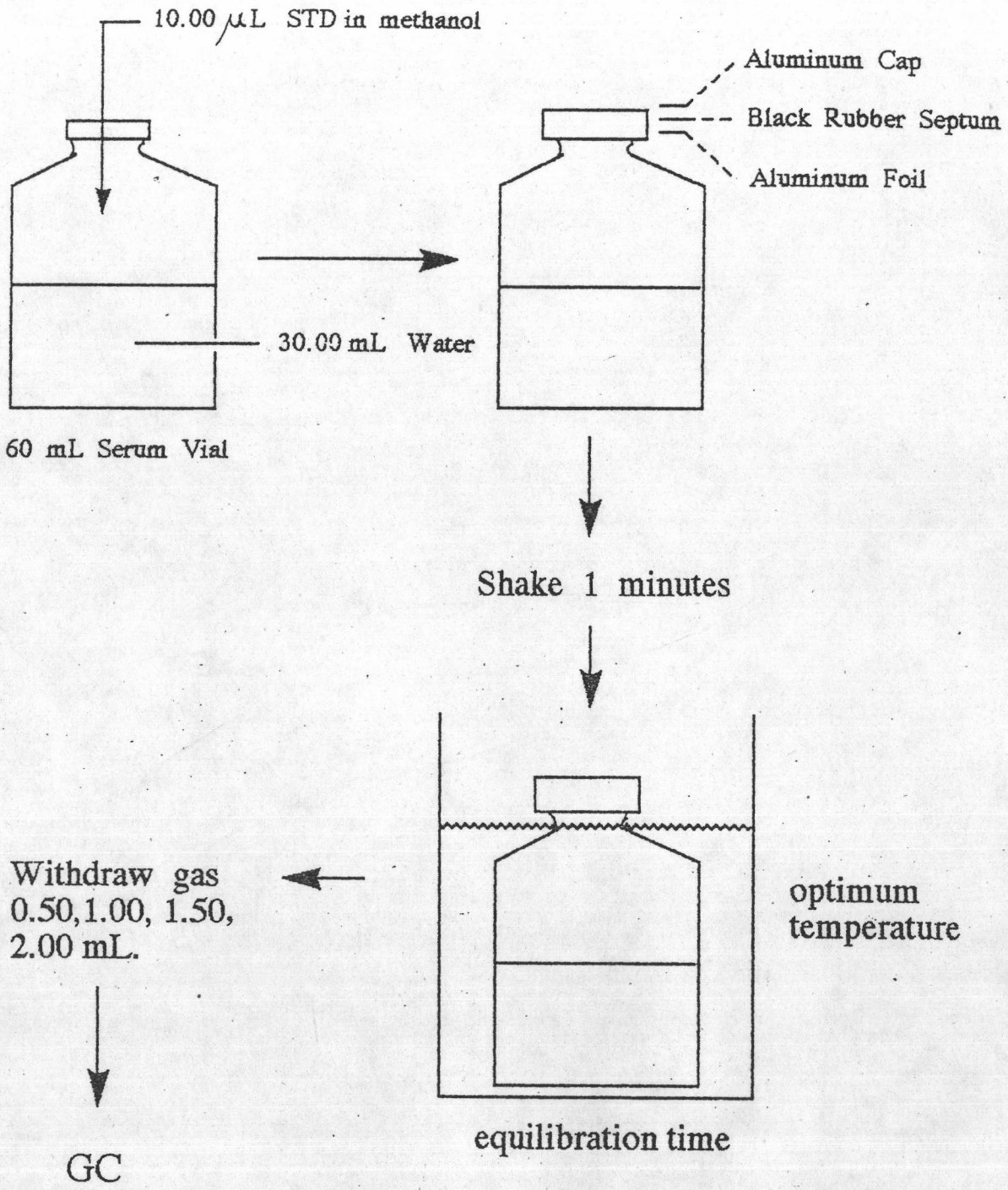

Figure 3.4 The procedure for the study of the effect of injection volume.

temperature is set at the optimum temperature as found in Section 3.5.2.

4. Withdraw 0.50, 1.00, 1.50 and 2.00 mL of vapor phase from each vial, respectively, with a 2.00 mL Pressure-Lok series A2 gas tight syringe and then inject into gas chromatograph under GC condition as described in Table 3.6 when it reaches the equilibration time.

5. Plot peak area of each studied compound (A) against the injection volume (mL).

6. Calculate the sensitivity(S) of each interested compound from equation (2.24) and then plot the sensitivity (S) of each compound against the injection volume (mL).

The optimum injection volume of each compound found in this study would be used in the study of salting out effect, minimum detectable level and sample analysis.

3.5.5 Salting Out Effect

The equilibration time, temperature, liquid to gas phase volume ratio and injection volume of each interested compound were studied and were evaluated to the optimum condition. Therefore, they would be used in the study of effect of adding salt on sensitivity of headspace technique. The study of the salting out effect was carried out with the saturated single component and mixture standard soultion and each study was consisted of four systems :

1. no addition of salt (not salting out)
2. 10.50 g of sodium chloride

3. 13.00 g of anhydous sodium sulfate

4. 0.10 g of calcium carbonate

The procedure for this study was shown in Figure 3.5 and described as follow :

1. Weigh 10.50 g of sodium chloride, 13.00 g of anhydrous sodium sulfate and 0.10 g of calcium carbonate in a series of 60 mL serum vials.

2. Pipet the volume of water which is equal to the optimum volume found in section 3.5.3 and inject 10.00 μL of standard solution in methanol into four 60 mL serum vials containing no salt, 10.50 g of sodium chloride, 13.00 g of anhydrous sodium sulfate and 0.10 g of calcium carbonate.

3. Close each vial with aluminum foil, black rubber septum and aluminum cap sequentially, and then tightly crimp it with Manual Hand Operated Crimper.

4. Shake vigorously the sealed serum vials for about 1 min and place them in a constant temperature water bath which the temperature is set at the optimum temperature as found in section 3.5.2.

5. Withdraw the vapor phase volume at the optimum quantity as found in section 3.5.4 from sample vial with a 2.00 mL Pressure -Lok series A2 gas tight syringe and then inject into gas chromatograph under GC condition as described in Table 3.6, 3.7 and 3.8 when it reaches the equilibration time.

6. Determine the distribution coefficient(K),the sensitivity (S) and percent recovery (% E) of each chlorinated hydrocarbon and compare the results of the four systems study i.e., no salt, 10.50 g

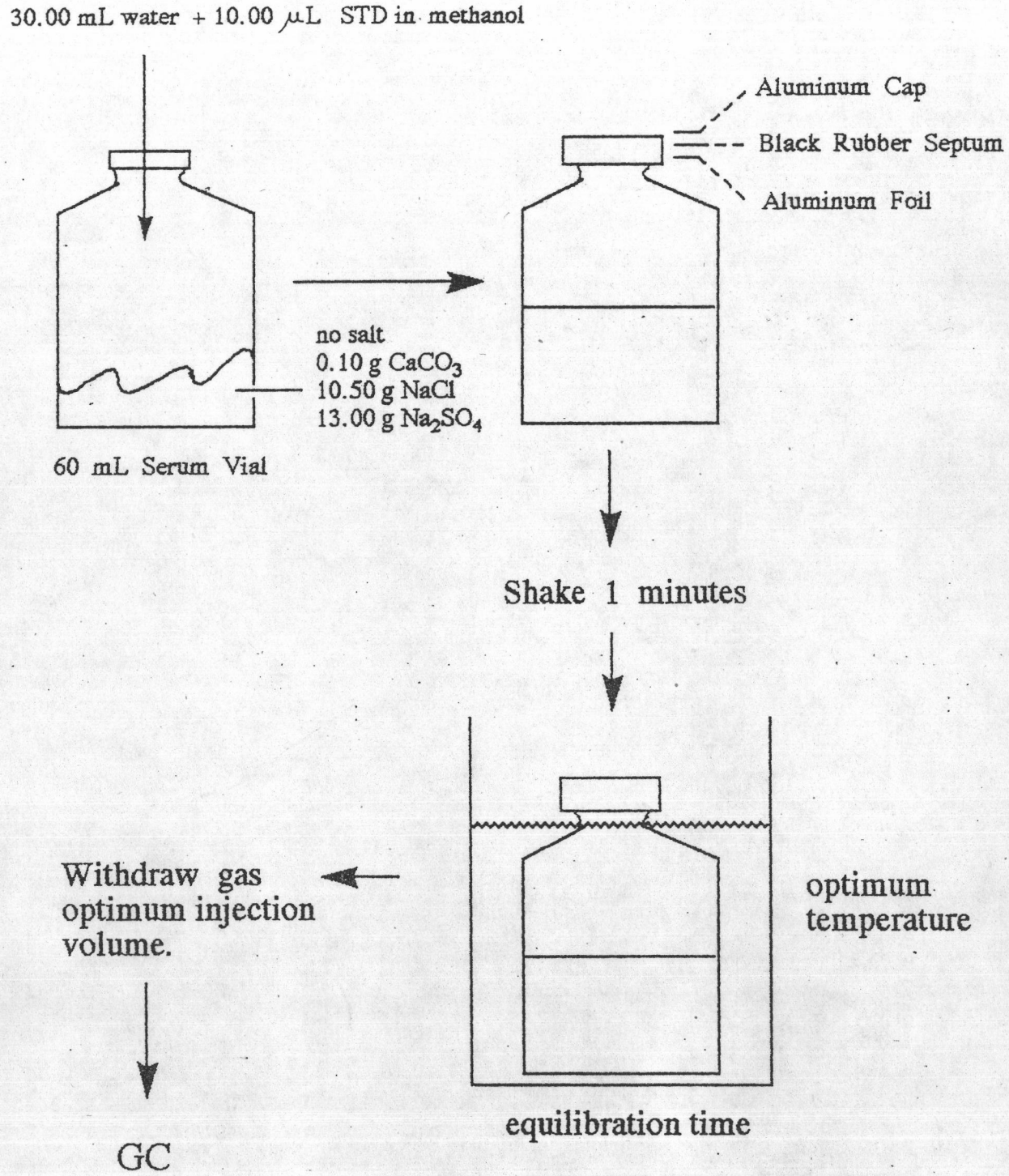

Figure 3.5 The procedure for the study of the effect of salting out effect.

of sodium chloride, 13.00 g of anhydrous sodium sulfate and 0.10 g of calcium carbonate.

7. Plot the distribution coefficient(K) and the sensitivity (S) against the salt.

The optimum salt of each compound found in this study would be used in the study of minimum detectable level and sample analysis.

3.6 Minimum Detectable Level (MDL).

Minimum detectable level of each chlorinated hydrocarbon was determined by using the optimum condition of time, temperature, liquid to gas phase ratio, injection volume and adding salt. The concentration of each interested compound which was studied by headspace technique would be decreased until the GC chromatogram showed the signal to noise ratio was equal to 2. That concentration was minimum detectable level of the component.

3.7 Quantitative Headspace Analysis

Any tradition quantitative gas chromatographic techniques can be used to determine the initial concentration (C^o_1) of a substance in solution from its concentration in the gas phase. The internal standardization was chosen as the quantitative methods for determination of the initial concentration of each chlorinated hydrocarbon in unknown aqueous solution. The principle of this quantitative method is described in the following clauses.

3.7.1 The 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 GC chromatograms are plotted against the weight or the concentration ratios to obtain a graph in figure 3.6, the plot should be linear for a particular system.

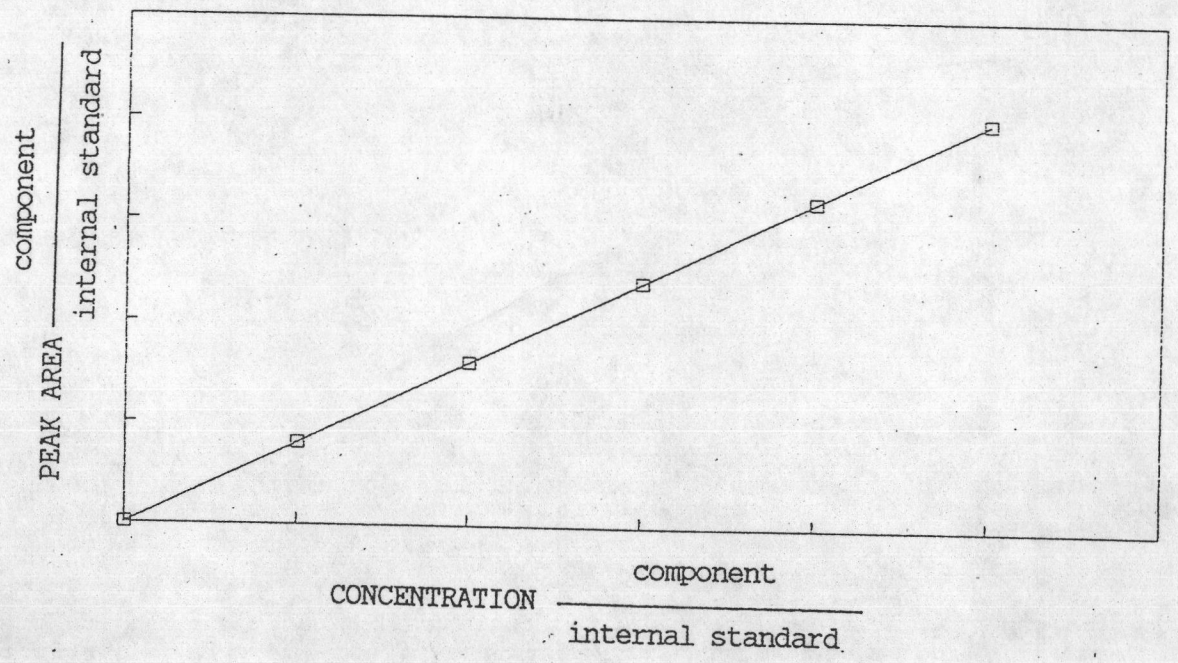

Figure 3.6 Relative calibration curve

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

$$\text{slope} = \frac{\text{peak area ratio}}{\text{concentration ratio}} = F \qquad (3.1)$$

$$F = \frac{A_c C_i}{A_i C_c} \qquad (3.2)$$

Where A_c and C_c are the peak area and concentration of the interested component.

and A_i and C_i are the peak area and concentration of the internal standard.

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

$$C_c = \frac{A_c C_i}{A_i F} \qquad (3.3)$$

For headspace analysis technique, the concentration in this equation (C_c and C_i) is the initial concentration (C^o_l), hence the unknown initial concentration of interested component could be determined as the following

$$C^o_{cl} = \frac{A_c C^o_{il}}{A_i F} \qquad (3.4)$$

Where C^o_{cl} and C^o_{il} are the initial concentration of the interested component and internal standard, respectively.

The internal standard is then chosen such that it must

1. Elute from column adequately separate from all sample components.

2. Elute as near as possible to the desired components and, ideally, before the last sample peak so that analysis time is not increased.

3. Be similar in functional group type in the components of interest.

4. Be stable under the required analytical conditions and non reactive with sample components.

3.8 The Determination of Equilibrium Concentration of the Interested Compound in Gas Phase Using External Standardization Method.

In order to determine the equilibrium concentration of the interested component in vapor phase ($C_{g,i}$), the calibration curve of each interested component must be constructed. thus, a series of standard solutions containing the known weight of the components were prepared and chromatographed under the identical GC condition as the analyzed sample. Then the peak area of each standard component obtained from the chromatogram (A) was plotted against the weight of the standard component (W). The curve should be linear for a particular system. The slope (m) and y-intercept (b) can be calculated by the linear least square method from a linear equation

$$A = m W + b \qquad (3.5)$$

The exact volume of the vapor phase from the equilibrated headspace sample (v_g) was then chromatographed. The computation of weight of the interested component i in gas phase ($W_{g,i}$) can be compared graphically to the constructed calibration curve of that interested component or calculated by substitution the peak area of the interested component i ($A_{g,i}$) into equation (3.1) and the equilibrium concentration of the interested component i in vapor phase can be calculated by dividing the weight of interested compound ($W_{g,i}$) by the injection volume of vapor phase (v_g), therefore :

$$C_{g,i} = \frac{W_{g,i}}{V_g} \qquad (3.6)$$

where $C_{g,i}$ = Equilibrium concentration of the interested component i in gas phase.

v_g = Injection volume.

The calibration curves of each standard compound including methylene chloride in heptane and chloroform, carbon tetrachloride, 1,1,1-trichloroethane and trichloroethylene in carbon disulfide used in the determination of the weight of the interested component were shown in Figures 3.7, 3.8, 3.9, 3.10 and 3.11, respectively for GC condition in Table 3.6, and Figures 3.12, 3.13, 3.14, 3.15 and 3.16, respectively for GC condition in Table 3.7. Figures 3.17, 3.18, 3.19, 3.20 and 3.21 have calibration curves of each standard compound in hexane using GC condition as described in Table 3.8.

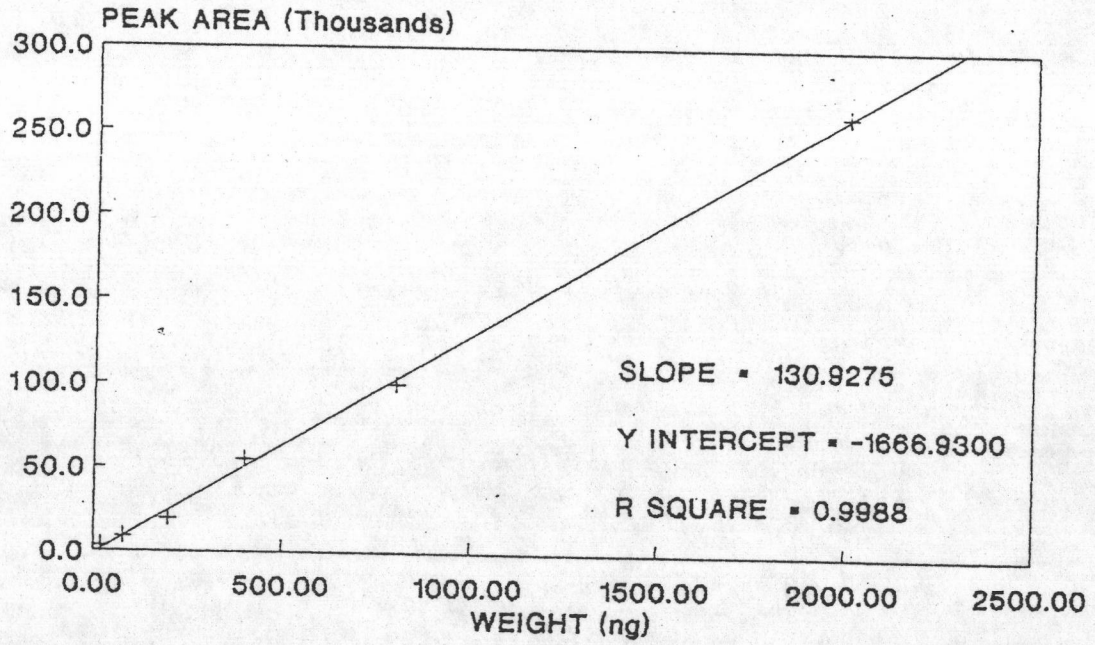

Figure 3.7 The calibration curve of methylene chloride in heptane using HP-1 capillary column with FID as a detector.

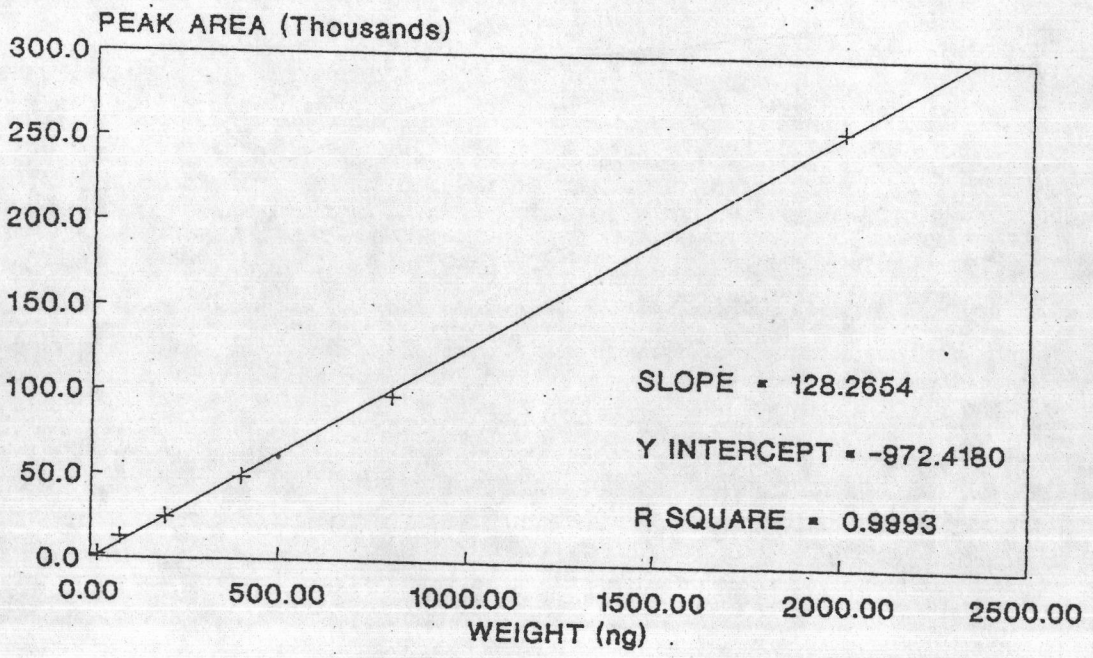

Figure 3.8 The calibration curve of chloroform in carbon disulfide using HP-1 capillary column with FID as a detector.

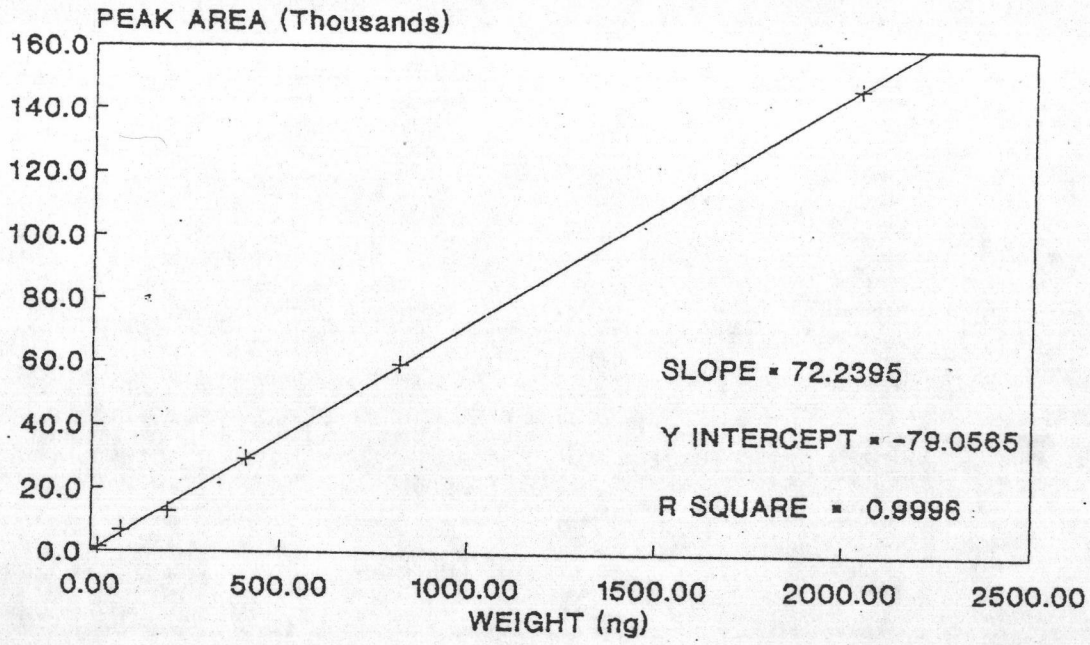

Figure 3.9 The calibration curve of carbon tetrachloride in carbon disulfide using HP-1 capillary column with FID as a detector.

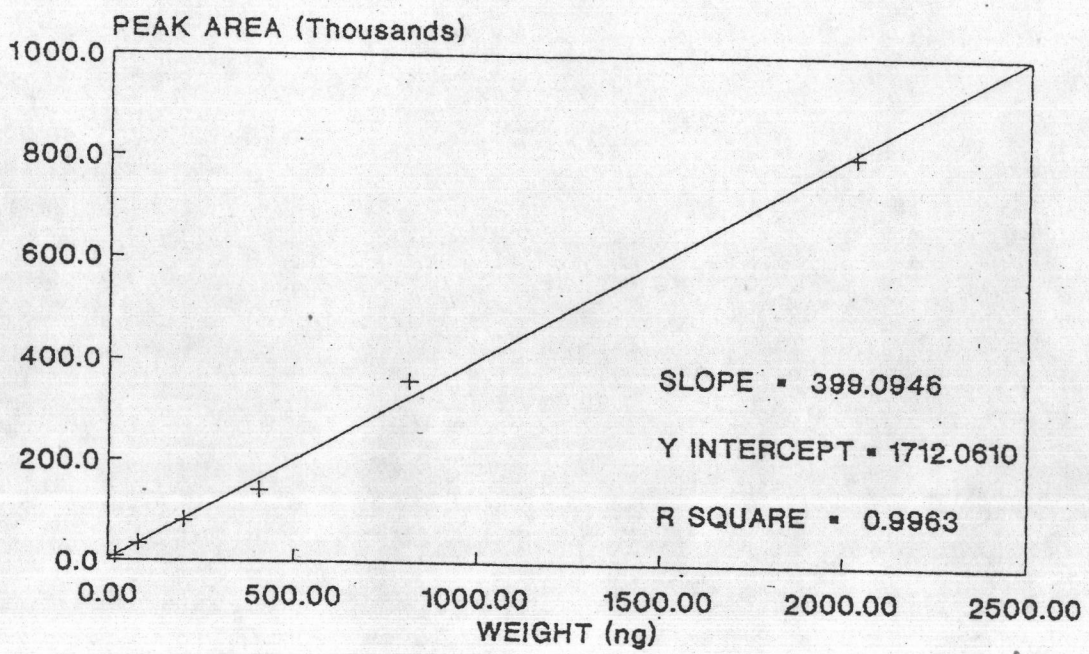

Figure 3.10 The calibration curve of 1,1,1-trichloroethane in carbon disulfide using HP-1 capillary column with FID a detector.

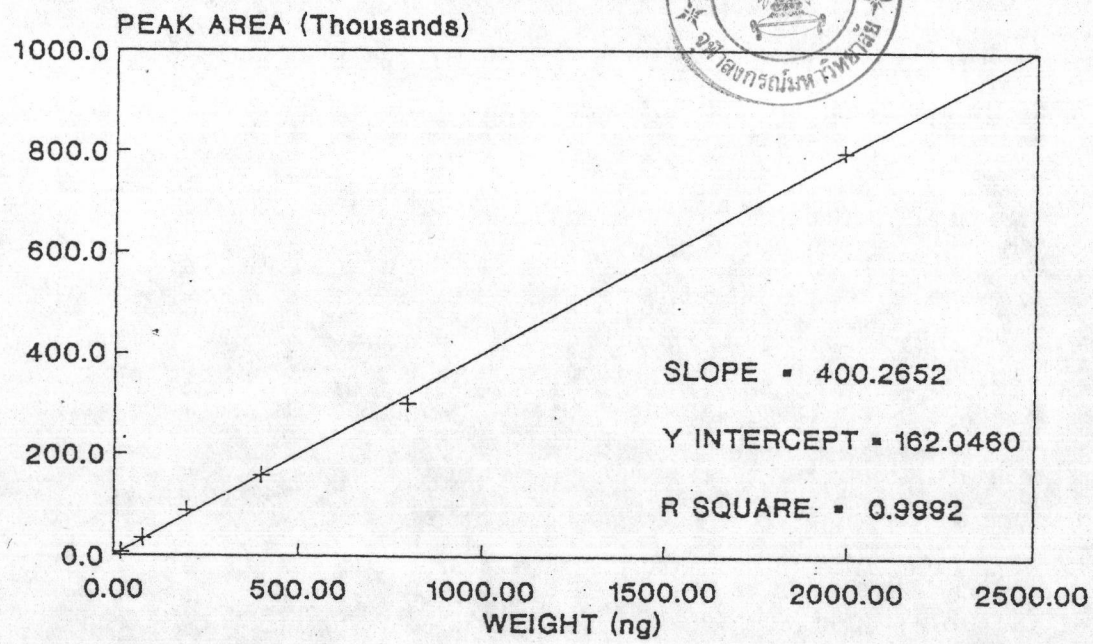

Figure 3.11 The calibration curve of trichloroethylene in carbon disulfide using HP-1 capillary column with FID as a detector.

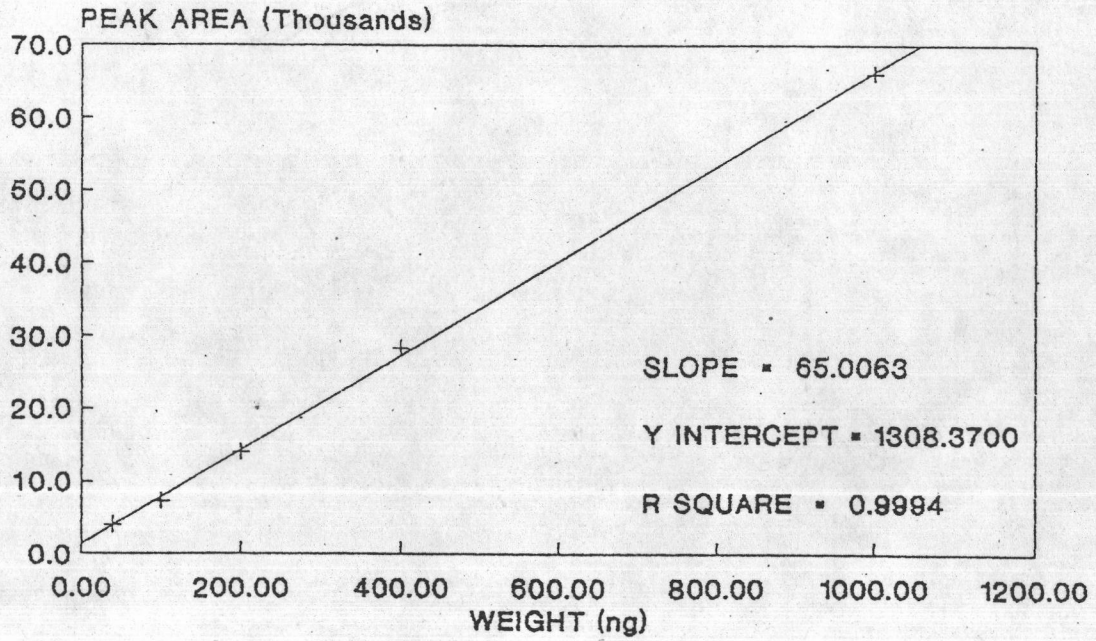

Figure 3.12 The calibration curve of methylene chloride in heptane using HP-5 capillary column with FID as a detector.

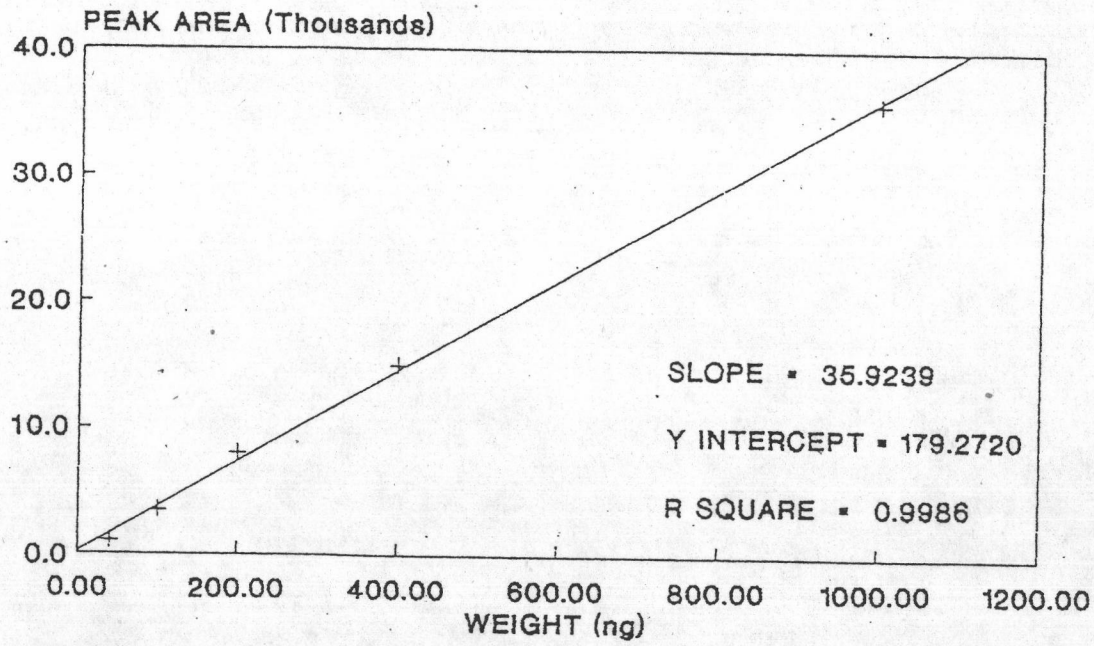

Figure 3.13 The calibration curve of chloroform in carbon disulfide using HP-5 capillary column with FID as a detector.

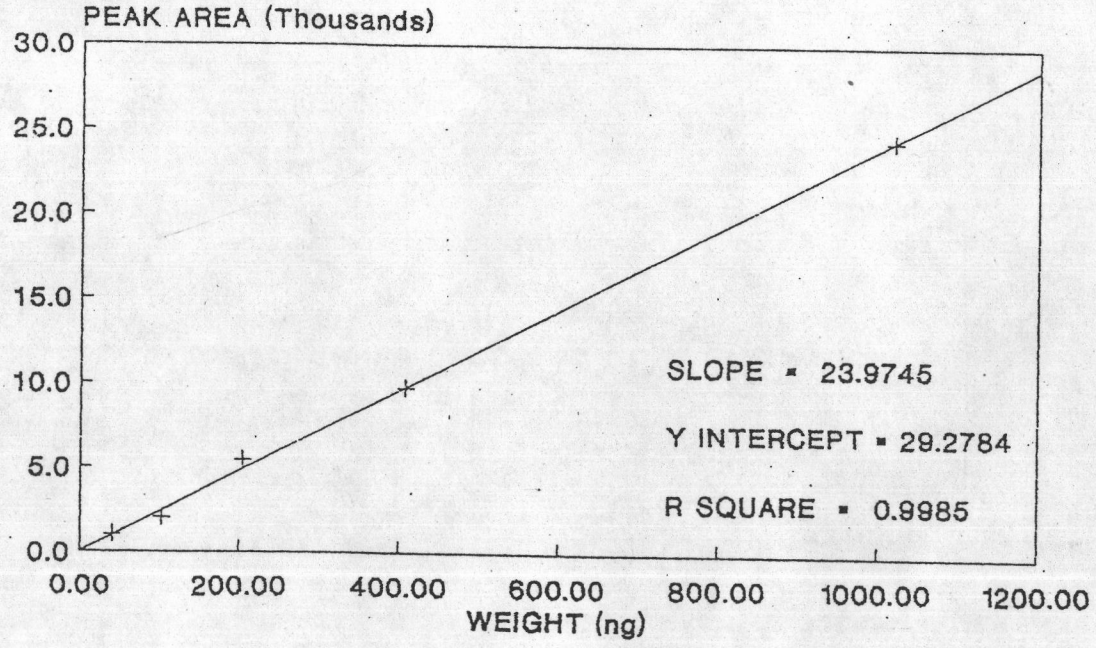

Figure 3.14 The calibration curve of carbon tetrachloride in carbon disulfide using HP-5 capillary column with FID as a detector.

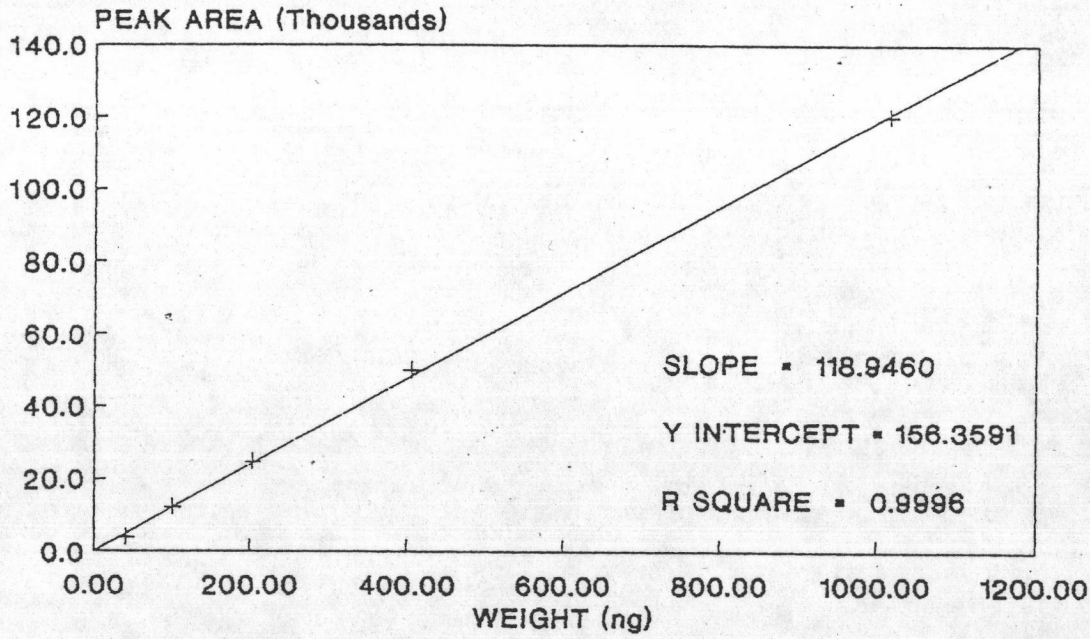

Figure 3.15 The calibration curve of 1,1,1-trichloroethane in carbon disulfide using HP-5 capillary column with FID a detector.

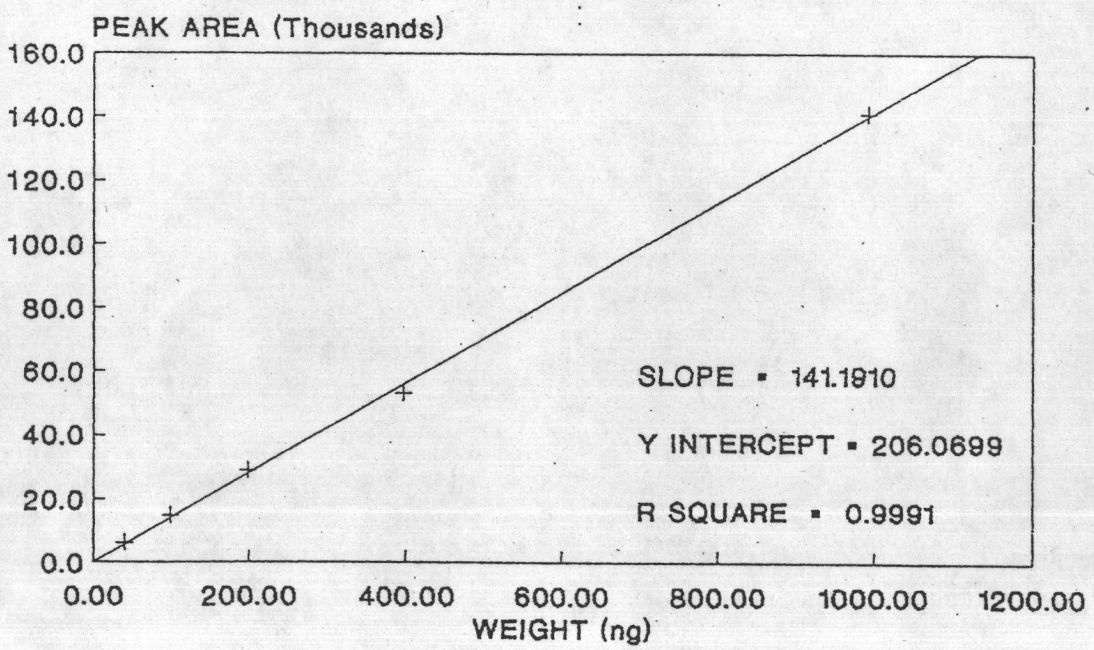

Figure 3.16 The calibration curve of trichloroethylene in carbon disulfide using HP-5 capillary column with FID as a detector.

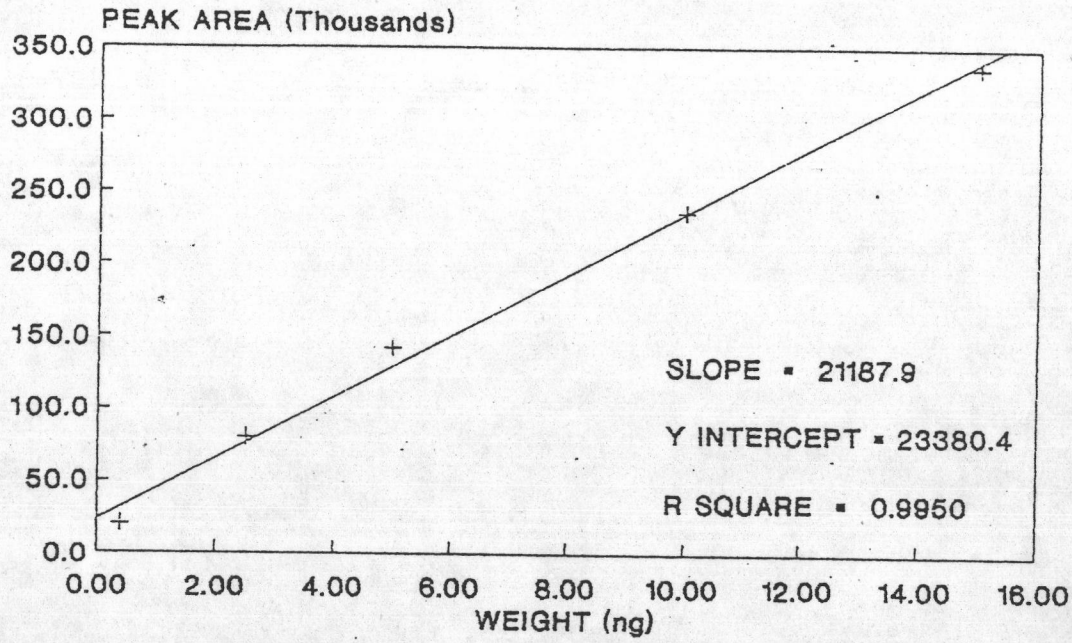

Figure 3.17 The calibration curve of methylene chloride in hexane using HP-5 capillary column with ECD as a detector.

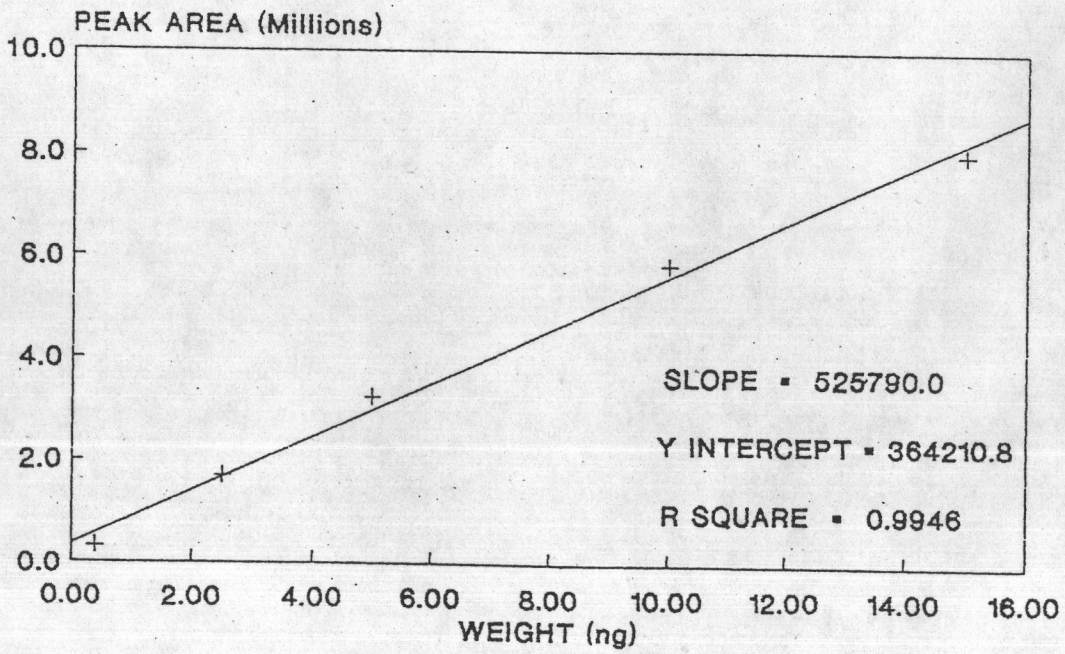

Figure 3.18 The calibration curve of chloroform in hexane using HP-5 capillary column with ECD as a detector.

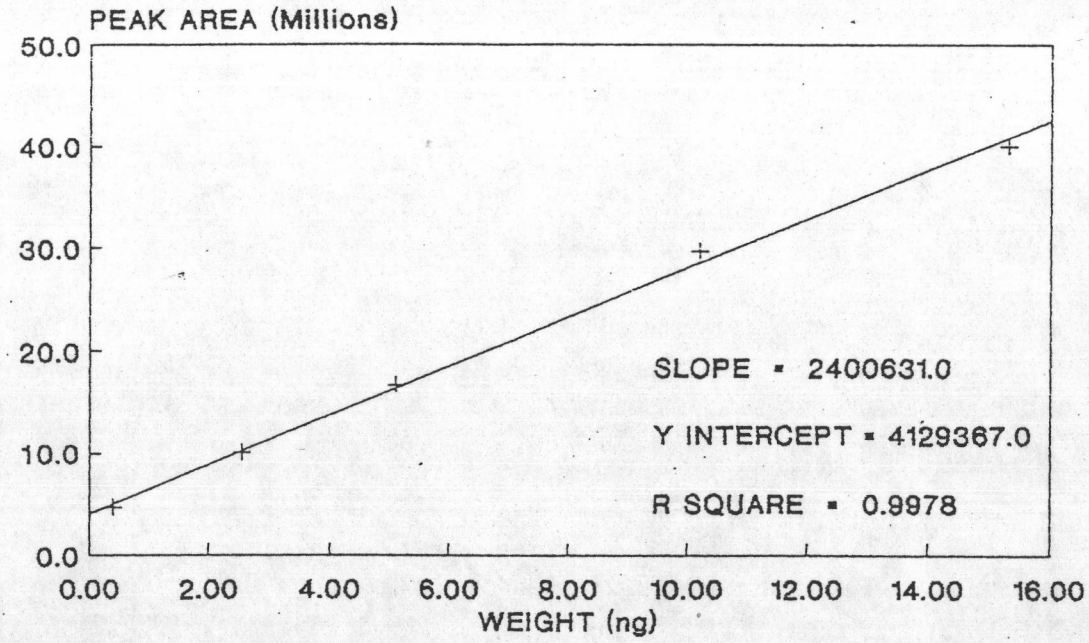

Figure 3.19 The calibration curve of carbon tetrachloride in hexane using HP-5 capillary column with ECD as a detector.

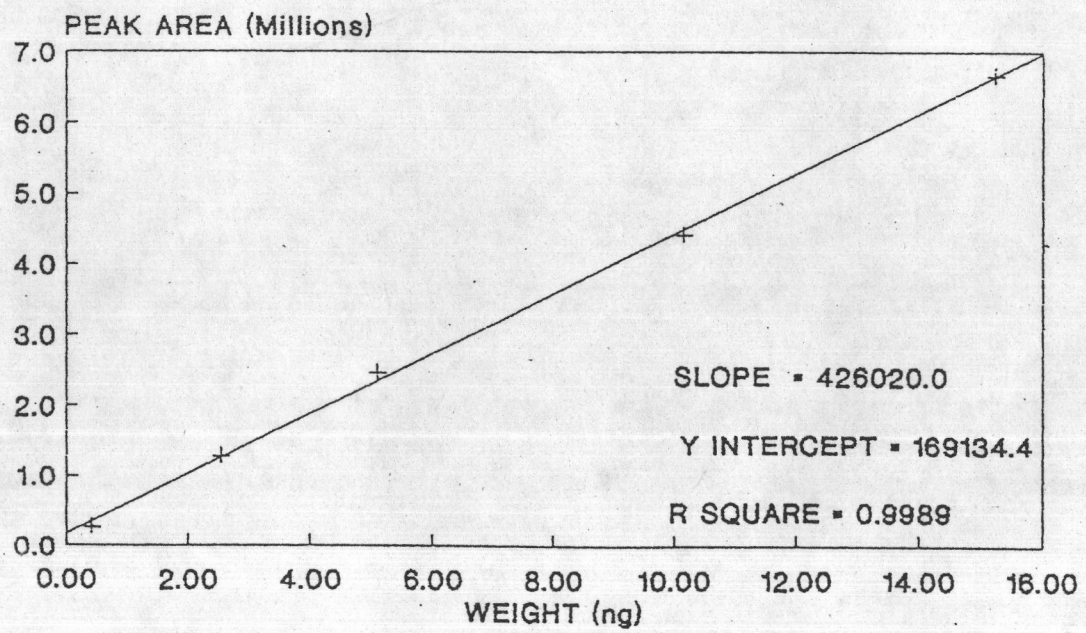

Figure 3.20 The calibration curve of 1,1,1-trichloroethane in hexane using HP-5 capillary column with FID a detector.

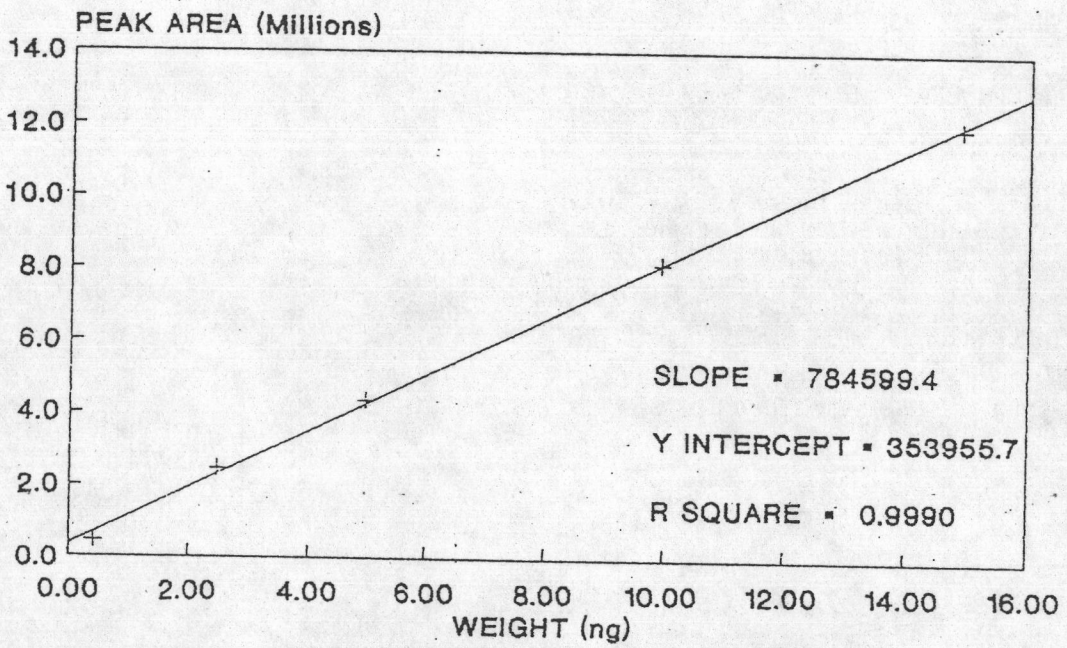

Figure 3.21 The calibration curve of trichloroethylene in hexane using HP-5 capillary column with ECD as a detector.

3.9 The Procedure for Checking the Accuracy of Headspace Analysis Technique.

The synthetic unknown mixture solutions in methanol were prepared to evaluate the accuracy of headspace analysis technique. The concentration of each volatile organic compound ,i.e., methylene chloride, chloroform, carbon tetrachloride, 1,1,1-trichloroethane and trichloroethylene was determined by means of the internal standardization method as described in section 3.6.1 using 2-bromo-1-chloropropane as the internal standard under the optimum headspace analysis condition found in the previous studies and GC condition in Table 3.8. The procedure was described as follow:

1. Pipet 30.00 mL of distilled water, inject 10.00 μL of 983.68 ppm 2-bromo-1-chloropropane and inject 10.00 μL of the synthetic unknown solution in methanol into a 60 mL serum vial containing 13.00 g anhydrous sodium sulfate.

2. Close the vial with aluminum foil, black rubber septum and aluminum cap sequentially, and then tightly crimp the vial with Manual Hand Operated Crimper.

3. Shake vigorously the sealed serum vial for about 1 min and place it into a constant temperature water bath which the temperature is set at 60.0 ^{o}C for 60 min.

4. Withdraw 1.50 mL of vapor phase from the serum vial by using 2.00 mL Pressure-Lok series A2 gas tigh syringe and inject into gas chromatograph under GC condition as described in Table 3.8.

5. Determine the initial concentration of each volatile

organic compound in aqueous solution (C^{o}_{1}) from the absolute internal standard calibration curves as shown in Figures 3.22 - 3.26 or using equation 3.4.

3.10 The Determination of Volatile Chlorinated Hydrocarbons in Real Samples.

Water samples collected from several places were analyze by headspace analysis technique under the optimum headspace condition and under the GC condition as mentioned in table 3.8. The concentration of the interested component in water samples were determined by means of internal standardization methods. The procedure was described as the follow:

1. Pipet 30.00 mL of water sample and inject 10.00 L of 983.68 ppm 2-bromo-1-chloropropane into a 60 mL serum vial containing 13.00 g anhydrous sodium sulfate.

2. Close the vial with aluminum foil, black rubber septum and aluminum cap sequentially, and then tightly crimp the vial with Manual Hand Operated Crimper.

3. Shake vigorously the sealed serum vial for about 1 min and place it into a constant temperature water bath which the temperature is set at 60.0°C for 60 min.

4. Withdraw 1.50 mL of vapor phase from the serum vial by using 2.00 mL Pressure-Lok series A2 gas tigh syringe and inject into gas chromatograph under GC condition as described in Table 3.8.

5. Determine the initial concentration of each chlorinated

hydrocarbon in aqueous solution (C^{o}_{1}) from the absolute internal standard calibration curves as shown in Figures 3.22 - 3.26 or using equation 3.4.

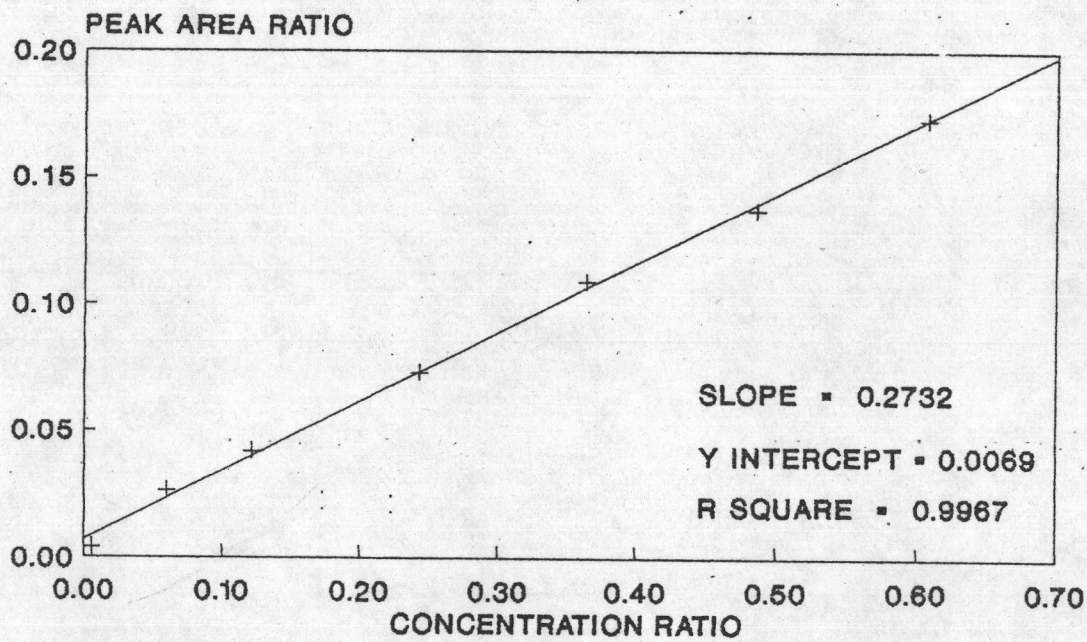

Figure 3.22 The internal standard calibration curve of methylene chloride.

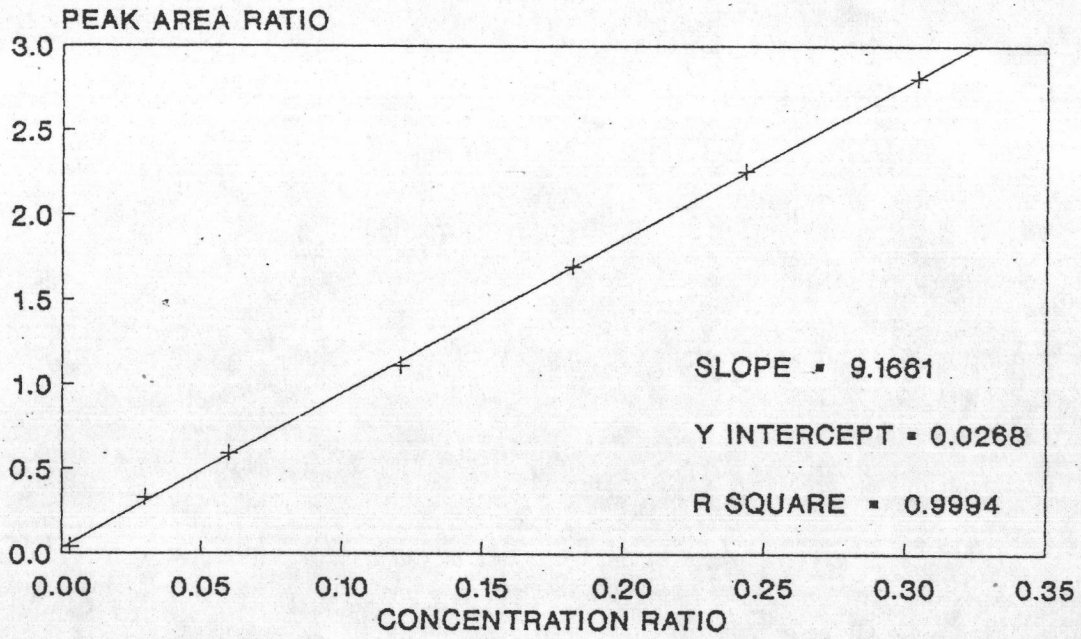

Figure 3.23 The internal standard calibration curve of chloroform.

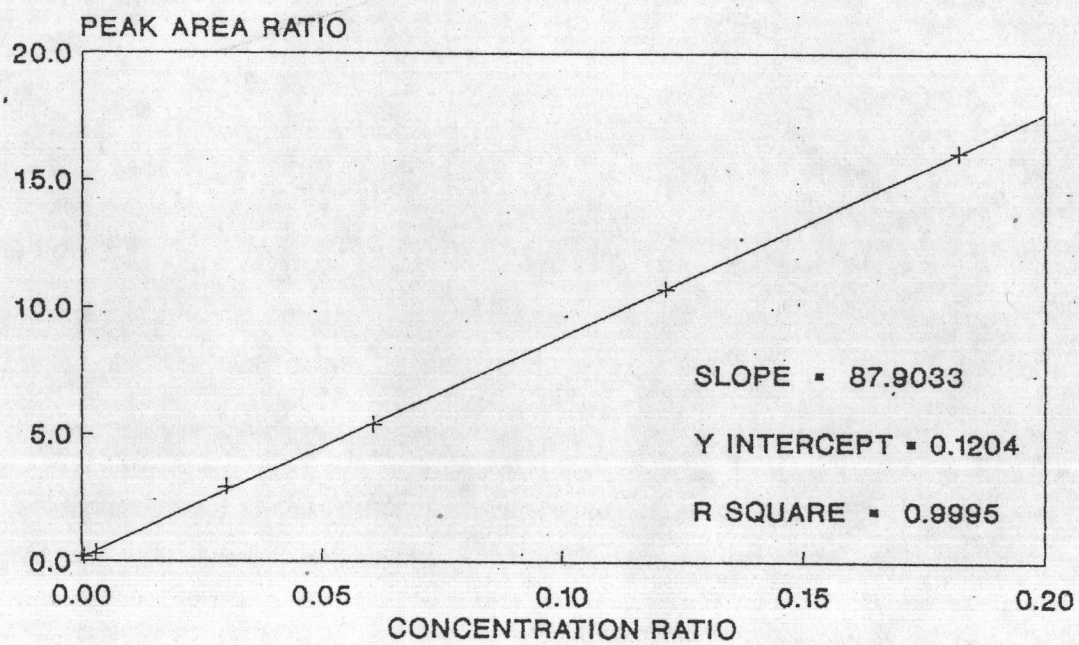

Figure 3.24 The internal standard calibration curve of carbon tetrachloride.

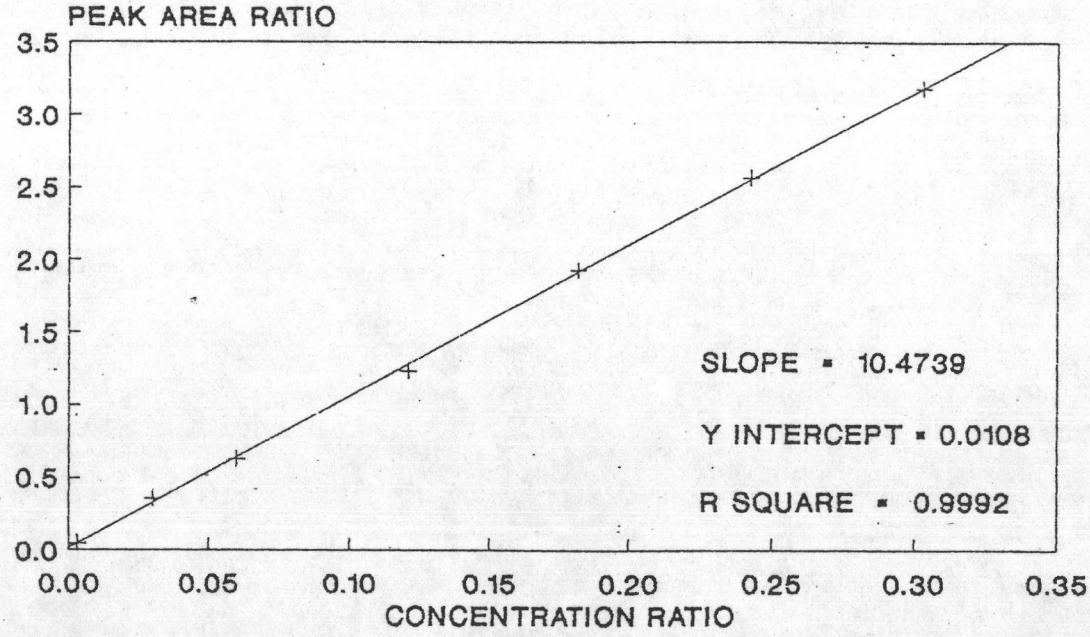

Figure 3.25 The internal standard calibration curve of 1,1,1-trichloroethane.

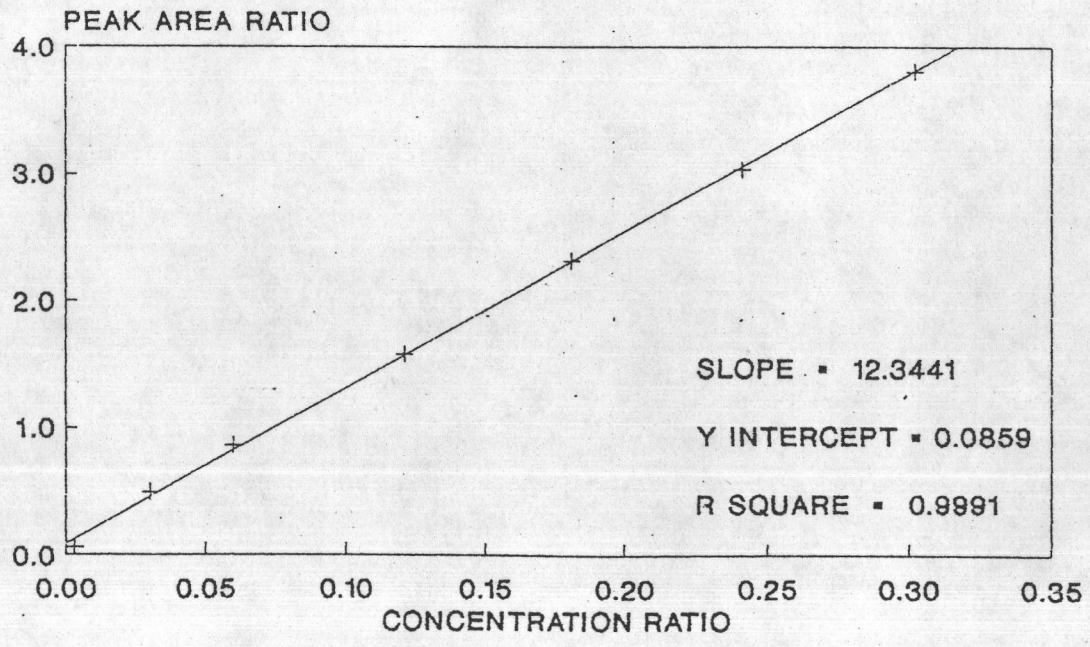

Figure 3.26 The internal standard calibration curve of trichloroethylene.