CHAPTER IV

RESULTS

4.1 Effect of column temperature on retention time

The results of the experiment are shown in Table 4.1 and plotted in Figure 4.1. It is obvious that the increase of the column temperature causes a decrease in the retention time.

Table 4.1 Effect of column temperature on retention time

Instrumental condition : injection port temperature 150°C

detector temperature 150°C

flow rate of carrier gas 50 $cm^3 min^{-1}$

temperature (°C)	retention time (min)
130	8.1 ± 0.1
135	7.0 ± 0.1
140	6.2 ± 0.1
145	5.3 ± 0.1
150	4.5 ± 0.1
155	4.1 ± 0.1
160	3.5 ± 0.1
165	3.2 ± 0.1
175	2.5 ± 0.1
185	2.0 ± 0.1

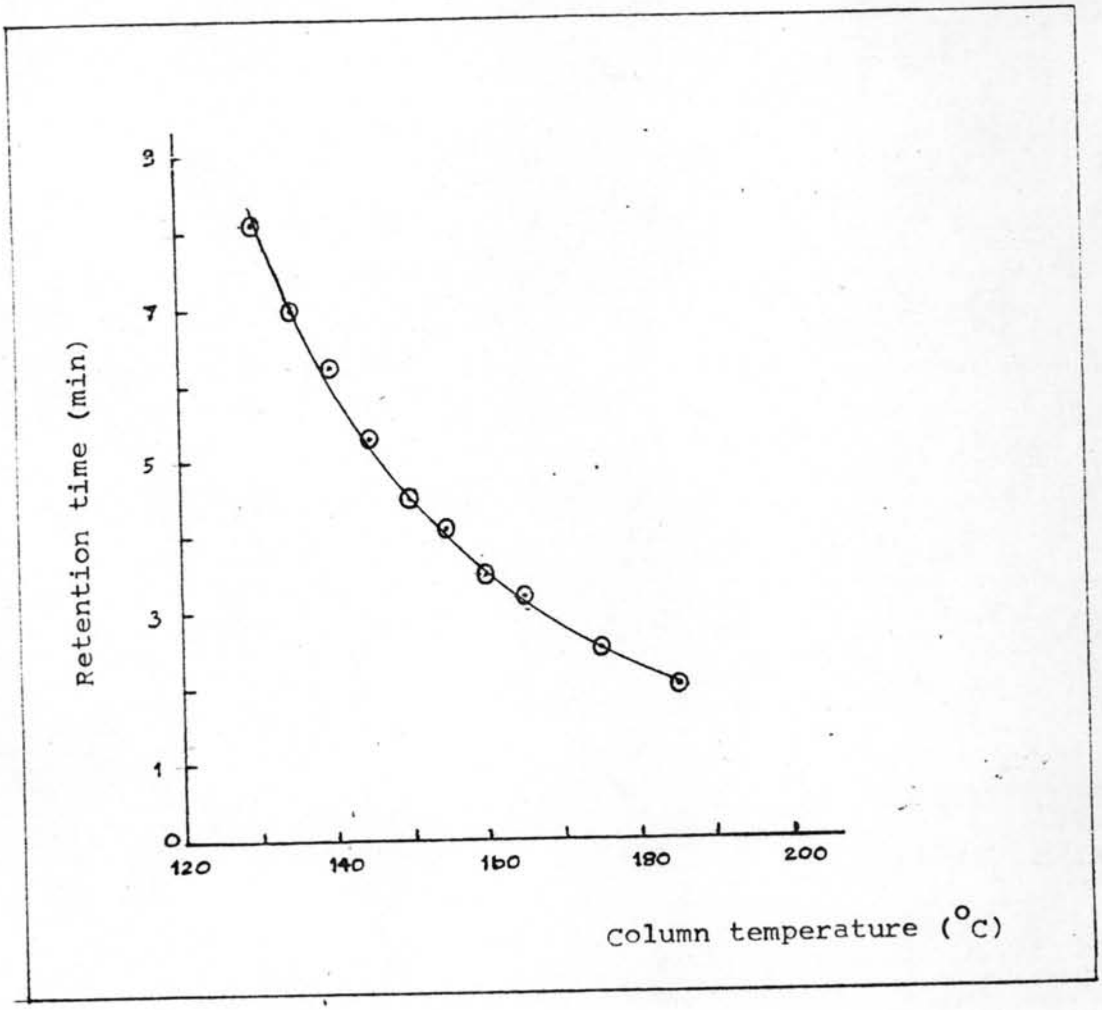

Figure 4.1 Effect of column temperature on retention time of methylmercury(II) chloride

The curve plotted between temperature against retention time is nonlinear but slightly exponential. To precure column life since the maximum tolerable temperature of the liquid phase is 190°C and to assure complete separation of methylmercury, the temperature between 135 - 140°C was chosen.

4.2 Effect of temperature of injection port and of detector on retention time

The results are tabulated in Table 4.2 and plotted in Figure 4.2. No significant change in the retention time was observed when the temperature of the injection port and of the detector were varied between 150°C and 300°C. The temperature of the injection port and of the detector were therefore kept constant at 150°C throughout the experiment.

Table 4.2 Effect of temperature of injection port and of detector on retention time

Instrumental condition : column temperature 135°C

flow rate of carrier gas 50 $cm^3 min^{-1}$

temperature (°C)	retention time (min)
150	7.1 ± 0.1
175	7.1 ± 0.1
200	7.0 ± 0.1
225	7.1 ± 0.1
250	7.1 ± 0.1
275	6.8 ± 0.1
300	6.9 ± 0.1

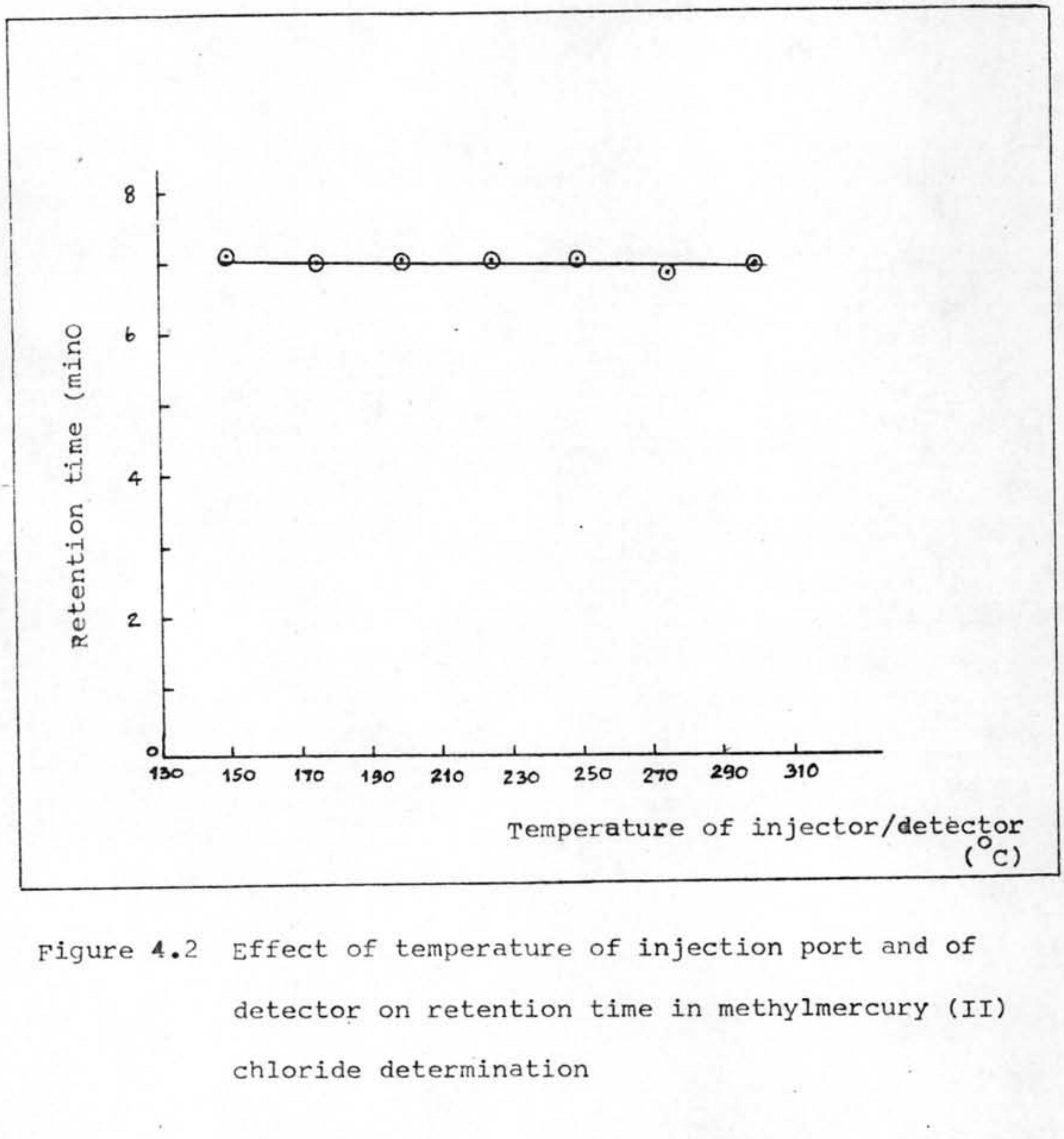

Figure 4.2 Effect of temperature of injection port and of detector on retention time in methylmercury (II) chloride determination

4.3 Effect of flow-rate of nitrogen carrier gas

The results are shown in Table 4.3 and plotted in Figure 4.3. The retention time decreases sharply when the flow-rate is increased from 10 $cm^3 min^{-1}$ to 50 $cm^3 min^{-1}$ after which the decrease is only slight.

Table 4.3 Effect of carrier-gas flow-rate on retention time

Instrumental condition :		
	column temperature	135°C
	injection port temperature	150°C
	detector temperature	150°C

flow rate ($cm^3 min^{-1}$)	retention time (min)
10	22.2 ± 0.1
30	14.2 ± 0.3
50	6.9 ± 0.1
70	5.6 ± 0.1
90	5.0 ± 0.1
110	4.5 ± 0.1
120	4.5 ± 0.1

The HETP for the elution of methylmercury (II) chloride from the column under various condition is calculated and tabulated in Table 4.4. The HETP is plotted against the flow rate of carrier gas to give the van Deemter plot as shown in Figure 4.4.

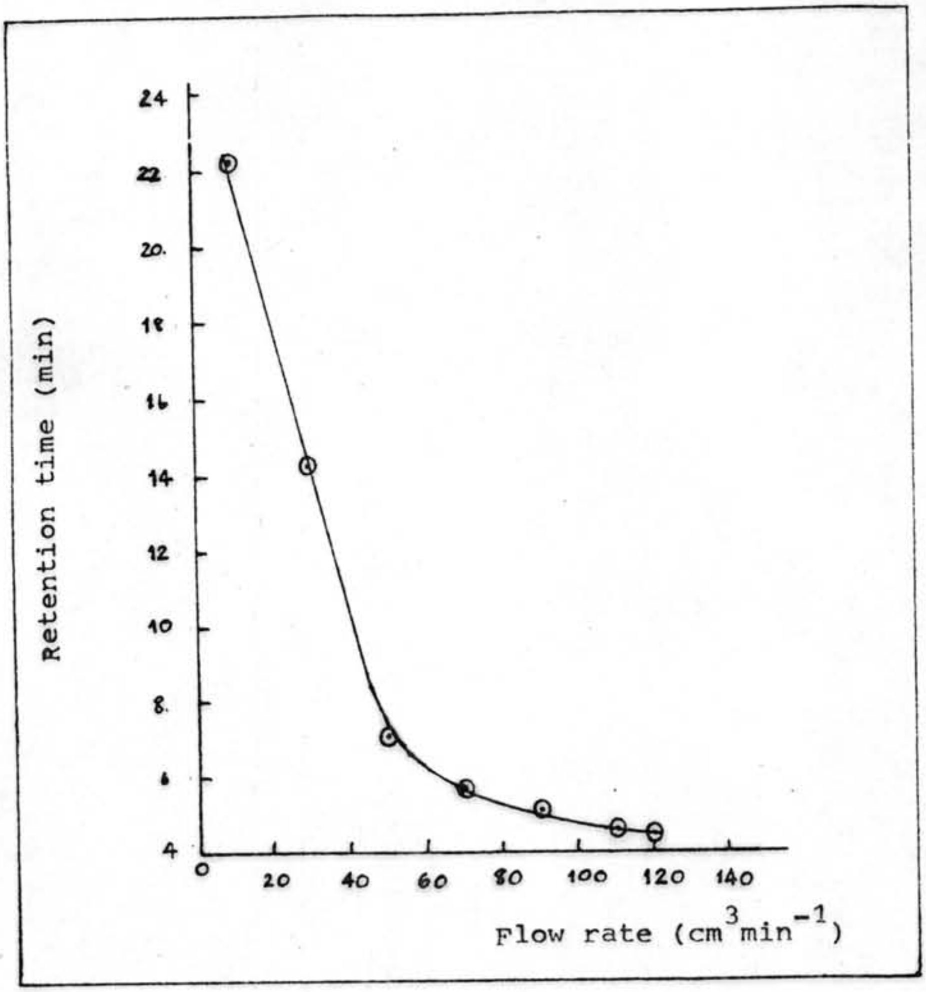

Figure 4.3 Effect of flow rate of carrier gas on retention time in methylmercury (II) chloride determination

Table 4.4 The HETP of the column

Instrumental condition : column temperature 135°C

injection port temperature 150°C

detector temperature 150°C

flow rate ($cm^3 min^{-1}$)	retention time (min)	peak width at base line (min)	HETP	average[a] HETP
10	22.1	3.6	0.33	0.32±0.01
	22.2	3.6	0.32	
	22.4	3.6	0.32	
30	14.0	2.0	0.25	0.28±0.03
	14.5	2.2	0.29	
	14.0	2.2	0.31	
50	7.0	1.0	0.25	0.26±0.01
	6.9	1.0	0.26	
	6.9	1.0	0.26	
70	5.6	0.8	0.25	0.25±0.01
	5.6	0.8	0.25	
	5.5	0.8	0.26	
90	4.9	0.8	0.33	0.32±0.01
	5.0	0.8	0.32	
	5.1	0.8	0.30	
110	4.4	0.8	0.41	0.40±0.02
	4.4	0.8	0.41	
	4.6	0.8	0.38	

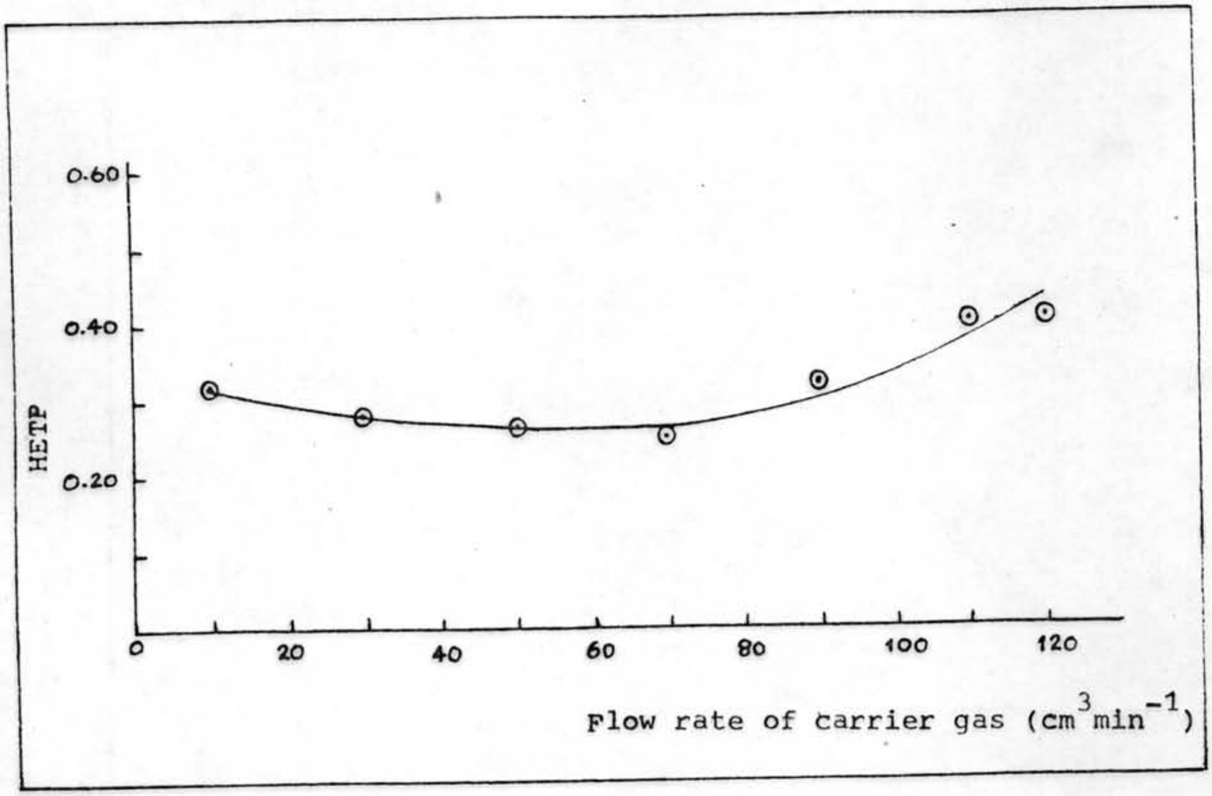

Figure 4.4 Van Deemter plot in methylmercury (II) chloride determination

Table 4.4 (continue) The HETP of the column.

flow rate ($cm^3 min^{-1}$)	retention time (min)	peak width at base line (min)	HETP	average[a] HETP
120	4.5	0.8	0.40	0.40 ± 0.01
	4.4	0.8	0.41	
	4.6	0.8	0.38	

(a) The standard deviation is calculated from SD = $\sqrt{\sum(x-\bar{x})^2/N-1}$

From the above figure, the optimum flow rate should be between 50 and 70 $cm^3 min^{-1}$. The flow rate at 70 $cm^3 min^{-1}$ is prefered as a shorter analytical time is required.

4.4 Determination of the minimum detectable quantity

The peak height at various methylmercury (II) chloride contents detected under optimal operation condition of the detector is given in Table 4.5. The minimum amount of methylmercury (II) chloride that can be detected, or the amount of methylmercury(II) chloride that gives a detector response equal to twice the average noise level, is approximately 52 pg. The chromatogram of 52 pg methylmercury (II) chloride is illustrated in Figure 4.5.

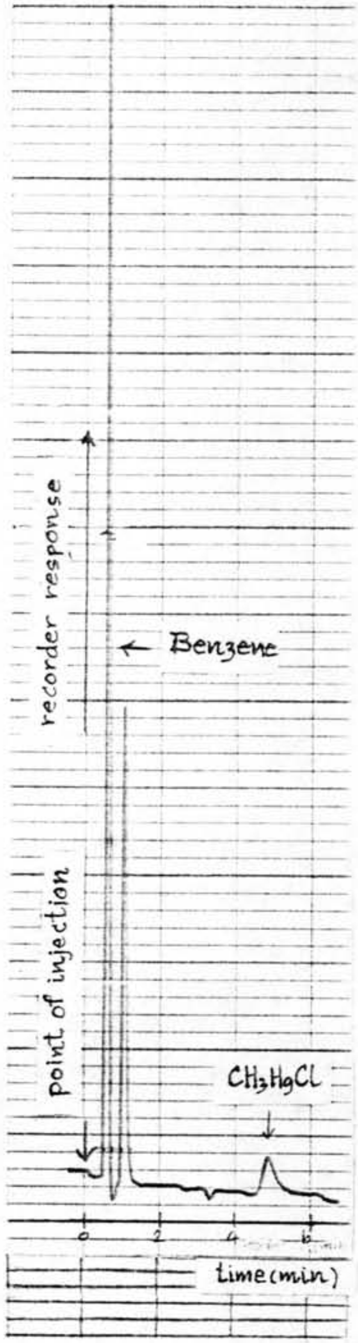

Figure 4.5 Typical chromatogram of 52 pg methylmercury (II) chloride

Table 4.5 The minimum detectable quantity of the detector

Instrumental condition : column temperature $140^{\circ}C$

injection port temperature $150^{\circ}C$

detector temperature $150^{\circ}C$

flow rate of carrier gas $70\ cm^{3} min^{-1}$

ng Hg in the form methylmercury (II) chloride	peak height (mm)
0.052	5.5 ± 0.7
0.130	17.0 ± 0.1
0.208	28.5 ± 0.7
0.416	43.0 ± 1.7
0.678	75.3 ± 1.5
0.936	98.6 ± 2.3
1.196	116.0 ± 2.0

A linear relationship between the concentration and the detector response is essentially required for an accurate quantitative analysis. The slope of the curve plotted between concentration and the detector response also defines the sensitivity. The dynamic range for the determination of methylmercury(II) chloride under the selected detector conditions is between 52 pg and 800 pg. The sensitivity of the detector was therefore approximately 100 mm ng^{-1}.

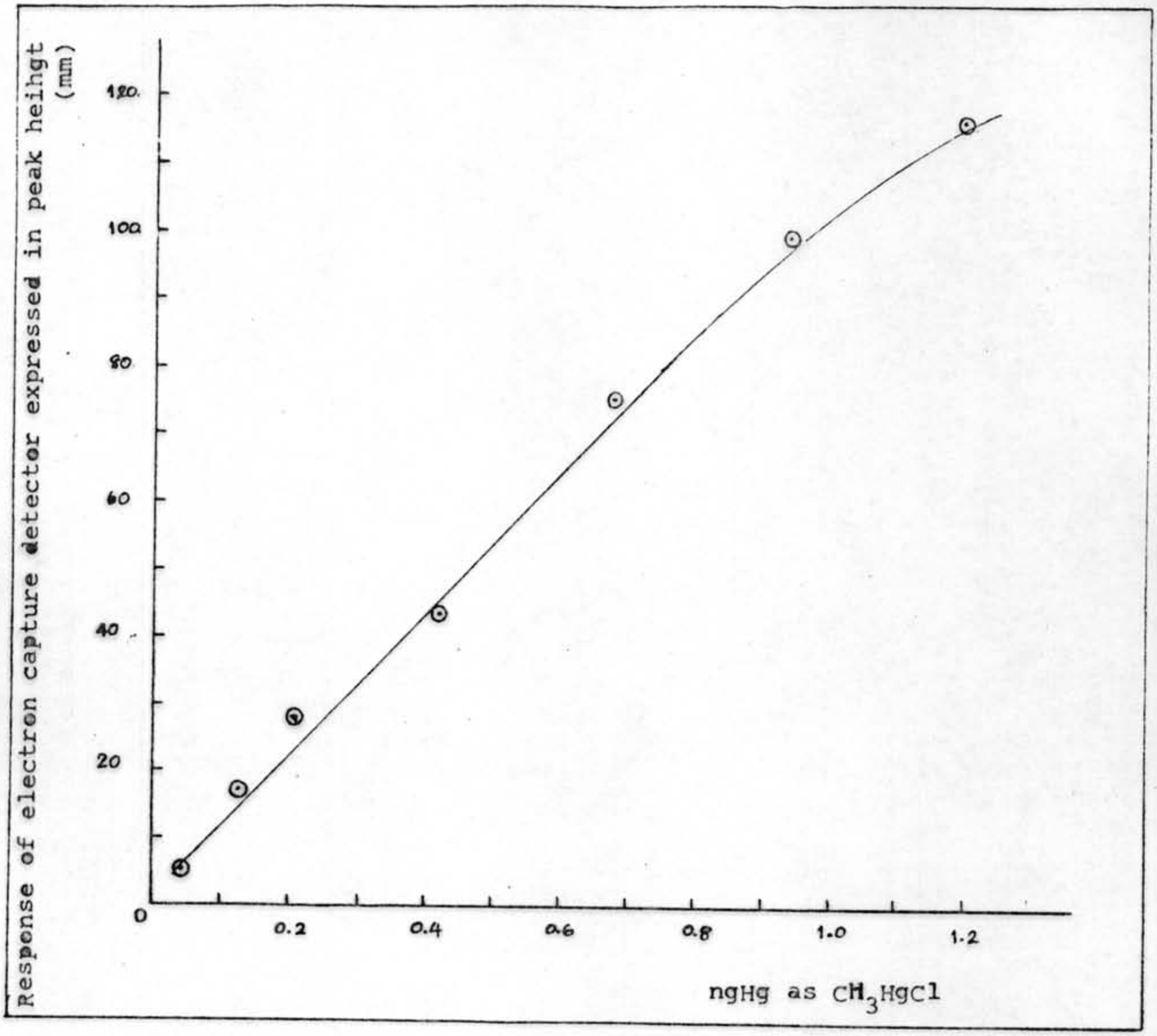

Figure 4.6 Response of electron capture detector for methylmercury(II) chloride

4.5 Determination of optimum time for sample digestion

The results of the experiment are shown in Table 4.6

Table 4.6 Recovery yield at different digestion time

Instrumental condition : column temperature 140°C
injection port temperature 150°C
detector temperature 150°C
flow rate of carrier gas 70 $cm^3 min^{-1}$

digestion time (min)	recovery yield (%)
15	62 ± 3
30	59 ± 2
45	66 ± 3
60	60 ± 3

The recovery yield is plotted against digestion time in Figure 4.7. The recovery yield are mostly in the order of 60%. Thirty minutes is prefered to ensure complete digestion

4.6 Determination of optimum temperature for sample digestion

The results are shown in Table 4.7 and plotted in Figure 4.8.

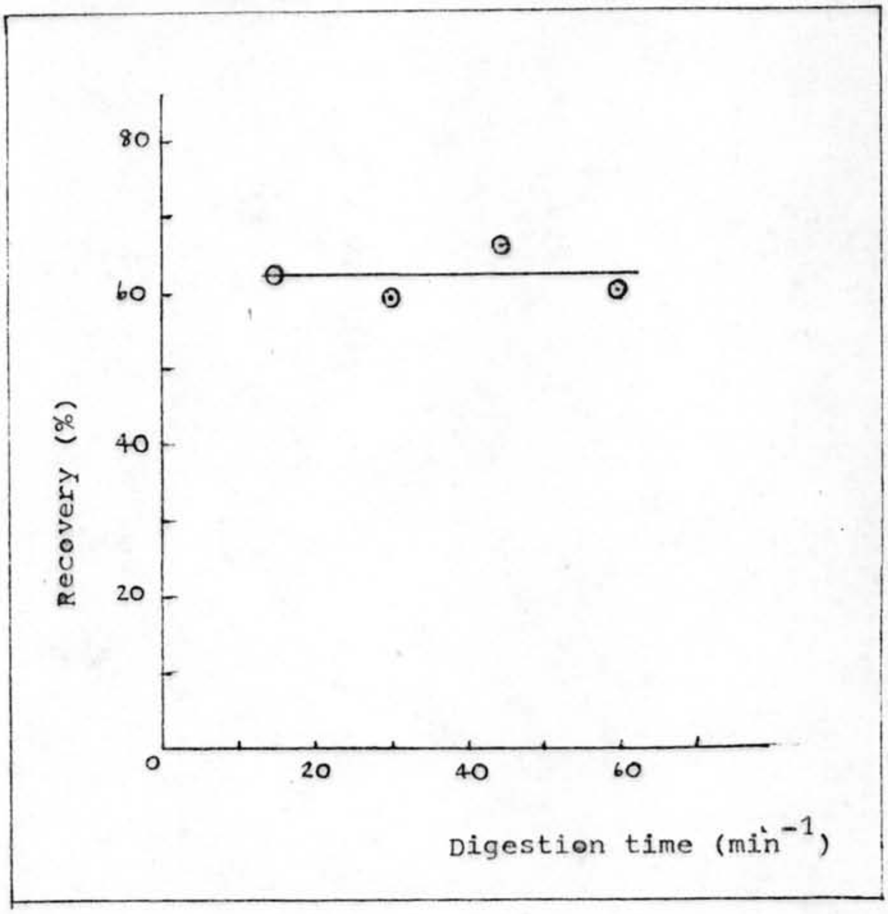

Figure 4.7 Effect of sample digestion time on recovery yield

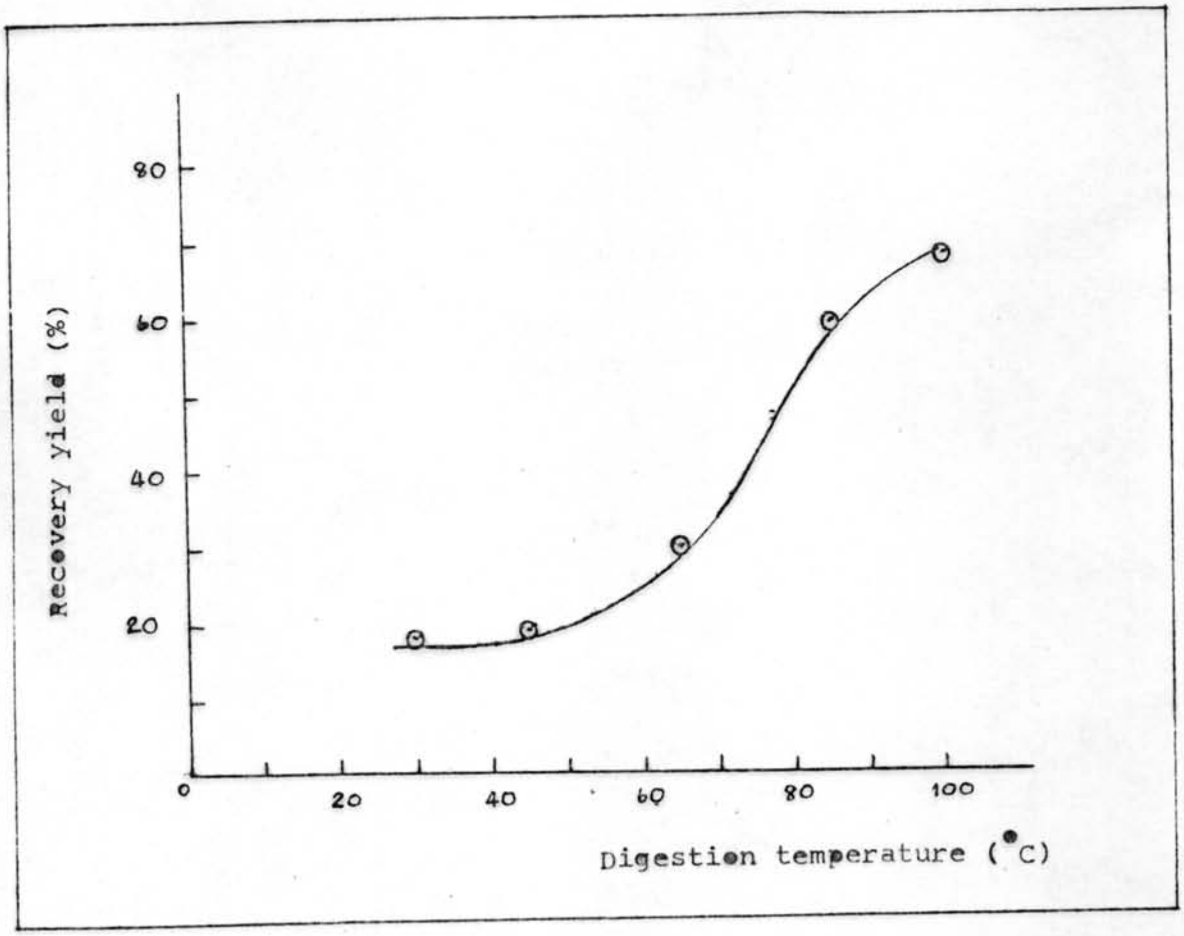

Figure 4.8 Effect of sample digestion temperature on recovery yield

Table 4.7 Recovery yield at different digestion temperature

Instrumental condition : column temperature 140° C

injection port temperature 150° C

detector temperature 150° C

flow rate of carrier gas 70 $cm^3 min^{-1}$

digestion temperature (°C)	recovery yield (%)
30	18 ± 4
45	19 ± 1
65	30 ± 2
85	59 ± 3
100	68 ± 6

From Figure 4.8, the recovery yield increases sharply when the temperature is increased from 50°C to 100°C. The digestion temperature at 100°C is prefered because it gives the highest recovery yield.

4.7 Determination of the recovery yield of methylmercury added to fish

The data for the recovery yield is summarized in Table 4.8. From seven experiments on different fish samples, an average recovery yield of 91% with a 10% relative standard diviation was obtained.

Table 4.8 Recovery yield of methylmercury

Instrumental condition : column temperature 140°C

injection port temperature 150°C

detector temperature 150°C

flow rate of carrier gas 70 $cm^3 min^{-1}$

Sample No.	μgHg as CH_3HgCl (blank)	CH_3HgCl added				CH_3HgCl recovered, with blank correction				recovery yield (%)
		μgHg as CH_3HgCl	μgHg as CH_3HgCl / g samples	μgHg as CH_3HgCl / cm^3 benzene	μgHg as CH_3HgCl / 5 μl benzene	μgHg as CH_3HgCl	μgHg as CH_3HgCl / g sample	μgHg as CH_3HgCl / cm^3 benzene	μgHg as CH_3HgCl / 5 μl benzene	
x_1	0.02	0.90	0.45	0.09	450	0.83	0.41	0.08	410	91.1
x_2	0.02	0.90	0.45	0.09	450	0.90	0.45	0.09	450	100.0
x_3	0.05	0.90	0.45	0.09	450	0.80	0.40	0.08	400	88.9
x_4	0.05	0.90	0.45	0.09	450	0.70	0.35	0.07	350	77.8
x_5	0.05	0.90	0.45	0.09	450	0.75	0.37	0.07	370	82.2
x_6	0.02	0.90	0.45	0.09	450	0.83	0.41	0.08	410	91.1
x_7	0.02	0.90	0.45	0.09	450	0.96	0.48	0.10	480	106.7

mean 91.1

standard deviation 9.9

4.8 Quantitative analysis of methylmercury in fish sample

Typical chromatograms of methylmercury(II) chloride from a sample is given in Figure 4.9 in comparison to that from a standard in Figure 4.10. The retention time of 4.2 min was obtained from both chromatograms. The analytical results are tabulated in Table 4.9 - 4.12. Condition for the instrumental set-up are as follows :

column temperature	140^{o} C
injection port temperature	150^{o} C
detector temperature	150^{o} C
flow rate of carrier gas	70 $cm^{3} min^{-1}$

Table 4.9 Concentration of methylmercury in Hairtail

Sample No.	concentration of methylmercury (II) chloride (ng CH_3Hg/g wet)			Average value	Source of sample
D_1	17.19	20.42	-	18.80 ± 2.28	market
D_2	20.42	15.05	-	17.73 ± 3.79	B_1[a]
D_3	22.57	32.25	25.79	26.87 ± 4.93	market
D_4	21.49	23.65	16.12	20.42 ± 3.88	market

[a] Station B_1, Figure 3.1

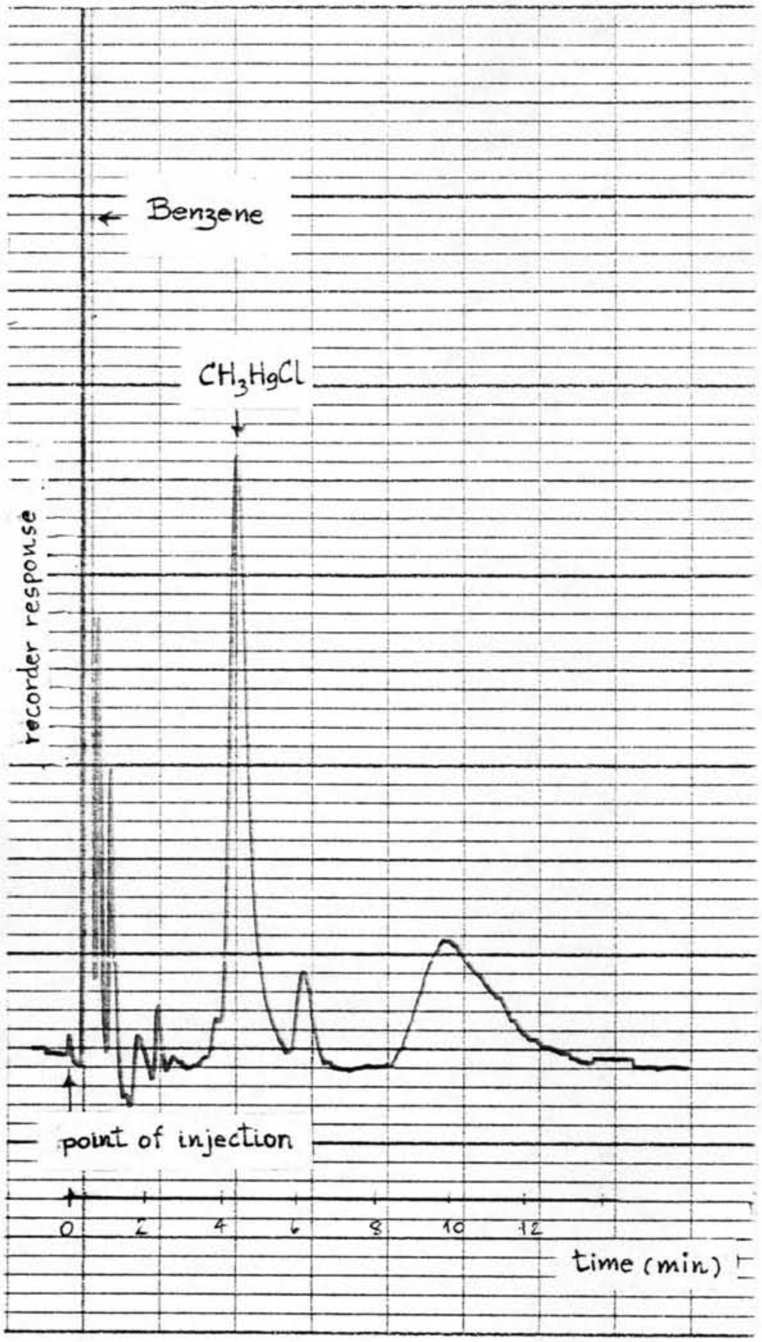

Figure 4.9 Typical chromatogram of methylmercury(II) chloride from sample

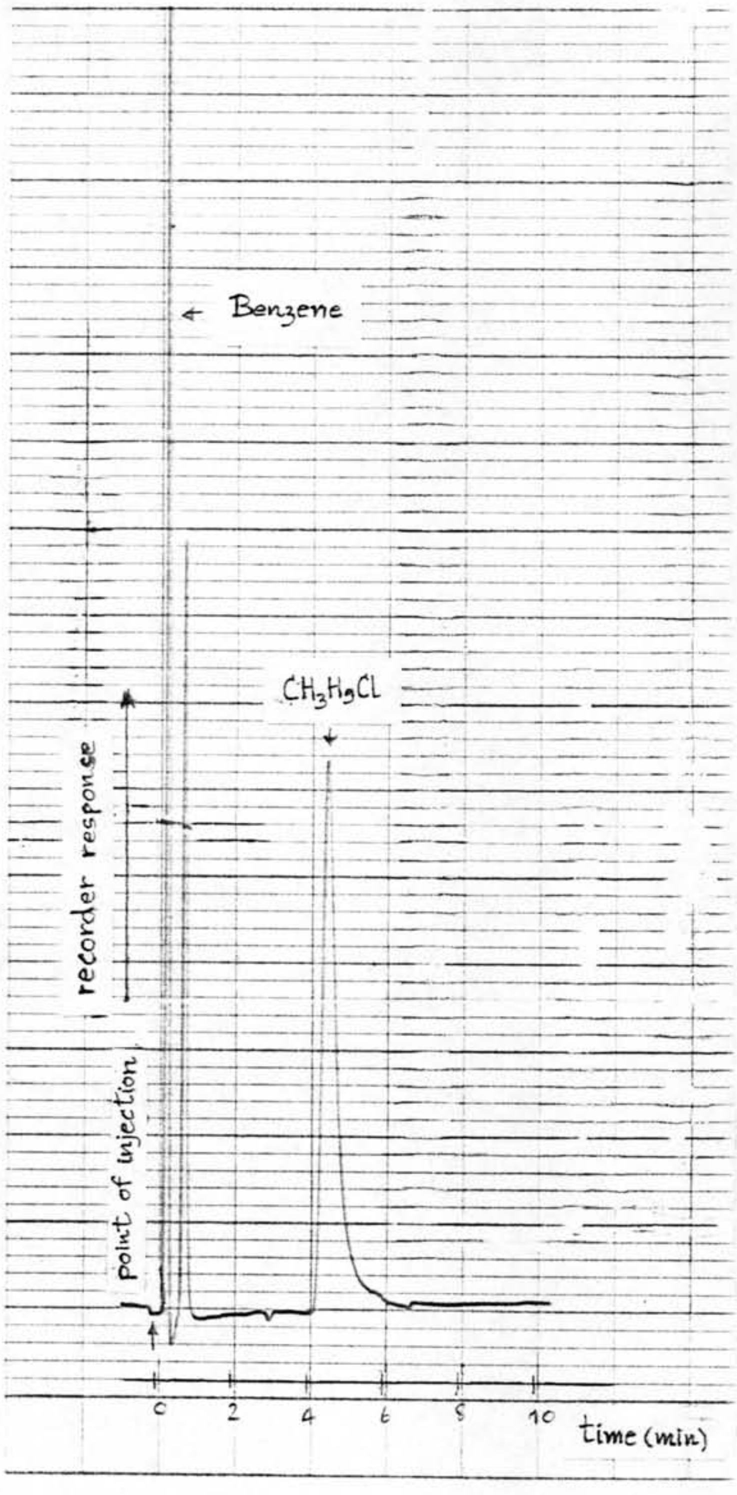

Figure 4.10 Typical chromatogram of standard methylmercury (II) chloride.

Table 4.10 Concentration of methylmercury in Barracuda

Sample No.	Concentration of methylmercury (II)chloride ($ngCH_3Hg/g$ wet)			Average value	Sourec of sample
N_1	24.72	26.87	31.17	27.59±3.28	market
N_2	31.17	38.69	32.25	34.04±4.06	market
N_3	22.57	20.42	26.87	23.29±3.28	market
N_4	18.27	23.63	-	20.95±3.79	market

Table 4.11 Concentration of methylmercury in Threadfin

Sample No.	Concentration of methylmercury (II) chloride (ng CH_3Hg/g wet)			Average value	Source of sample
T_1	39.77	37.62	-	38.69 ± 1.52	market
T_2	58.05	53.75	-	55.90 ± 3.04	market
T_3	66.65	68.75	63.42	66.29 ± 2.70	market
T_4	49.45	46.22	50.52	48.73 ± 2.24	market

Table 4.12 Concentration of methylmercury in Scad

Sample No.	Concentration of methylmercury (II) chloride (ng CH_3Hg/g wet)		Average value	Source of sample[b]
SA_1	97.82	82.77	90.29±10.64	A_1
SA_2	56.97	65.65	61.31±6.13	A_1
SB_1	7.52	13.97	10.74±4.56	B_1
SB_2	11.82	12.89	12.35±0.76	B_1

b Station A_1, B_1, Figure 3.1