CHAPTER II

EXPERIMENTAL

2.1 Apparatus

The studies of microextraction were performed by Shimadzu gas chromatograph model 9A equipped with electron capture detector (ECD) and chromatograms were recorded on Shimadzu CR-3A integrator. The contents of the extraction vials were shaken by Kika-Werk, Janke & Kunket HS-500.

2.2 Chemicals

The solvents, i.e., hexane, cyclohexane, isooctane and absolute methanol which were A.R. Grade were purchased from J.T. Baker Chemical Company, Phillipsberg, New Jersey, U.S.A. They were refluxed with sodium metal for 6-8 hours and were then distilled twice with the all glass distillation apparatus prior to use as the solvent for an extraction.

The standard chemicals i.e., methylene chloride, chloroform, 1,1,1-trichloroethane, carbontetrachloride, 1,2-dichloropropane and 1,2-dichloroethane were purchased from various chemical companies.

There were :

- 1. Methylene chloride (A.R. Grade) of Farmitalia carlo erba, Italy.
 - 2. Chloroform (A.R. Grade) of Chem Service, U.S.A.
 - 3. Carbontetrachloride (A.R. Grade) of BDH Chemicals Ltd.,

England.

- 4. 1,1,1-trichloroethane (A.R.Grade) of Fluka A.G., Switzerland.
- 5. 1,2-dichloroethane (A.R. Grade) of Fluka A.G, Switzerland.
- 6. 1,2-dichloropropane (A.R. Grade) of Fluka A.G., Switzerland.

The purity of the standard chemicals used in this study were determined by GC-ECD and the standard chemicals used in checking for the purity of them were provided by EPA. The results were shown in Table 2.1.

Table 2.1 The purity of the standard chemicals used in the microextraction study.

Name of compound	% purity of chemicals from EPA	% purity of Standard compounds
methylene chloride	99.80%	99.50%(<u>+</u> 1.24)
chloroform	96.00%	96.58%(<u>+</u> 0.11)
1,1,1-trichloroethane	99.90%	99.81%(+0.18)
Carbontetrachloride	99.97%	99.83%(+0.17)
1,2-dichloropropane		>99.00%
1,2-dichloroethane	_	>99.50%

Sodium chloride (A.R. Grade) and anhydrous sodium sulfate (A.R. Grade) were obtained from J.T. Baker Chemicals Co., Deventer, Holland. They were heated in an oven at 160°C for 8 hours and kept in desiccator prior to use in the study.

2.3 Reagents

Distilled water used in this study was obtained from a redistillation of a double distilled water (the distillator, Fistreem of Fisson made in England) with all glass distillation apparatus before used.

2.3.1 The Standard Solutions of Halogenated Alkanes

2.3.1.1 The Standard Solutions of Halogenated Alkanes in Methanol

ppm of Methylene Chloride

9.95 mg of the methylene chloride standard was dissolved in methanol and was then diluted to the mark with methanol in 10.00 mL volumetric flask.

ppm of Chloroform

10.04 mg of the chloroform standard was dissolved in methanol and was then diluted to the mark with methanol in 10.00 mL volumetric flask.

ppm of 1,1,1-Trichloroethane

9.94 mg of 1,1,1-trichloroethane standard was dissolved in methanol in 10.00 mL volumetric flask and was diluted to the mark with methanol.

2.3.1.1.4 <u>The Standard Solution of 1000.00</u> ppm of Carbontetrachloride

10.00 mg of carbontetrachloride standard was dissolved in methanol in 10.00 mL volumetric flask and was diluted to the mark with methanol.

2.3.1.1.5 The Standard Solution of 994.00 ppm of 1,2-Dichloropropane

9.94 mg of 1,2-dichloropropane standard was dissolved in methanol in 10.00 mL volumetric flask and was diluted to the mark with methanol.

The Standard Solution of Mixture

The standard solution of the

mixture of 982.00 ppm,991.00 ppm, 981.00 ppm, 95.00 ppm and 981.00

ppm of methylene chloride, chloroform, 1,1,1-trichloroethane,

carbontetrachloride and 1,2-dichloropropane, respectively, was

prepared by dissolving 9.82, 9.91, 9.81, 0.95 and 9.81 mg of

methylene chloride, chloroform, 1,1,1-trichloroethane,

carbontetrachloride and 1,2-dichloropropane, respectively in

methanol and was then diluted to the mark with methanol in 10.00 mL

volumetric flask.

2.3.1.2 The Standard Aqueous Solutions of Halogenated Alkanes.

2.3.1.2.1 The Standard Aqueous Solutions of Methylene Chloride.

The standard solutions containing

0.99 and 9.95 ppm of methylene chloride in water were prepared by transfering 10.00 and 100.00 µL of the standard solution from 2.3.1.1.1 into 100.00 mL volumetric flasks and were diluted to the marks with distilled water.

2.3.1.2.2 The Standard Aqueous Solutions of Chloroform

The 1.00 and 10.04 ppm standard solutions of chloroform in water were prepared by transfering 10.00 and 100.00 µL of the standard solution from 2.3.1.1.2 into 100.00 mL volumetric flasks and were diluted to the marks with distilled water.

2.3.1.2.3 The Standard Aqueous Solutions of 1,1,1-Trichloroethane

The standard solutions containing 0.99 and 9.94 ppm of 1,1,1-trichloroethane in water were prepared by transfering 10.00 and 100.00 µL of the standard solution from 2.3.1.1.3 into 100.00 mL volumetric flasks and were then diluted to the marks with distilled water.

2.3.1.2.4 The Standard Aqueous Solutions of Carbontetrachloride

The 1.00 and 10.00 ppm of standard solutions of carbontetrachloride in water were prepared by transfering 10.00 and 100.00 µL of the standard solution 2.3.1.1.4 into 100.00 mL volumetric flasks and were diluted to the marks with distilled water.

2.3.1.2.5 The Standard Aqueous Solutions of

1,2-Dichloropropane

The standard solutions containing 1.00 and 10.05 ppm of 1,2-dichloropropane in water were prepared by transfering 10.00 and 100.00 µL of standard solution from 2.3.1.1.5 into 100.00 mL volumetric flasks and were then diluted to the marks with distilled water.

2.3.1.2.6 The Standard Aqueous Solutions of

Mixture

The standard solution containing 0.98, 0.99, 0.98, 0.09 and 0.98 ppm of methylene chloride, chloroform, 1,1,1-trichloroethane, carbontetrachloride and 1,2-dichloropropane in distilled water was prepared by transfering 10.00 μ L of the solution from 2.3.1.1.6 into 100.00 μ L volumetric flask and was then diluted to the mark with distilled water.

The standard solution containing 9.82, 9.91, 9.81, 0.95 and 9.84 ppm of methylene chloride, chloroform, 1,1,1-trichloroethane, carbontetrachloride and 1,2-dichloropropane in distilled water was prepared by transfering 100.00 µL of the solution from 2.3.1.1.6 into 100.00 mL volumetric flask and was then diluted to the mark with distilled water.

2.3.1.3 The Standard Solutions of Halogenated Alkanes in Extracting Solvents.

2.3.1.3.1 The Standard Solutions of 995.00 ppm of Methylene Chloride.

The standard solutions of

methylene chloride were prepared by dissolving 9.95 mg of methylene chloride standard with the extracting solvent e.g., hexane, cyclohexane and isooctane in 10.00 mL volumetric flask and was finally diluted to the mark with the extracting solvent.

2.3.1.3.2 The Standard Solutions of 1004.00 ppm of Chloroform

The standard solutions of chloroform in each extracting solvent e.g., hexane , cyclohexane and isooctane were prepared by dissolving 10.04 mg of chloroform standard in each extracting solvent in 10.00 mL volumetric flask and was then diluted to the mark with the extracting solvent.

2.3.1.3.3 The Standard Solutions of 994.00 ppm of 1,1,1-Trichloroethane

The standard solutions of 1,1,1-trichloroethane in each extracting solvent e.g., hexane, cyclohexane and isooctane were prepared by dissolving 9.94 mg of 1,1,1-trichloroethane standard with each extracting solvent i.e., hexane, cyclohexane and isooctane in 10.00 mL volumetric flask and was then diluted to the mark with the extracting solvent.

2.3.1.3.4 <u>The Standard Solutions of 1000.00</u> ppm of Carbontetrachloride

The standard solutions of carbontetrachloride in each extracting solvent e.g., hexane, cyclohexane and isooctane were prepared by dissolving 10.00 mg of carbontetrachloride with each extracting solvent e.g., hexane, cyclohexane and isooctane in 10.00 mL volumetric flask and

was then diluted to the mark with the extracting solvent.

2.3.1.3.5 <u>The Standard Solutions of</u> 994.00 ppm of 1,2-Dichloropropane

The standard solutions of 1,2-dichloropropane in each extracting solvent e.g., hexane ,cyclohexane and isooctane were prepared by dissolving 9.94 ppm of 1,2-dichloropropane with each extracting solvent in 10.00 mL volumertric flask and was then diluted to the mark with the extracting solvent.

2.3.1.3.6 The Standard Solutions of Mixture The standard solutions

containing of 982.00 , 991.00 , 981.00 , 95.00 and 981.00 ppm of methylene chloride, chloroform, 1,1,1-trichloroethane, carbontetrachloride and 1,2-dichloropropane in each extracting solvent e.g., hexane, cyclohexane and isooctane were prepared by dissolving 9.82, 9.91, 9.81, 0.95 and 9.81 mg of methylene chloride, chloroform, 1,1,1-trichloroethane, carbontetrachloride and 1,2-dichloropropane with the extracting solvent in 10.00 mL volumetric flask and was then diluted to the mark with the extracting solvent.

2.3.2 Internal Standard Solutions

2.3.2.1 <u>Internal Standard Solutions for Single</u>

Component Solutions

2.3.2.1.1 Methylene chlcride

The internal standard used for an analysis of methylene chloride was 1,2-dichloroethane . To prepare

988.00 ppm of 1,2-dichloroethane standard solutions, 9.88 mg. of 1,2-dichloroethane standard was dissolved in each extracting solvent i.e., hexane,cyclohexane and isooctane in 10.00 mL volumetric flask and was then diluted to the mark with the extracting solvent.

2.3.2.1.2 Chloroform

The internal standard used for the determination of chloroform was 1,1,1-trichloroethane. The preparation of 994.00 ppm of 1,1,1-trichloroethane standard solutions would be the same as section 2.3.1.3.3

2.3.2.1.3 1,1,1-Trichloroetane

The internal standard used for the quantification of 1,1,1-trichloroethane was carbontetrachloride and chloroform. The preparation of 1000.00 ppm of carbontetrachloride standard solutions would be the same as section 2.3.1.3.4. The preparation of 1004.00 ppm of chloroform standard solutions would be the same as section 2.3.1.3.2.

2.3.2.1.4 Carbontetrachloride

The internal standard used in the analysis of carbontetrachloride was chloroform. The preparation of 1004.00 ppm of chloroform standard solutions would be the same as section 2.3.1.3.2.

Note * The 1000.00 ppm of carbontetrachloride standard solutions was used as the internal standard only for the analysis of 0.99 ppm of 1,1,1-trichloroethane solution and for the sample-to-hexane ratio of 9:1.



2.3.2.1.5 1,2-Dichloropropane

The internal standard used in the analysis of 1,2-dichloropropane was 1,2-dichloroethane. The preparation of 988.00 ppm of 1,2-dichloroethane standard solutions would be the same as section 2.3.2.1.1.

2.3.2.2 <u>Internal Standard Solutions for Mixture</u> Solutions.

The internal standard used in the analysis of the mixture solutions was 1,2-dichloroethane. The preparation of 988.00 ppm of 1,2-dichloroethane standard solutions would be the same as section 2.3.2.1.1.

The internal standard solutions in 2.3.2.1 and 2.3.2.2 were diluted to a suitable concentration prior to be used in the determination of the concentration of each component at the two different levels of concentrations and at the various sample-to-solvent ratios were shown in the Table 2.2 and the Table 2.3.

Table 2.2 Internal standard used in the single component extraction.

Halogenated alkanes	initial conc.of the compound (ppm)	sample-to -solvent ratio	internal standard	conc.of internal standard(ppm)
CH ₂ Cl ₂	9.95	9:1	с ₂ н ₄ с1 ₂	47.08
		5:5		9.78
		2:8		9.78
	0.99	9:1	C2H4C12	9.78
		5:5		1.80
		2:8		1.80
CHC13	10.04	9:1	с ₂ н ₃ с1 ₃	47.82
		5:5		9.94
		2:8		9.94
	1.00	9:2	C2H3C13	9.94
		5:5		4.99
		2:8		4.99
C2H3C13	9.94	9:1	CHC13	47.32
		5:5		9.84
		2:8		9.84
	0.99	9:1	*cc14,cHC13	5.02,9.84
		5:5		4.94
		2:8		4.94

Halogenated alkanes	initial conc.of the compound (ppm)	sample-to -solvent ratio	internal	conc.of internal standard(ppm
cc1 ₄	10.00	9:1 5:5	CHC13	90.33
		2:8		90.33
,	1.00	9:1	снсіз	90.33
		5:5		24.23
	La Establish	2:8		24.23
^C 3 ^H 6 ^{Cl} 3	9.94	9:1	с ₂ н ₄ с1 ₂	47.05
	*	5:5		24.09
		2:8		24.09
	0.99	9:1	c2H4c15	24.09
		5:5	100	1.81
		2:8		1.81

Note * 5.02 ppm of carbontetrachloride solution was used as the internal standard only for the study of 0.99 ppm of 1,1,1-trichloroethane solution and for the sample-to-hexane ratio of 9:1.

Table 2.3 Internal standard used in the mixture extraction.

Conc.of each	Sample-to-solvent	Conc. of
nalogenated alkane	ratio	internal standard
in the mixture(ppm)		(ppm)
9.82 ppm of CH ₂ Cl ₂ ,		
9.91 ppm of CHCl ₃ ,	9:1	2857.37
9.81 ppm of C ₂ H ₃ Cl ₃ ,	5:5	474.40
0.95 ppm of CCl ₄ and	2:8	474.40
9.81 ppm of C ₃ H ₆ Cl ₂		
0.98 ppm of CH ₂ Cl ₂ ,		
0.99 pmm of CHCl ₃	9:1	474.40
0.98 pmm of C ₂ H ₃ Cl ₃ ,	5:5	239.08
0.09 ppm of CCl ₄ and	2:8	239.08
0.98 ppm of C ₃ H ₆ Cl ₂		

2.4 Gas Chromatographic Conditions

2.4.1 The Gas Chromatographic Conditions for Single Component Solutions.

2.4.1.1 The gas chromatographic condition used in the study of halogenated alkanes (this condition was not used in 2.4.1.2 and 2.4.1.3) in three extracting solvents i.e., hexane, cyclohexane and isooctane was:

column : 6' x 3/8" O.D. glass column

column packing : 1% AT 1000 on Graphpac GB 60/80 mesh

oven temperature : 150°C

injection port temperature: 180°C

detector temperature : 180°C

detector : ECD

N₂ flowrate : 30 mL/min

2.4.1.2 The gas chromatographic condition used in the study of 0.99 ppm of 1,1,1-trichloroethane solution at sample-to-hexane ratio of 9:1 was:

column : 6' x 1/4" O.D. stainless steel column

column packing : 15% Apeizon L on chromosorb PAW

100/120 mesh

oven temperature : 70°C

injection port temperature : 120°C.

detector temperature : 120°C

detector : ECD

N₂ flowrate : 30 mL/min

2.4.1.3 The gas chromatographic condition used in the study of 1,2-dichloropropane solution with isooctane was:

column : 6' x 3/8" O.D. glass column

column packing : 1% AT1000 on Graphpac GB 60/80 mesh

oven temperature : 170°C

injection port temperature : 200°C

detector temperature : 200°C

detector : ECD

N₂ flowrate : 30 mL/min

2.4.2 The Gas Chromatographic Condition for Mixture Solutions.

The gas chromatograms of mixture solutions in various solvents obtained from the gas chromatographic condition as mention below were shown in Fig 2.1-2.3.

2.4.2.1 The gas chromatographic condition used in the study of the standard mixture solution with hexane and cyclohexane was:

column : 6' x 3/8" O.D. glass column

column packing : 1% AT1000 on Graphpac GB 60/80 mesh

oven temperature: 110°C

injection port temperature : 200°C

detector temperature : 200°C

detector : ECD

N₂ flowrate : 30 mL/min

2.4.2.2 The gas chromatographic condition used in the study of the standard mixture solution with isooctane was:

column : 6' x 3/8" O.D. glass column

column packing : 1% AT1000 on Graphpac GB 60/80 mesh

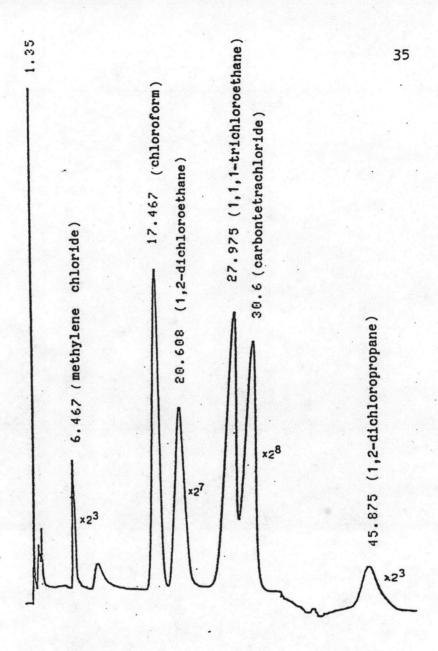
oven temperature : 120°C

injection port temperature : 200°C

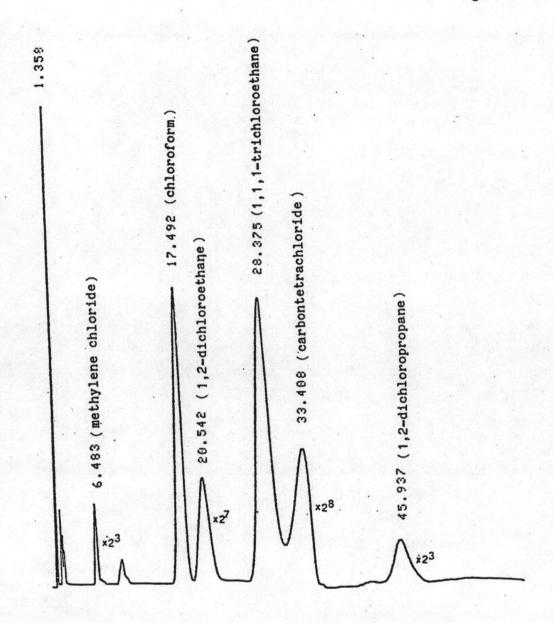
detection temperature : 200°C

detector : ECD

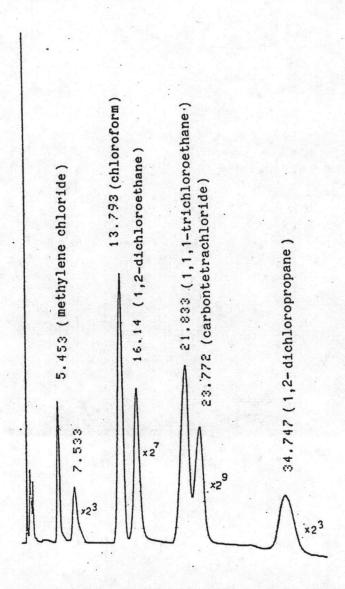
N₂ flowrate : 30 mL/min



The Ni⁶³ ECD gas chromatogram of 3.95 ppm of 2.1 Figure methylene chloride, 5.02 ppm of chloroform, 1,1,1-trichloroethane, of 0.80 ppm of 1,2-4.02 carbontetrachloride and ppm of 474.40 dichloropropane with 1,2ppm dichloroethane (internal standard) in using 6'x 3/8" O.D. glass column with 1% AT1000 on Graphpac GB 60/80 mesh; oven temperature port temperature 200°C; detector ;injection temperature 200 $^{\circ}$ C and N₂ flowrate 30 mL/min.



Ni⁶³ ECD gas chromatogram of 2.49 2.2 Figure chloride, 5.03 ppm of chloroform, methylene 1,1,1-trichloroethane, 0.81 of ppm 4.03 1,2carbontetrachloride and ppm 239.08 1,2dichloropropane with ppm dichloroethane (internal standard) in cyclohexane, using 6'x 3/8" O.D. glass column with 1% AT1000 on Graphpac GB 60/80 mesh; oven temperature 110°C 200°C; detector injection port temperture temperature 200 °C and N2 flowrate 30 mL/min.



ECD gas chromatogram of 3.92 ppm 2.3 Figure methylene chloride, 4.06 ppm of chloroform, of 1,1,1-trichloroethane, 0.40 ppm 1,2-4.07 ppm carbontetrachloride and 1,2-239.08 ppm with dichloropropane dichloroethane (internal standard) in using 6'x 3/8" 0.D. glass column with 1% AT1000 on Graphpac GB 60/80 mesh; oven temperature 120°C injection port temperture 200°C temperature 200°C and N2 flowrate 30 mL/min.

2.5 Procedure

2.5.1 The Study of Shaking Time

The shaking time of each halogenated alkane i.e., methylene chloride, chloroform, 1,1,1-trichloroethane, carbontetrachloride and 1,2-dichloropropane at different sample-to-solvent ratio e.g. 9:1, 5:5 and 2:8 in each extracting solvent i.e.,hexane, cyclohexane and isooctane were studied.

The procedure for the study of the shaking time was described as the follow:

- The solvent and standard solution was added into the vial sequentially.
- 2. The contents in the vial were shaken for 2, 5, 10, 20 and 30 min by a mechanical shaker.
- 3. Allow it to stand until the two phases were completely separated
- 4. Remove 200.00 uL of the extract by microsyringe and transfer it to another vial.
- 5. 5.00 uL of internal standard solution was added into the vial.
- 6. 0.7 uL of the solution was injected into gas chromatograph
- 7. The concentration of a solute was determined by using an internal standardization method.

Once the concentration of the solute in the extract was known, the percentage of recovery of each solute could be

calculated. The equilibration time of each solute in each solvent for each sample-to-solvent ratio can be evaluated from the graph plotting between the percent recovery and time in min and this equilibration time would be used in the next section.

2.5.2 Microextraction Procedure

Three different sample-to-solvent ratios were used in this study, i.e., 9:1, 5:5 and 2:8, respectively. The effect of adding salts on percent recovery was also studied, the salts used were 2.00g of Na_2SO_4 and 2.00g of NaCl. Therefore each extraction consisted of three systems. The conditions of the three systems were described below.

- 1. Non salting out (no salt),
- 2. 2.00 g of Na SO, and
- 3. 2.00 g of NaCl

The procedure for the study of microextraction was described as the follow:

- Solid salt, extracting solvent and standard sample solutions were added into the vial sequentially.
- 2. The contents in the vial were shaken for the times as found in the section (2.5.1) by a mechanical shaker.
- 3. Allow the contents to stand until they were completely separated.
- 4. Remove 200.00 uL of the extract by microsiringe and transfer it into another vial.
- 5. 5.00 uL of internal standard solution was added into the vial.

- 6. 0.7 uL of this solution was injected into the gas chromatograph.
- 7. The concentration of a solute was determined from the calibration curves as shown in Fig 2.5-2.9. by the internal standardization method.

The concentration obtained from the internal standardization method was used to calculated the percent recovery of the solute .

2.5.3 Internal Standardization Method.

This technique may be understood by assuming that only the component C is of interest for analysis and that the unknown contains no component A. If a standard containing known weights of both A and C is prepared and chromatographed, the In practice several response factor, F, can be determined. standards should be made and a plot of weight ratio as abscissa and peak area ratio as ordinate is made. This plot must be linear for the particular system. Once the linearity is established for a given sample type and system only one standard mix need to be used to define the slope of that plot. Therefore the standard is actually used to determine the factor F. The unknown is now ready to be run. Since no A is present in the unknown, a known weight of A component is added to a known weight of the sample. This mixture is then chromatographed and the peak area ratio of component C to A is Knowing F, the ratio of the peak area ratio and weight measured. ratio and the peak area ratio in the unknown, the weight ratio can be calculated.

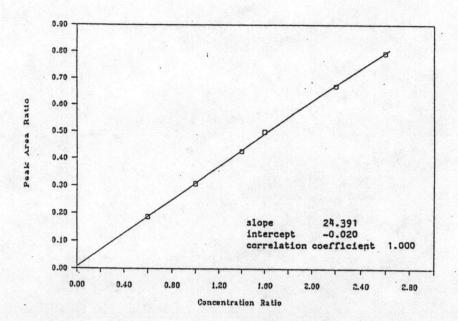


Fig 2.4 Calibration curve

$$\frac{W_C}{W_\Delta} = \frac{A_C}{A_\Delta} \times F \qquad (27)$$

Where W_C and W_A are weights of C and A, respectively, and A_C and A_A are peak areas of C and A, respectively, F is response factor.

Since the weight of A added to a sample is known, the weight of C in the sample can be calculated.

$$W_{C} = \frac{A_{C} \times W_{A} \times F}{A_{A}}$$
 (28)

and since the weight of the sample is known.

% C =
$$\frac{W_C \times 100}{\text{sample weight.}}$$
 (29)

where % C is the percent of the component C in the solution.

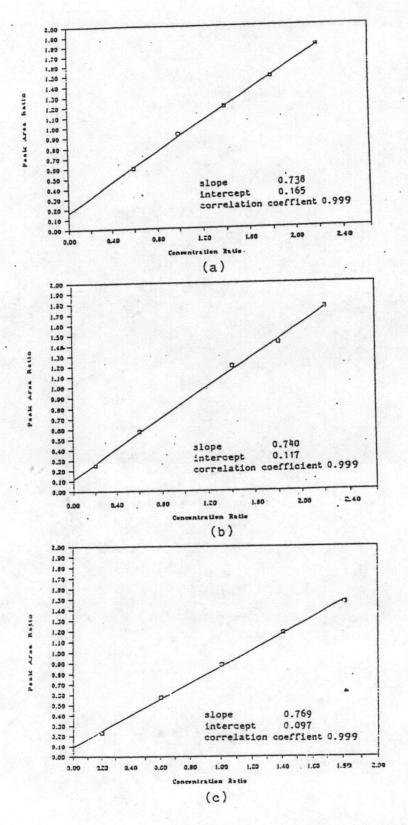


Figure 2.5 The calibration curve of methylene chloride in various solvents.

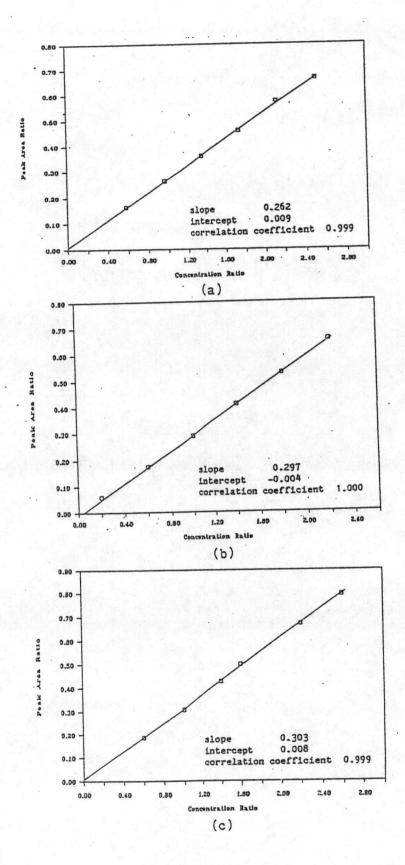


Figure 2.6 The calibration curve of chloroform in various solvents.

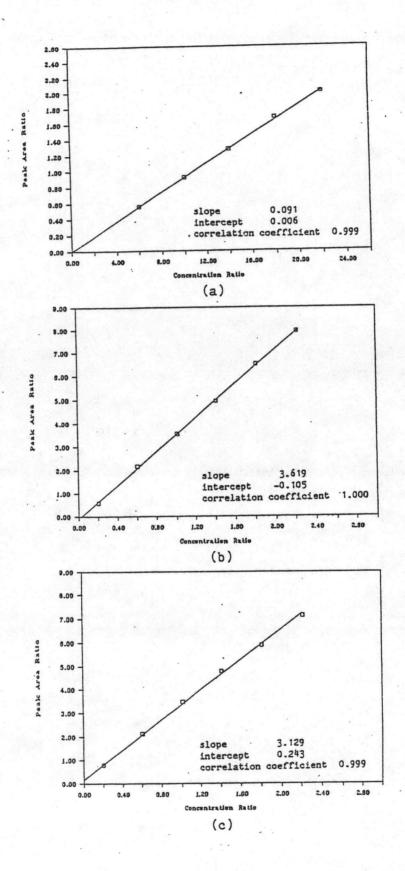


Figure 2.7 The calibration curve of 1,1,1-trichloroethane in various solvents.

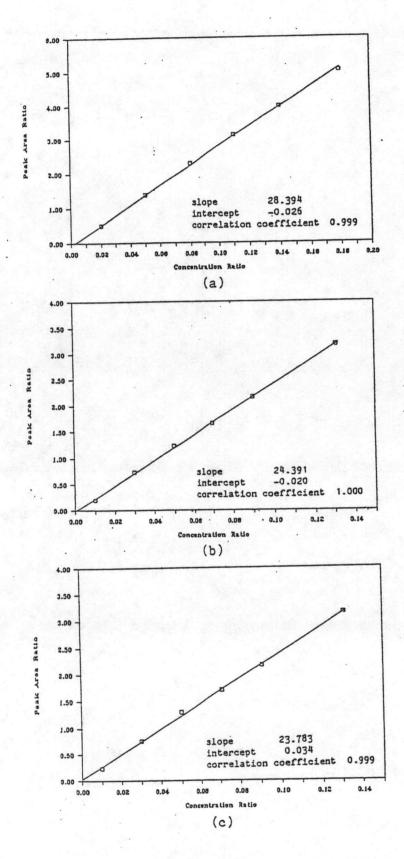


Figure 2.8 The calibration curve of carbontetrachloride in various solvents.

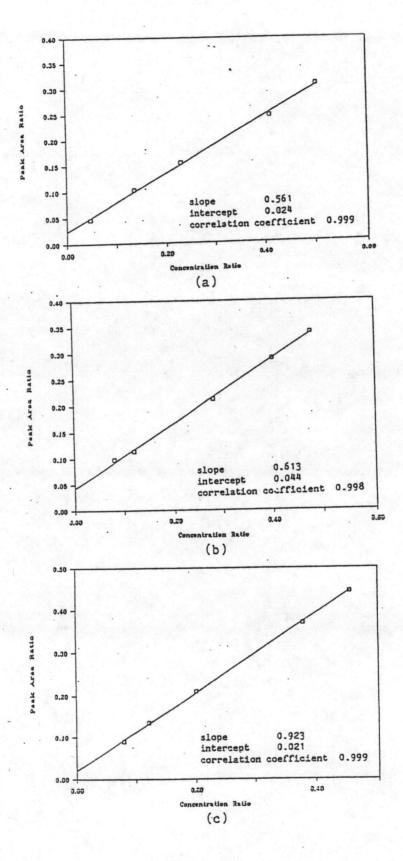


Figure 2.9 The calibration curve of 1,2-dichloropropane in various solvents.