CHAPTER III

EXPERIMENTAL

Apparatus

A Hewlett Packard HP 5890 gas chromatrograph equipped with flame Ionization Detector (FID) , Electron Capture Detector (ECD) and Hewlett Packard HP 3394A integrator.

A Hewlett Packard HP 19395A automated headspace.

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

Gas tight syringe 1 mL (Hamilton Company , Switzerland).

Graduated pipettes 1.00, 2.00 and 5.00 mL.

Volumetric pipettes 1.00 , 2.00 , 5.00 , and 10.00 mL.

Vials , 20.00 mL.

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

10 mL. vials for headspace (see appendix A).

Gray septa , aluminum cap. (see appendix A).

Manual Hand Operated Crimper (Hewlett Packard). (see Appendix A)

Adapter , vial (for 10 mL.)

All glasswares , including vials and vial for headspace were cleaned with detergent , diluted HNO_3 (1:1) , water and rinsed with double distilled water respectively and drived in an oven at 150 °C for 3 hours.

Chemicals

1. The Standard of Volatile Organic Compounds

Methylene chloride , Chloroform and Benzene which were analytical reagent grade were purchased from J.T. Baker Chemical Company , Deventer , Holland.

Trichloroethylene and 1,4-Dioxane which were analytical reagent grade were purchased from Carlo Erba , Italy.

All of the standard chemical which had percent purity more than 99.5 and they were checked for the purity by gas chromatograph prior to use in the study.

Table 3.1 The density of the standard of volatile organic compounds used in the headspace study.

Name of Compound	Density at 20 °C (g/mL)
Methylene Chloride (CH_2Cl_2)	1.316
Chloroform ($CHCl_3$)	1.473
Benzene (C_6H_6)	0.874
Trichloroethylene (TCE)	1.462
1,4-Dioxane ($C_4H_8O_2$)	1.034

2. Organic Solvents

Dimethyl Sulfoxide which were analytical reagent grade (AR Grade) was purchased from Fuka A.G. , Switzerland. It was checked for the purity by gas chromatograph prior to use in the study.

3. Salts

Anhydrous sodium sulfate (AR Grade) was obtained from J.T. Baker Chemical Company , Deventer , Holland. They were heated in an oven at 150 °C for 2 hours and were kept in descicator before used.

4. Double Distilled Water

The double distilled water was distilled by the Yamato distillation model WA-52R (Yamato Scientific Co.,Ltd. Tokyo , Japan).

Preparation of the Standard Solution

1. The Standard Solution for the Headspace Study Using GC with FID as a detector

1.1 The Single Component Standard Stock Solution of Methylene Chloride , Chloroform , Benzene , Trichloroethylene and 1,4-Dioxane in Dimethyl Sulfoxide (DMSO)

A single component standard stock solution of each volatile organic compound i.e. , methylene chloride , chloroform , benzene , trichloroethylene and 1,4-dioxane in DMSO were prepared measuring each volatile organic compounds by microsyringe and diluting it with DMSO to the mark in 25.00 mL. volumetric flasks. The measuring volume of each standard volatile organic compound was calculated from the density as shown in Table 3.1 and its concentration were shown in Table 3.2

Table 3.2 The measuring volume standard volatile organic compounds and the concentration of the single component standard stock solution in DMSO

Compounds	The volume of standard (μL)	The concentration of the single standard stock solution (ppm)
Methylene chloride	20.00	1052.80
Chloroform	18.00	1060.56
Benzene	30.00	1048.80
Trichloroethylene	18.00	1052.64
1,4-Dioxane	80.00	3308.80

1.2 The Single Standard Solution of Methylene chloride . Chlorofom , Benzene and Trichloroethylene in DMSO.

A single standard solution containing 210.56 , 212.11, 209.76 and 210.53 ppm of methylene chloride ,chloroform , benzene and trichloroethylene , respectively in DMSO were prepared by pipetting 20.00 mL of methylene chloride , chloroform , benzene and trichloroethylene of each single standard stock solution as shown in Table 3.2 into a 100.00 mL volumetric flask and diluting to the mark with DMSO.

1.3 The Standard Mixture Solution of Methylene chloride .Chlorofom , Benzene .Trichloroethylene and 1.4- Dioxane in Water.

A standard mixture solution containing 21.06 , 21.21, 20.98 , 21.05 and 496.32 ppm of methylene chloride , chloroform , benzene trichloroethylene and 1,4- Dioxane, respectively in water were prepared by pipetting 10.00 mL of methylene chloride ,chloroform ,benzene and trichloroethylene of each single standard solution in section 3.3.1.2 and15.00mL of 1,4-Dioxane single stock standard solution in Table 3.2 into a 100.00 mL volumetric flask and diluting to the mark with water.

2. The Standard solutions for the Headspace Studv Using GC with ECD as a detector.

2.1 The Single Component Standard Stock Solution of Methylene chloride , Chloroform , Benzene , Trichloroethylene and 1,4-Dioxane in DMSO .

The Single Component Standard Stock Solution of Methylene chloride , Chloroform , Benzene , Trichloroethylene and 1,4-Dioxane in DMSO were prepared by measuring the standard volatile organic compounds by microsyringe and diluting it with DMSO to the mark in 25.00 mL volumetric flasks. the volume of each volatile organic compound was calculated from the density as shown in Table 3.1 and its concentration were shown in Table 3.3.

Table 3.3 The measuring volume of standard volatile organic compounds and the concentration of the single component standard stock solution in DMSO

Compound	The Volume of Standard (μL)	The Concentration of the Single Standard Solution (ppm)
Methylene Chloride	20.00	1052.80
Chloroform	18.00	1060.56
Benzene	150.00	5244.00
Trichloroethylene	18.00	1052.64
1,4 Dioxane	130.00	5376.80

2.2 The Single Standard Solution of Methylene Chloride, Chloroform and trichloroethylene in DMSO.

The standard single solutions of 105.28 ppm methylene chloride, 106.05 ppm chloroform and 105.26 ppm trichloroethylene in DMSO were prepared by pipetting 10.00 mL methylene chloride , chloroform and trichloroethylene of each single standard stock solution as shown in Table 3.3 into a 100.00 mL volumetric flask and diluting to the mark with DMSO.

2.3 The Standard Mixture Solution of Methylene Chloride, Chloroform ,Benzene ,trichloroethylene and 1,4-Dioxane in Water

The standard mixture solutions of 4.21 ppm methylene chloride 4.24 ppm chloroform , 2097.60 ppm benzene , 4.21 ppm trichloroethylene and 2150.72 ppm 1,4-dioxane in water were prepared by pipetting 4.00 mL methylene chloride , chloroform and trichloroethylene of each single standard solution form 3.3.2.2 and pipetting 40.00 mL benzene and 1,4-dioxane of each standard stock solution as shown in Table 3.3 into a 100.00 mL volumetric flask and diluting to the mark with water.

3. The Standard Solution for the Calibration Curve using GC with FID as a detector.

3.1 The Single Component Standard Stock Solution of Methylene chloride , Chloroform , Benzene , Trichloroethylene and 1,4-Dioxane in DMSO.

The single component standard stock solution of 1052.8 ppm methylene chloride , 1060.56 ppm chloroform , 1048.80 ppm benzene, 1052.64 ppm trichloroethylene and 3308.80 ppm 1,4-dioxane in DMSO were prepared by transfering 20.00 , 18.00 , 30.00 , 18.00 and 80.00 μL of methylene chloride, chloroform , benzene , trichloroethylene and 1,4-dioxane into each 25.00 mL volumetric flask containing DMSO about 10.00 mL , diluting each flask to the mark and mixing throughly.

3.4 The Standard Solution for the Calibration Curve Using GC with ECD as a Detector .

The single component standard component stock solution of 1052.80 ppm methylene chloride , 1060.56 ppm chloroform and 1052.64 ppm trichloroethylene in DMSO were prepared by transfering 20.00 μL of methylene chloride , 18.00 μL of chloroform and 18.00 μL of trichloroethylene into each 25.00mL volumetric flask containing DMSO about 10.00 mL , diluting each flask to the mark and mixing throughly.

Gas Chromatographic Conditions

Table 3.4 The gas chromatographic condition used FID as a detector for the study of mixture component.

GC Parameter	GC Condition
Analytical Column	25 m x 0.32 mm x 1.05 μm film thickness., HP-5 (5% Phenyl Methyl Silicone) Capillary column
Temperature Program	A. 35 °C(5 min) to 100 °C(0 min) at 8 °C/min B. 100 °C(0min) to 260 °C(5min) at 30 °C/min
Split Ratio	20:1
Splitless Time	0.0 min
Flow Rate of Carrier Gas (He)	1.5 mL/min
H_2	40.0 mL/min
Air	380.0 mL/min
N_2	30.0 mL/min
Detector	Flame Ionization Detector (FID)
Detector Temperature	260 °C
Inlet Temperature	180 °C

Table 3.5 The gas chromatographic condition used ECD as a detector for the study of mixture component.

GC Parameter	GC Condition
Analytical Column	25 m x 0.32 mm x 1.05 μm film thickness., HP-5 (5% Phenyl Methyl Silicone) Capillary column
Temperature Program	A. 35 °C(5 min) to 100 °C(0 min) at 8 °C/min B. 100 °C(0min) to 260 °C(5min) at 30 °C/min
Split Ratio	20:1
Splitless Time	0.0 min
Flow Rate of Carrier Gas (He)	1.5 mL/min
N_2	40.0 mL/min
Detector	Electron Capture Detector (ECD)
Detector Temperature	300 °C
Inlet Temperature	180 °C

The Study of the Various Parameters on the Sensitivity of Headspace Technique by using FID as a detector.

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

1. Temperature

The procedure for the study of effect of the temperature i.e., 60 , 70 ,80 and 90 °C on the sensitivity of headspace technique was described as the follow:

1. Pipet 4.00 mL of water into a series of 10 mL vial for headspace.
2. Pipet 1.00 mL of the standard mixture solution in water into the series of 10 mL vial for headspace .
3. Close each vial with gray septum and aluminum cap , and then tightly crimp it with Manual Hand Operated Crimper.
4. Shake the sealed vial about 1 min and place in constant temperature automated headspace which is set at various temperature i.e., 60, 70, 80 and 90.0 °C for 40 minutes.
5. When it reaches the time as set in step 4 , inject 1.00 mL of vapor phase from sample vial with programming from the automated headspace into gas chromatograph under the GC condition as described in Table 3.4 .
6. Determine the concentration of interested volatile component in vapor phase (C_g) by means of external standardization method , then calculate the distribution coefficient of each interested compound (K).

7. Calculate the sensitivity (s) of interested volatile compound from equation (2.19).

8. Plot the sensitivity (s) and the distribution coefficient (K) against the Temperature (°C).

The optimun temperature of each volatile organic compound found in this section would be used in the next study.

2. Equilibration Time

The procedure for the study of effect of the temperature i.e., 20 , 30 , 40 , 50 , 60 , 70 , 80 , 100 and 120 minutes on the sensitivity of headspace technique was described as the follow:

1. Pipet 4.00 mL of water into a series of 10 mL vial for headspace.

2. Pipet 1.00 mL of the standard mixture solution in water into the series of 10 mL vial for headspace .

3. Close each vial with gray septum and aluminum cap , and then tightly crimp it with Manual Hand Operated Crimper.

4. Shake the sealed vial about 1 min and place in constant temperature automated headspace which is the temperature is set at 70.0 °C for 20 30 , 40 , 50 , 60 , 70 , 80 , 100 and 120 minutes.

5. When it reaches the time as set in step 4 , inject 1.00 mL of vapor phase from sample vial with programming from the automated headspace into gas chromatograph under the GC condition as described in Table 3.4.

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

The optimun temperature of each volatile organic compound found in this section would be used in the next study.

3. Liquid to Gas Phase Volume Ratio

The liquid to gas phase volume ratio , i.e.,2:8 , 4:6 , 5:5 , 6:4 and 8:2 of each volatile organic compound were studied in order to be able to determine the optimum phase ratio. The procedure for this study was described as the follow:

1. Pipet 2.00 , 4.00 , 5.00 ,6.00 and 8.00 mL of the standard mixture solution in water into a series of 10 mL vial for headspace.
2. Close each vial with gray septum and aluminum cap , and then tightly crimp it with Manual Hand Operated Crimper.
3. Shake the sealed vial about 1 min and place in constant temperature automated headspace which is set at the optimum temperature section 3.5.1 .
4. When it reaches the time as set in step 3 , inject 1.00 mL of vapor phase from sample vial with programming from the automated headspace into gas chromatograph under the GC condition as described in Table 3.4.
5. Determine the concentration of interested volatile compound in vapor phase (C_g) by means of external standardization method , then calculate the distribution coefficient of each interested component (K).
6. Calculate the sensitivity (s) of interested volatile compound from equation (2.19).
7. Plot the sensitivity (s) and the distribution coefficient (K) against the phase ratio (V_l / V_g).

The optimun liquid to gas phase ratio of each volatile organic compound found in this section would be used in the next study.

4. Salting-out Effect

The temperature , equilibration time and liquid to gas phase volume ratio of each interested compound were studied and were calculated 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 mixture standard solution and each study was consisted of two systems:

1. No addition of salt (no salting-out).
2. 1.00g of anhydrous sodium sulfate .

The procedure for this study was described as follow:

1. Weight 1.00g of anhydrous sodium sulfate in a series of 10 mL vials for headspace .

2. Pipet the volume of water which is equal to the optimum volume found in section 3.5.3 and Pipet 1.00 mL (for FID as a detector) and 24.00 μL (for ECD as a detector) of the standard mixture solution in water into the series of 10 mL vial for headspace containing no salt and 1.00 g of anhydrous sodium sulfate.

3. Close each vial with gray septum and aluminum cap , and then tightly crimp it with Manual Hand Operated Crimper.

4. Shake the sealed vial about 1 min and place in constant temperature automated headspace which is the temperature is set at the optimum temperature as found in section 3.5.1 .

5. When it reaches the time as set in step 4 , inject 1.00 mL of vapor phase from sample vial with programming from the automated headspace into gas chromatograph under the GC condition as descriebed in Table 3.4 and Table 3.5.

6. Determine the distribution coefficient (K) , sensitivity (S) and percent recovery (%E) of each volatile organic compound and compare the results of the two systems study , no salt and 1.00 g anhydrous sodium sulfate .

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

The optimum no salt or 1.00g anhydrous sodium sulfate of each compound found in this study would be used in the study of minimum detectable level and sample analysis.

Method Detection Limit (MDL)

Method detection limit of each volatile organic compound was determined by using the optimum condition of temperature, time ,liquid to gas phase ratio and salting-out effect. 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 method detection limit of the component.

Quantitative Headspace Analysis

Any tradition quantitative gas chromatographic techniques can be used to determine the initial concentration (C^{o}_{l}) of a substance in solution from its concentration in the gas phase . The method of external standardisation was chosen as the quantitative method for the determination of the initial concentration of each volatile organic compound in unknown aqueous solutions. The principle of this quantitative methods is described in the following causes.

1. The External Standardisation Method

The quantitative analysis in the headspace technique can be carried out in two different methods:

1.1 When the Distribution Coefficient (K) of the Interested Compounds is Known.

The intial concentration of each interested compound in aqueous solution , C^{o}_{l} , can be determined by using Equation (3.1). Since the volume V_l of the liquid phase , the volume of the gas phase V_g , the distribution coefficient K , of each interested compound is known and the equilibrium concentration of each interested compound , C_g , in the equilibrated unknown sample can be determined by means of external standardisation method as described in section 3.8, hence , by substitution the value of C_g , K , V_g ,and V_l into Equation (3.1) , the initial concentration of the interested compound , C^{o}_{l} , in the aqueous solution can be easily calculated.

$$C^{o}_{l} = C_g \left(K + \frac{V_g}{V_l} \right)$$

1.2 When the Distribution Coefficient (K) of the Interested Compounds is not Known.

A various concentrations of standard mixture of interested compounds in aqueous solution are prepared for constructing the standard calibration curves of each interested compound . These standard mixtures in aqueous solution are analyzed by the headspace technique under the identical condition and GC condition as the analysed sample. Then the peak area of each standard compound obtained from the chromatogram ($A_{g,st}$) is plotted against the initial concentration of the standard ($C^{o}_{l,st}$). 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

$$A_{g,st} = m C^{o}_{l,st} + b \qquad (3.2)$$

The exact volume of the vapor phase from the equilibrated unknown sample is then chromatographed . Finally , the initial concentration of the interested compound i in the aqueous solution ($C^{o}_{l,i}$) can be determined by substitution the peak area $A_{g,i}$ of the interested compound into the equation (3.2)

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

In order to determine the equilibrium concentration of the interested compound in vapor phase ($C_{g,i}$) , the calibration curve of each interested compound must be constructed . Thus , a series of standard solution containing the known weight of the components in DMSO were prepared and chromatographed under the identical GC condition as analysed 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.

$$A = mW + b \qquad (3.3)$$

The exact volume of the vapor phase from the equilibrated headspace sample (V_g) was then chromatographed . The computation of the interested component i in gas phase ($W_{g,i}$) can be compared graphically to the constructed by substitution the peak area of the interested component i ($A_{g,i}$) into equation (3.2) and the equilibration 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

$$C_{g,i} = \frac{W_{g,i}}{C_g} \qquad (3.4)$$

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

V_g = Injection volume of gas phase

The calibration curve of each standard compound including methylene chloride , chloroform , benzene , trichloroethylene and 1,4-dioxnne in DMSO used in the determination of the weight of the interested component were in Figure 3.1 , 3.2 , 3.3 ,3.4 and 3.5 , respectively, for GC Condition in Table 3.4 and Figure 3.6 , 3.7 and 3.8 of methylene chloride , chloroform and trichloroethylene , respectively for GC Condition in Table 3.5.

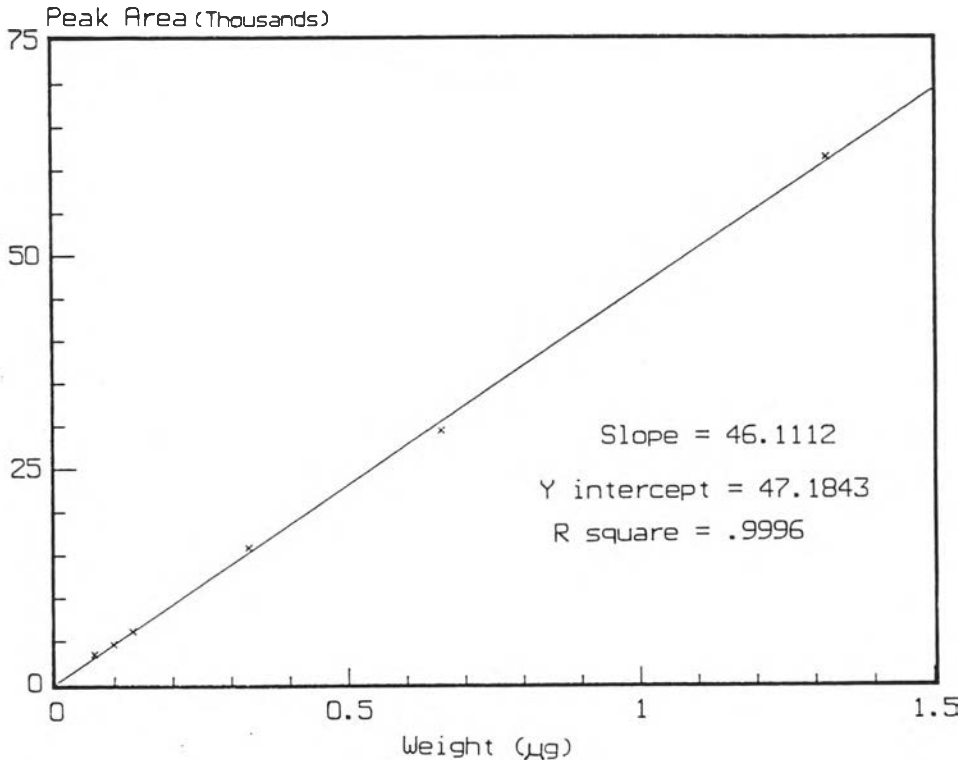

Figure 3.1 The calibration curve of methylene chloride in dimethyl sulfoxide (DMSO) using FID as a detector.

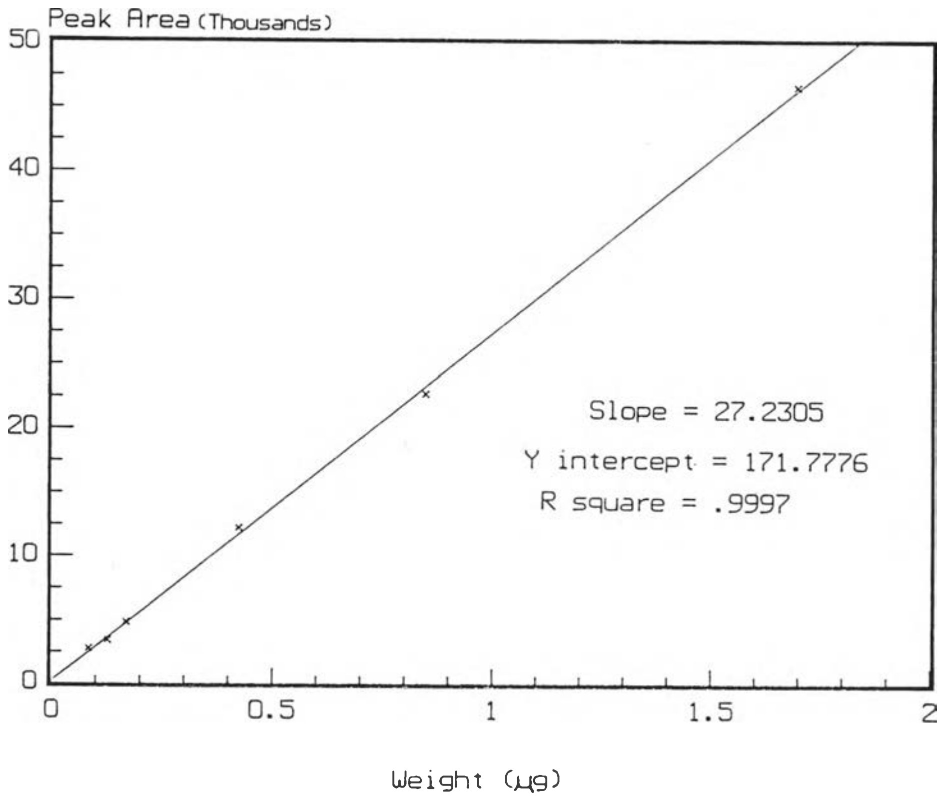

Figure 3.2 The calibration curve of chloroform in dimethyl sulfoxide (DMSO) using FID as a detector.

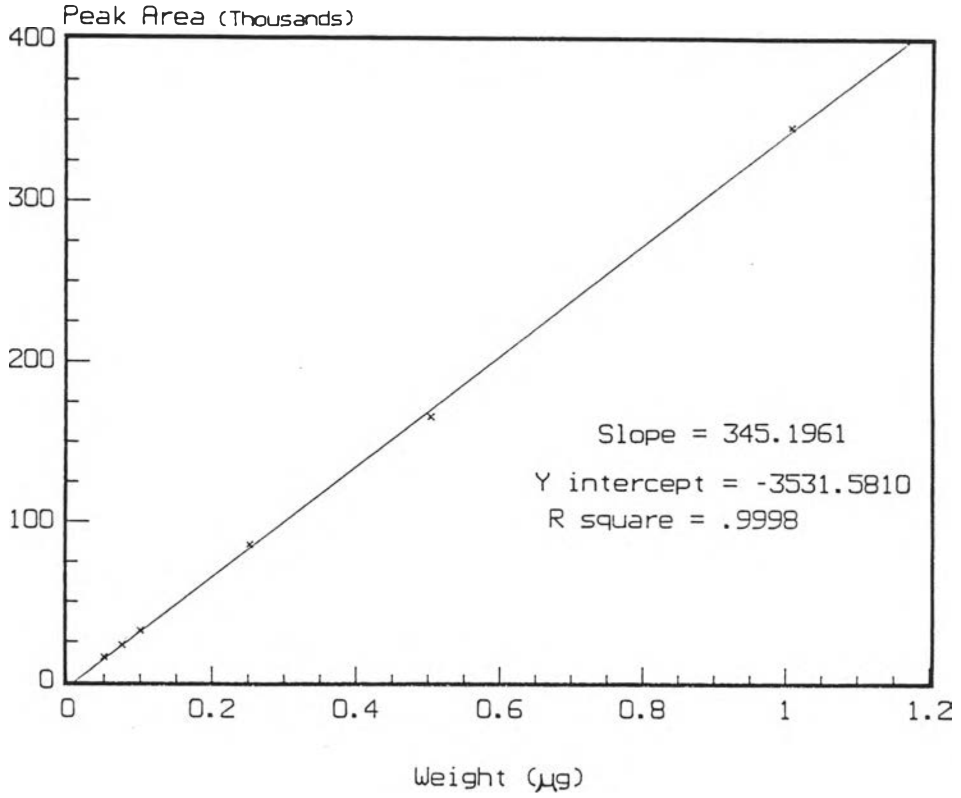

Figure 3.3 The calibration curve of benzene in dimethyl sulfoxide (DMSO) using FID as a detector.

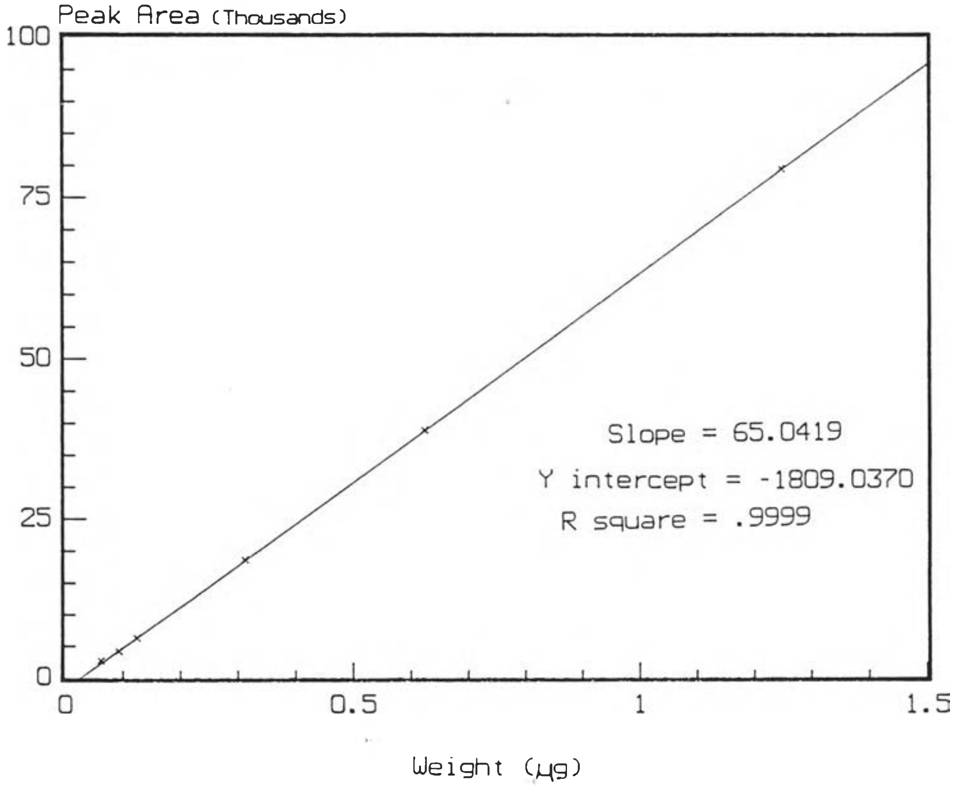

Figure 3.4 The calibration curve of trichloroethylene in dimethyl sulfoxide (DMSO) using FID as a detector.

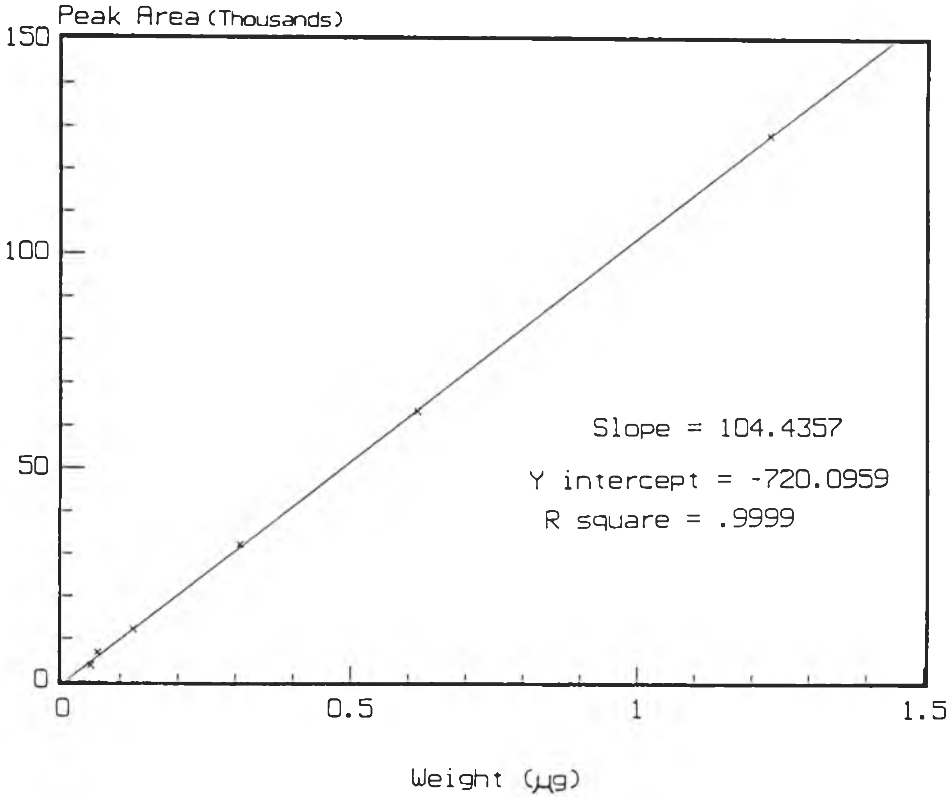

Figure 3.5 The calibration curve of 1,4-dioxane in dimethyl sulfoxide (DMSO) using FID as a detector.

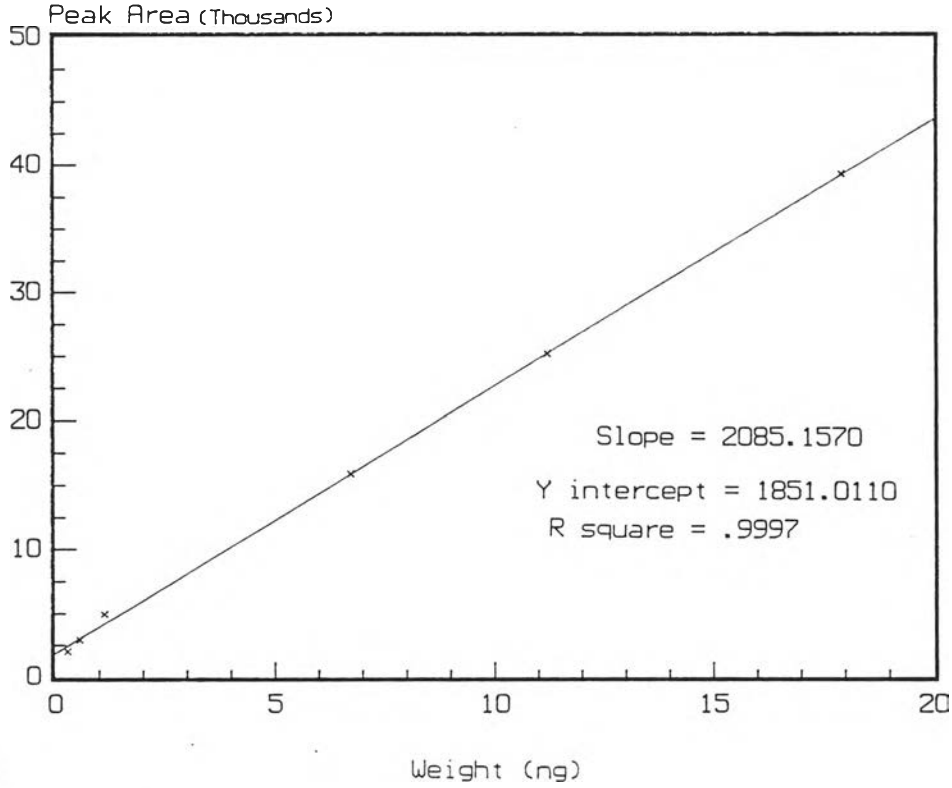

Figure 3.6 The calibration curve of methylene chloride in dimethyl sulfoxide (DMSO) using ECD as a detector.

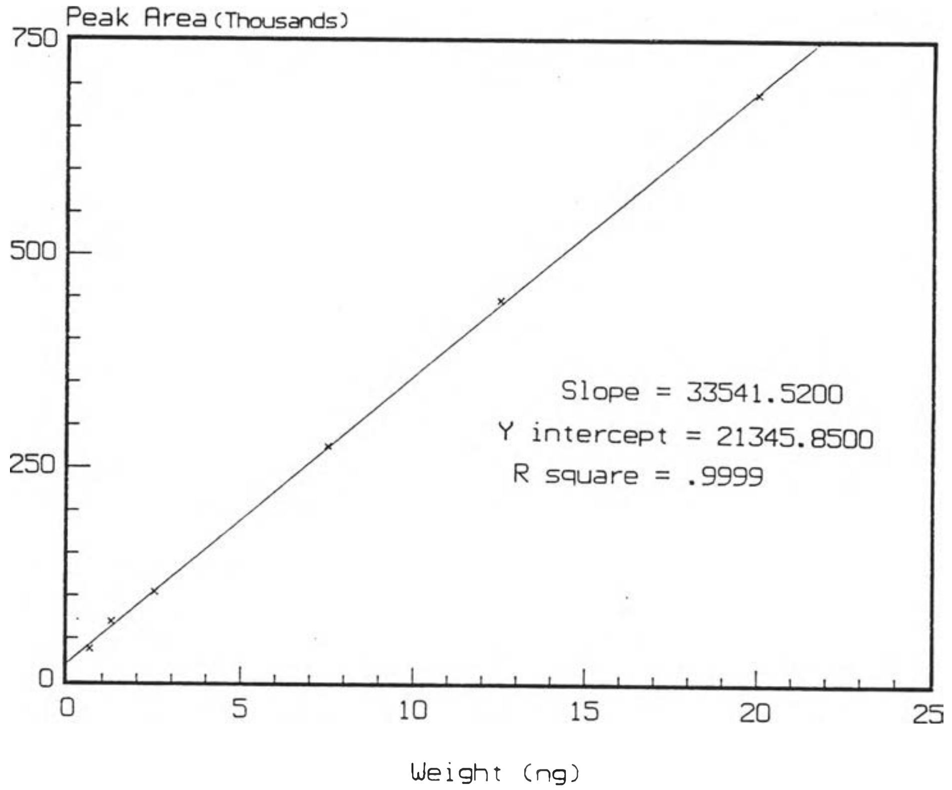

Figure 3.7 The calibration curve of chloroform in dimethyl sulfoxide (DMSO) using ECD as a detector.

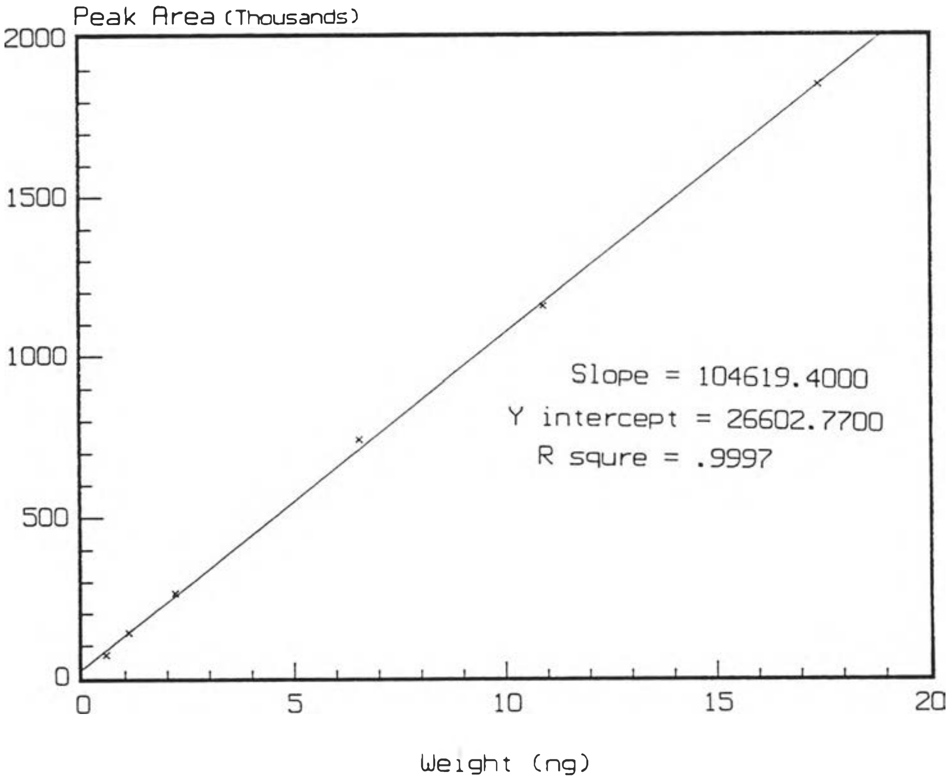

Figure 3.8 The calibration curve of trichloroethylene in dimethyl sulfoxide (DMSO) using ECD as a detector.

The Study of Precision of Automated Headspace Technique for Determination Volatile Organic Compounds in Aqueous Solution

The study of precision has been used the optimum conditions from the previous studies in Table 4.31 and the GC condition in Table 3.4 and 3.5 have been employed for this study . The procedure for the study of precision can be described as follows:

1. The Procedure for the Study of Precision of Each Volatile Organic Compound Using Using GC with FID as a Detector.

1. Ten 5.00 mL of the standard mixture solution of 4.21 ppm methylene chloride , 4.24 ppm chloroform , 4.19 ppm benzene , 4.21 ppm trichloroethylene and 99.26 ppm 1,4-dioxane in water into a series of 10 mL vial for headspace containing 1.00 g anhydrous sodium sulfate.
2. Close each vial with gray septum and aluminum cap , and then tightly crimp it with Manual Hand Operated Crimper.
3. Shake the sealed vial about 1 min and place in constant temperature automated headspace which the temperature is set at 70 °C for 40 minutes .
4. Inject 1.00 mL of vapor phase from sample vial with programmig from the automated headspace into gas chromatograph under the GC condition in Table 3.4.
5. Determine percent relative standard deviation (%RSD).

2. The Procedure for the Study of Precision of Each Volatile Organic Compound Using GC with ECD as a Detector.

1. Ten 5.00 mL of the standard mixture solution of 20.21 ppb methylene chloride , 20.36 ppb chloroform and 20.36 ppb trichloroethylene in water into a series of 10 mL vial for headspace containing 1.00 g anhydrous sodium sulfate.

2. Close each vial with gray septum and aluminum cap , and then tightly crimp it with Manual Hand Operated Crimper.

3. Shake the sealed vial about 1 min and place in constant temperature automated headspace which the temperature is set at 70 °C for 40 minutes .

4. Inject 1.00 mL of vapor phase from sample vial with programmig from the automated headspace into gas chromatograph under the GC condition in Table 3.4 .

5. Determine percent relative standard deviation (%RSD).

The Study of Linearity of Automated Headspace Technique for Determination Volatile Organic Compounds in Aqueous Solution

The linearity of each volatile organic compound was determined using the optimum condition of temperature, time, liquid to gas phase ratio and salting-out effect. The concentration of each interested compound which was studied by headspace technique used from minimum detectable level concentration to about 200 ppm by using FID as a detector and about 2 ppm by using ECD as a detector and studied the linearity range of each volatile organic compound.

1. The Procedure for the Study of Linearity of Each Volatile Organic Compound Using GC with FID as a Detector.

1. Pipet 5.00 mL of each concentration of the standard mixture solution in water into a series of 10 mL vial for headspace containing constaining 1.00 g anhydrous sodium sulfate.
2. Close each vial with gray septum and aluminum cap , and then tightly crimp it with Manual Hand Operated Crimper.
3. Shake the sealed vial about 1min and place in constant temperature automated headspace which the temperature is set at 70 °C for 40 minutes .
4. Inject 1.00 mL of vapor phase from sample vial with programmig from the automated headspace into gas chromatograph under the GC condition in Table 3.4 .
5. Plot the concentration against peak area.

2. The Procedure for the Study of Linearity of Each Volatile Organic Compound Using GC with ECD as a Detector.

1. Pipet 5.00 mL of each concentration of the standard mixture solution in water into a series of 10 mL vial for headspace containing 1.00 g anhydrous sodium sulfate.
2. Close each vial with gray septum and aluminum cap , and then tightly crimp it with Manual Hand Operated Crimper.
3. Shake the sealed vial about 1 min and place in constant temperature automated headspace which the temperature is set at 70 °C for 40 minutes .
4. Inject 1.00 mL of vapor phase from sample vial with programmig from the automated headspace into gas chromatograph under the GC condition in Table 3.5.
5. Plot the concentration against peak area

The Study of Accuracy of Automated Headspace Technique for Determination Volatile Organic Compounds in Aqueous Solution

To evaluate the accuracy of this technique , the synthetic unknown mixture solution in water were prepared . The concentration of each volatile organic compound i.e. , methylene chloride , chloroform , benzene , trichloroethylene and 1,4-dioxane for GC with FID as a detector was determined by means of the external standardization method as described in section 3.7.1 under the optimum headspace analysis condition found in the previous studied in Table 4.35 and GC condition in Table 3.4. The procedure for the study of accuracy can be described as follows:

1. The Procedure for the Study of Accuracy of Each Volatile Organic Compound Using GC with FID as a detector.

1. Pipet 5.00 mL of the synthetic unknown solution into a 10 mL vial for headspace containing 1.00 g anhydrous sodium sulfate .
2. Close the vial with gray septum and aluminum cap , and then tightly crimp it with Manual Hand Operated Crimper.
3. Shake the sealed vial about 1 min and place in constant temperature automated headspace which the temperature is set at 70 °C for 40 minutes .
4. Inject 1.00 mL of vapor phase from sample vial with programming from the automated headspace into gas chromatograph under the GC condition in Table 3.4.
5. Determine the initial concentration of each interested volatile component in aqueous solution(C_l^o) from the absolute calibration curve as shown in Figures 3.9 - 3.13 or using equation 3.2 .

The Determination of Volatile Organic Compound in Real Samples

Drug samples bought from serveral drugs and several companies are described as follows:

1. Amoxycillin from the Beechem Co.,Ltd.
2. Aspirin form the Unichem Co., Ltd.
3. Aspent from the Unichem Co.,Ltd.
4. B1,6,12 from the Merck Co.,Ltd.
5. B1,6,12 from the Olic Co.,Ltd.
6. B1,6,12 from the P.P.Lab Co.,Ltd.
7. B1,6,12 from the Takeda Co.,Ltd.
8. Brofen from the Pake-Davis Co.,Ltd.
9. Ibuprofen from the Pake-Davis Co.,Ltd.
10. Clamosin from the Biolab Co.,Ltd.
11. Erythromycin from the Pharmaceutical Organization
12. Erythromycin from the Abbott Co.,Ltd.
13. Erythromycin from the Servipharm Co.,Ltd.
14. Fuben 500 from the TO Co.,Ltd.
15. Stresstab 600+Zinc from the F.E. Zuellig Co.,Ltd.

Drug samples collected from several drugs and serveral companies were analyzed by headspace analysis technique under the optimum headspace condition are under the GC condition as mentioned in Table 3.4 and 3.5 . The concentration of the interested compound in drug samples were determined by means of the external standardization methods. The procedure was described as the follows:

1. Weigh 0.50 g of drugs powder into a 10 mL vial for headspace containing 1.00g anhydrous sodium sulfate.

2. Pipet 5.00 mL of water into a series of 10.00 mL vial for headspace containing drugs powder and anhydrous sodium sulfate from step 1.

3. Close the vial with gray septum and aluminum cap , and then tightly crimp it with Manual Hand Operated Crimper.

4. Shake the sealed vial about 1 min and place in constant temperature automated headspace which the temperature is set at 70 °C for 40 mintues .

5. inject 1.00 mL of vapor phase from sample vial with programmig from the automated headspace into gas chromatograph under the GC condition shown in Table 3.4 and 3.5.

6. Determine the initial concentration of each interested volatile component in aqueous solution(C^{o}_{l}) from the external standard calibration curve as shown in Figures 3.9- 3.13 using FID as a detector and Figures 3.14-3.16 for using ECD as a detector.

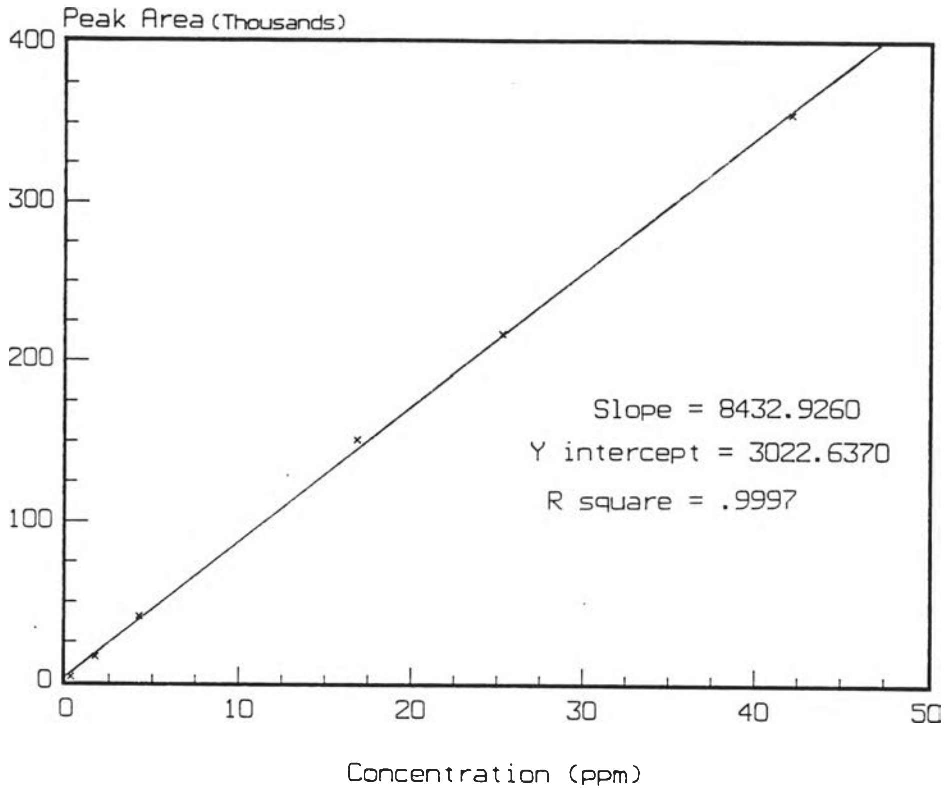

Figure 3.9 The external standard calibration curve of methylene chloride using FID as a detector.

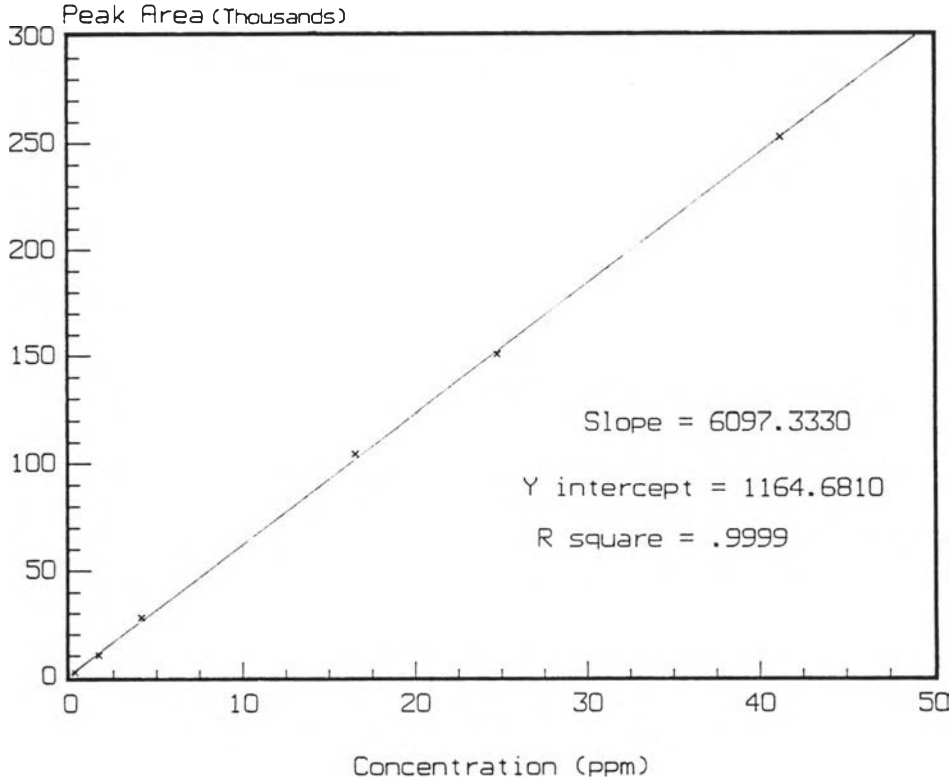

Figure 3.10 The external standard calibration curve of chloroform using FID as a detector.

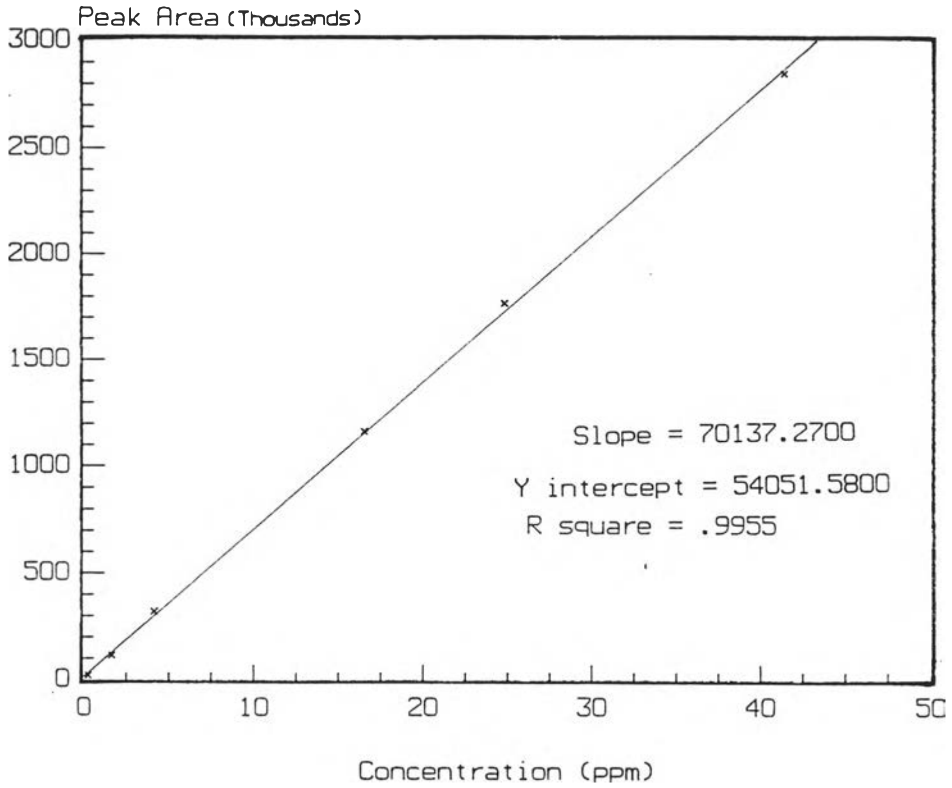

Figure 3.11 The external standard calibration curve of benzene using FID as a detector.

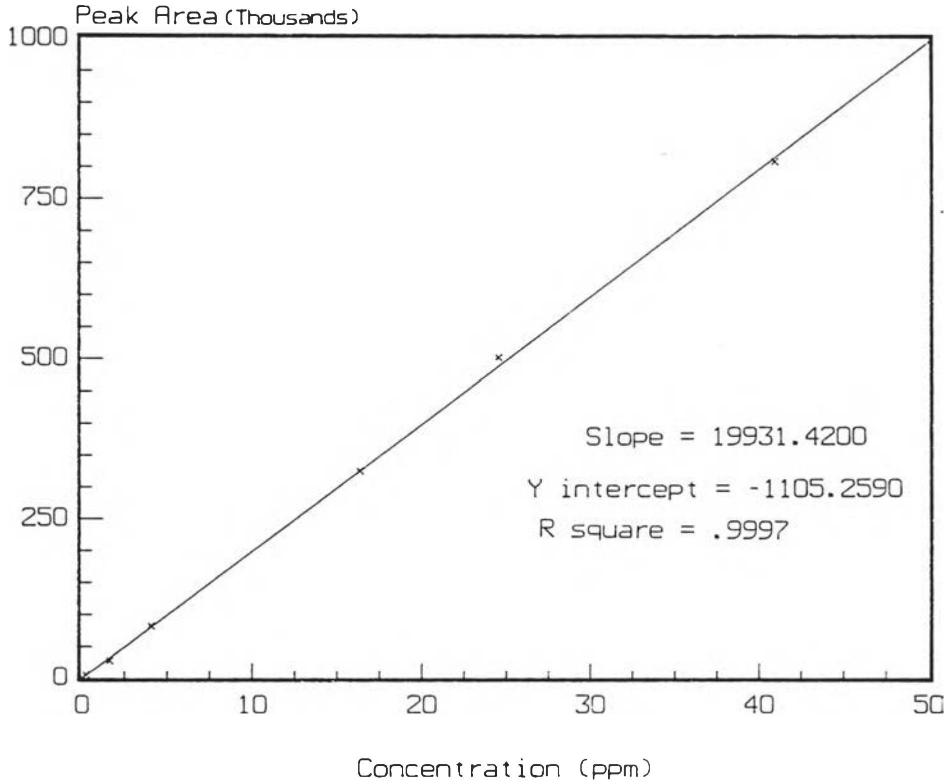

Figure 3.12 The external standard calibration curve of trichloroethylene using FID as a detector.

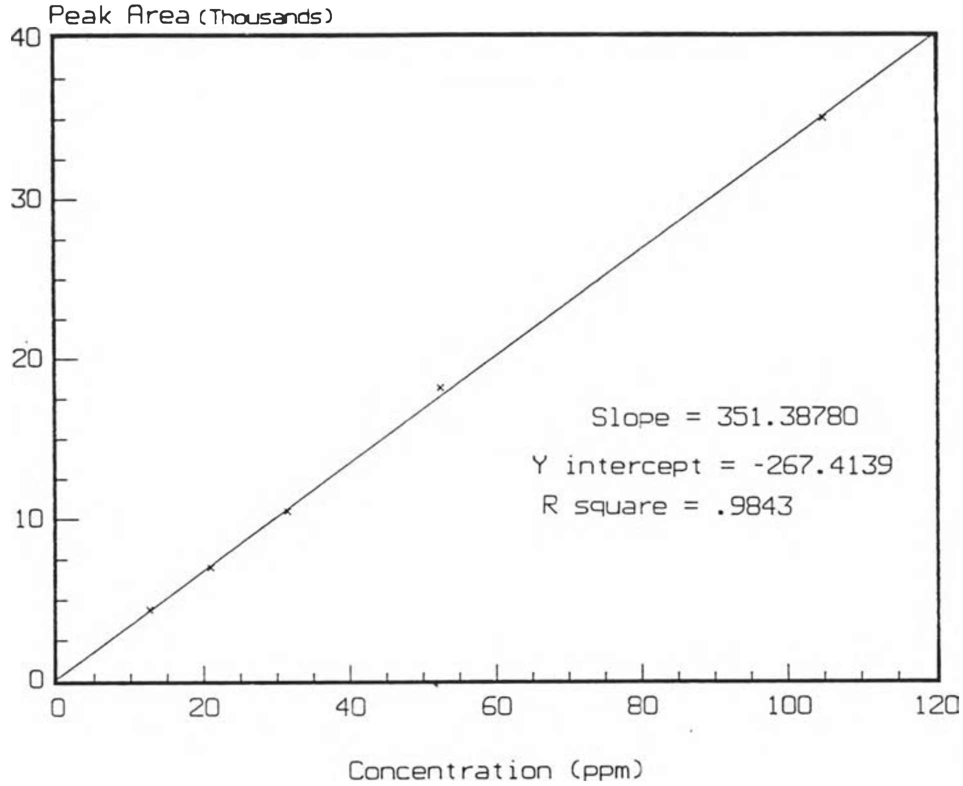

Figure 3.13 The external standard calibration curve of 1,4-dioxane using FID as a detector.

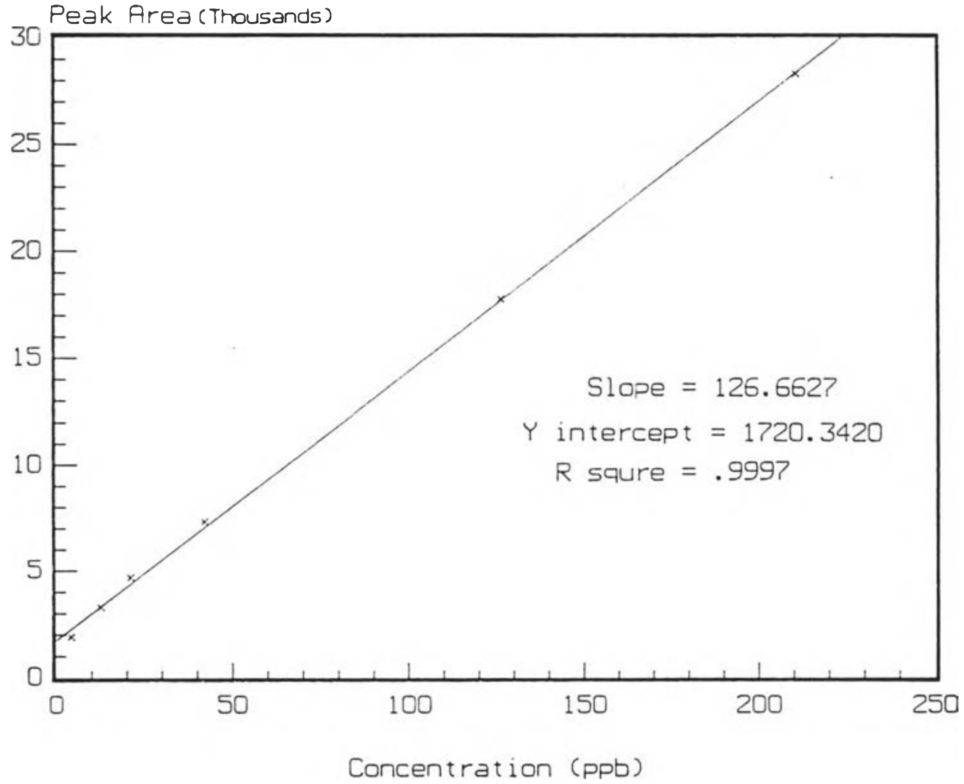

Figure 3.14 The external standard calibration curve of methylene chloride using ECD as a detector.

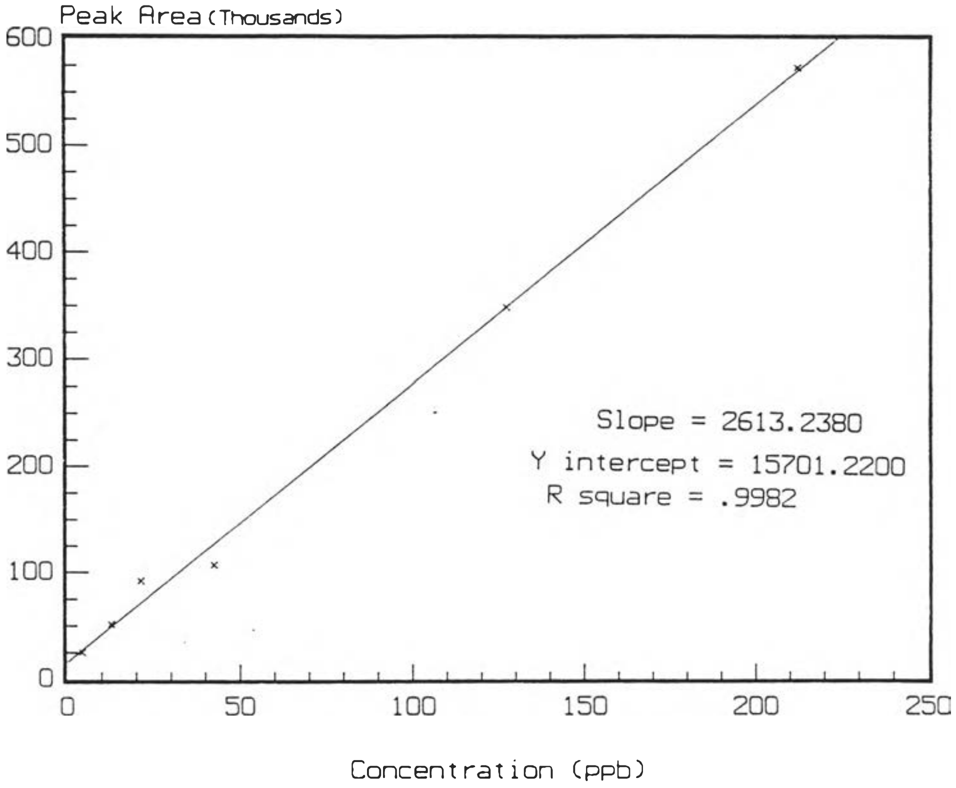

Figure 3.15 The external standard calibration curve of chloroform using ECD as a detector.

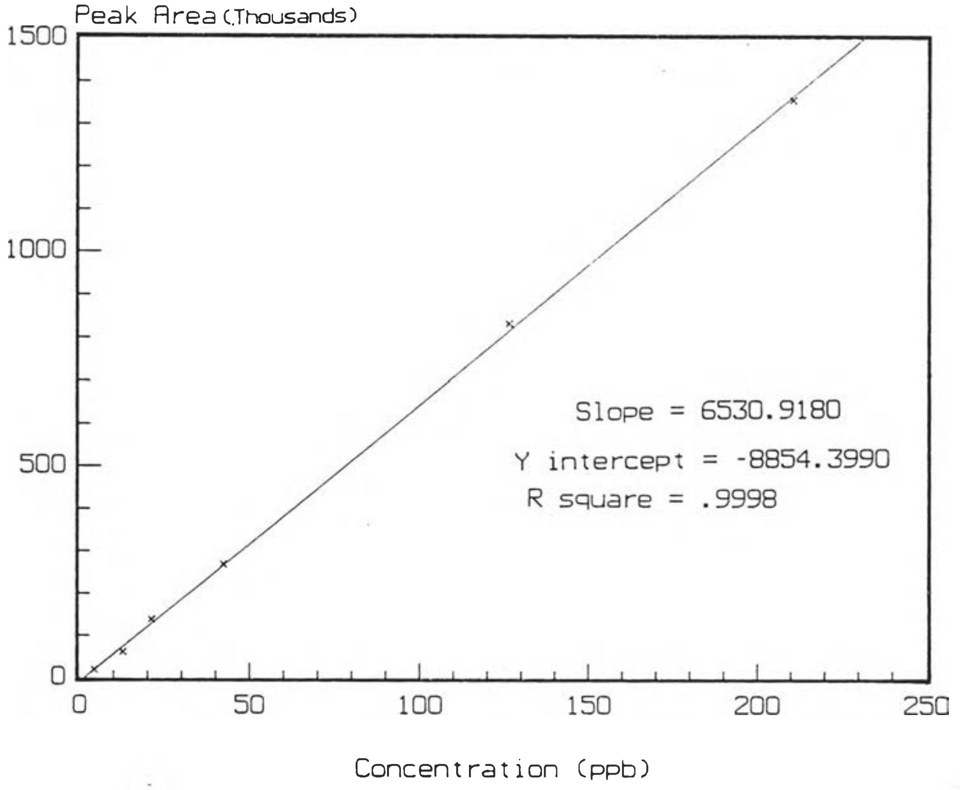

Figure 3.16 The external standard calibration curve of trichloroethlyene using ECD as a detector.

Confirmation on the Structure of Volatile Organic Impurities and Other Residual Solvents by GC/MSD

The headspace drug samples were injected into GC/MSD under GC/MSD conditions in Table 3.6 .

Table 3.6 The GC/MSD condition for the confirmation on structure of volatile organic impurities in drug.

GC Parameter	GC/MSD Condition
Analytical column	30 m x 0.2 mm. x 1.05 μm film thickness., HP-5MS (Low-beed 5% Phenyl Methyl Silicone)
Temperature Program	A. 35 °C (5 min) to 100 °C (0 min) at 8 °C/min B. 100 °C (0 min) to 260 °C (5 min) at 30 °C/min
Split Ratio	20:1
Splitless Time	0.0 min
Flow Rate of Carrier Gas (He)	1.5 mL/min
Dector	Mass Selective Detector (MSD)
Inlet Temperature	180 °C
Transfer Line Temperature	280 °C
Ion Source	EI (Electron-Impact) ; 70eV
Ion Analyzer	Quadrupole