CHAPTER IV

RESULTS

1. The effect of some organic solvents on oleoresin extraction.

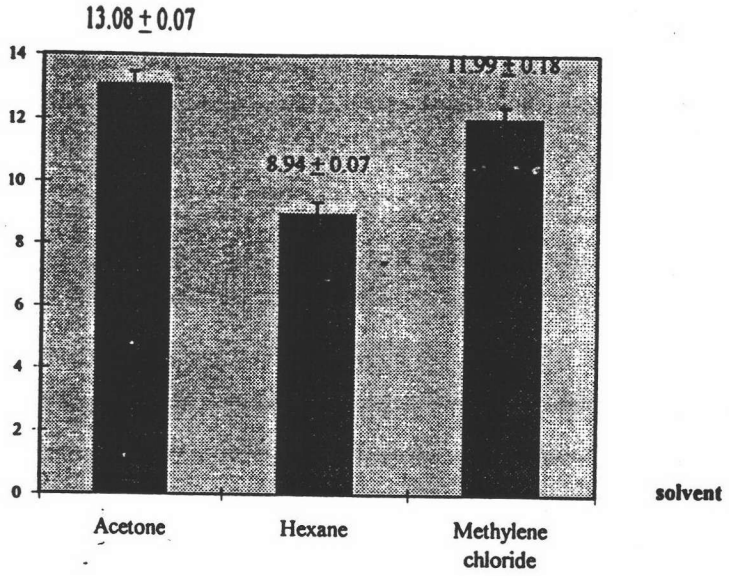
When 330 grams of Thai black pepper grade D was macerated separately in 1,000 ml of 3 different organic solvents : acetone, hexane and methylene chloride, it was found that acetone was the best among the three solvents in extracting oleoresin, volatile oil and piperine from the black pepper. As shown in Figure 6 and Table 5, acetone could extract 13.1%w/w of oleoresin, 24.1 % v/w of volatile oil and 31.6% w/w of piperine followed by hexane 8.9 % w/w oleoresin, 29.7 % v/w volatile oil and 38.3 % w/w piperine, respectively and methylene chloride 12% w/w oleoresin, 24.6 % v/w volatile oil and 36.1 % w/w piperine, respectively.

Table 5 The effect of some organic solvents on oleoresin extraction.

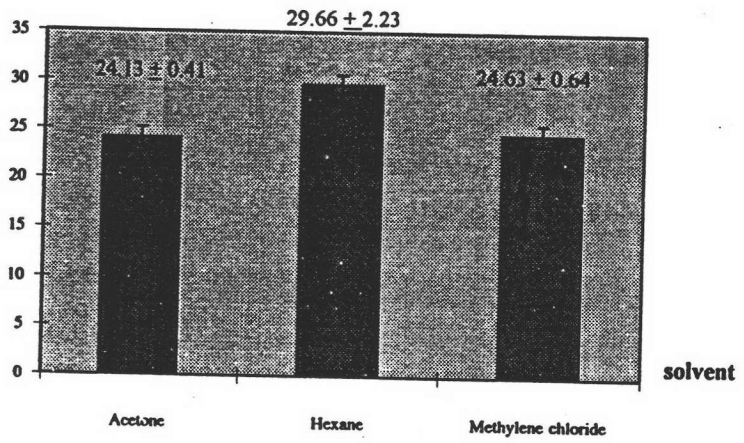
Solvent	Oleoresin (% w/w)	Volatile oil (% v/w)	Piperine (% w/w)
Acetone	13.08 $\pm$ 0.07	24.13 $\pm$ 0.41	31.63 $\pm$ 1.55
Hexane	8.94 $\pm$ 0.07	29.66 $\pm$ 2.23	38.33 $\pm$ 1.43
Methylene chloride	11.99 $\pm$ 0.18	24.63 $\pm$ 0.64	36.13 $\pm$ 0.09

(each value represents the mean $\pm$ SD for 3 separated preparations)

oleoresin (% w/w)



volatile oil (% v/w)



piperine (% w/w)

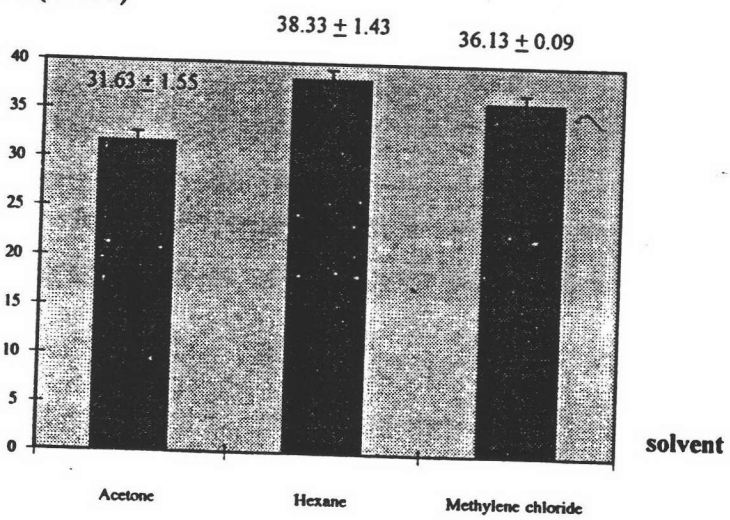


Figure 6 : The effect of some organic solvents on oleoresin extraction

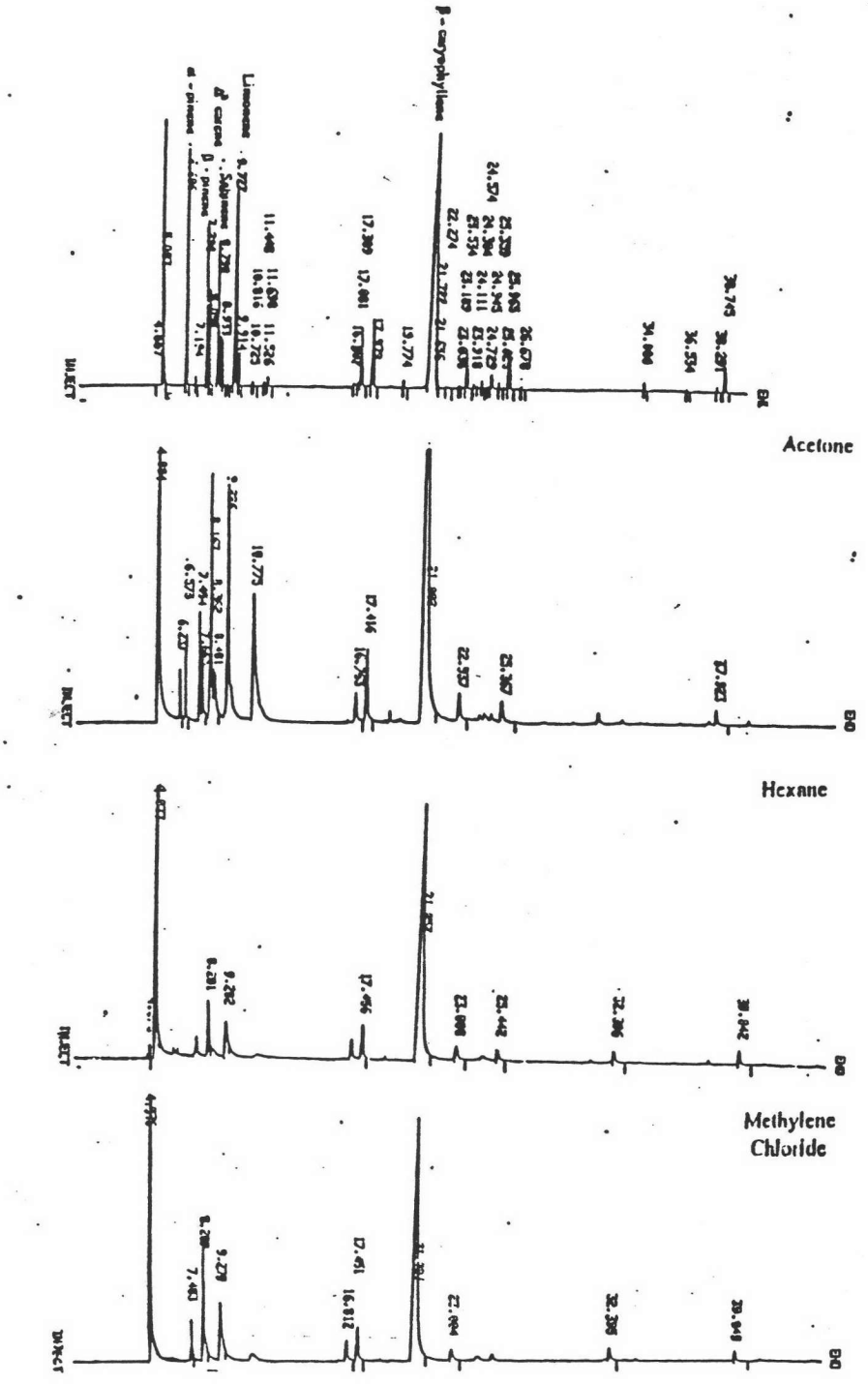


Figure 7 GC chromatograms of volatile oil components on the effect of some organic solvents on oleoresin extraction

With respect to volatile oil composition, the analysis by GC revealed that the oleoresin extraction by acetone gave GC chromatogram of the volatile oil very similar to the chromatogram of natural black pepper oil (Figure7). On the other hand, the extraction by hexane and methylene chloride caused the disappearance of some volatile oil components. According to the Retention time (RT) of various standards, it was found that there were 6 major components which was extracted from oleoresin by acetone. These included α-pinene, β-pinene, sabinene, Δ^3-carene, limonene and β-caryophyllene in pepper oil. For methylene chloride extraction, there were 4 components extracted including β-pinene, Δ^3-carene, limonene and β- caryophyllene. For hexane there were 3 main components extracted : Δ^3-carene, limonene and β-caryophyllene.(Figure7)

For piperine content

Based on a spectrophotometric method the calibration curve of piperine was found to be linear in the range from 16 to 265 μg/ml of concentration. This calibration had the correlation coefficient of 0.9995. (Figure 8)

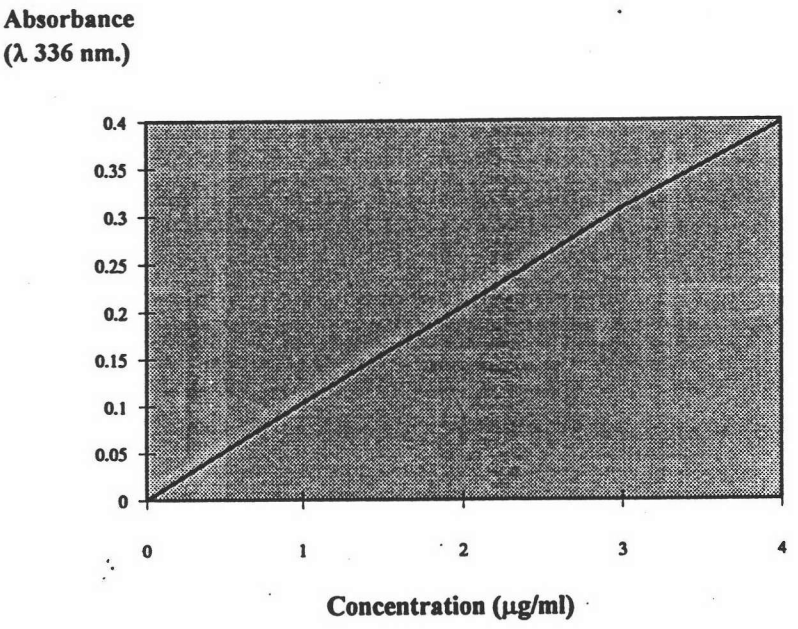
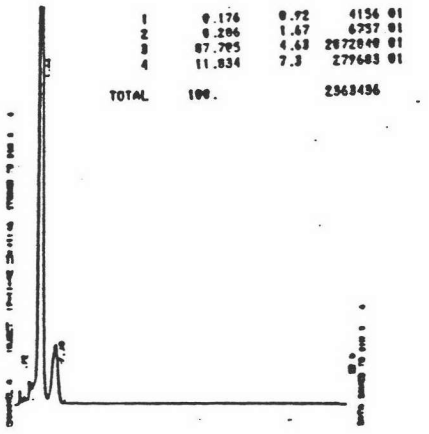


Figure 8 Calibration curve of piperine

With the established calibration curve, it was found that the oleoresins prepared from the solvent maceration of acetone, hexane and methylene chloride had very similar content of piperine, 31.6, 38.3 and 36.1 % w/w respectively (Table 5). This indicated that these 3 solvents had the same efficiency in extracting piperine from the black pepper powder.

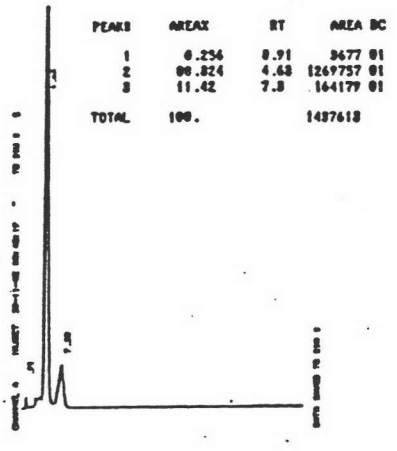
In order to check whether the oleoresin had piperine as a major constituent, we analysed the extracts of the different solvents by HPLC. It was found that all the oleoresin extracts had similar HPLC chromatograms which showed that piperine was the major constituent (Figure 9).



1	0.176	0.92	4156 01
2	0.286	1.67	6757 01
3	87.795	4.63	2072849 01
4	11.834	7.3	279683 01
TOTAL	100.		2363436

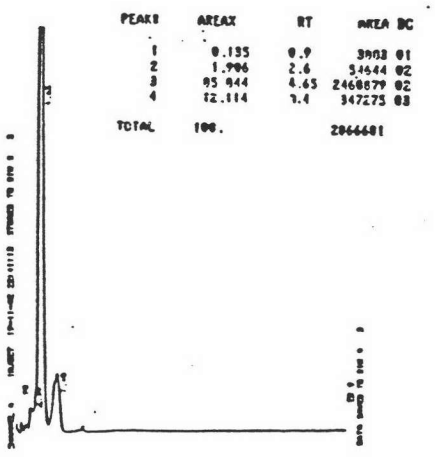
solvent

acetone



PEAK#	AREA%	RT	AREA DC
1	0.256	0.91	3677 01
2	99.824	4.63	1269757 01
3	11.42	7.3	164179 01
TOTAL	100.		1437618

hexane



PEAK#	AREA%	RT	AREA DC
1	0.135	0.9	3903 01
2	1.996	2.6	54644 02
3	85.944	4.65	2468879 02
4	12.114	7.4	347275 03
TOTAL	100.		2866681

methylene chloride

.**Figure 9** : HPLC chromatograms of piperine on the effect of some organic solvents on oleoresin extraction

2. The effect of different grades of pepper on the quality and quantity of pepper.

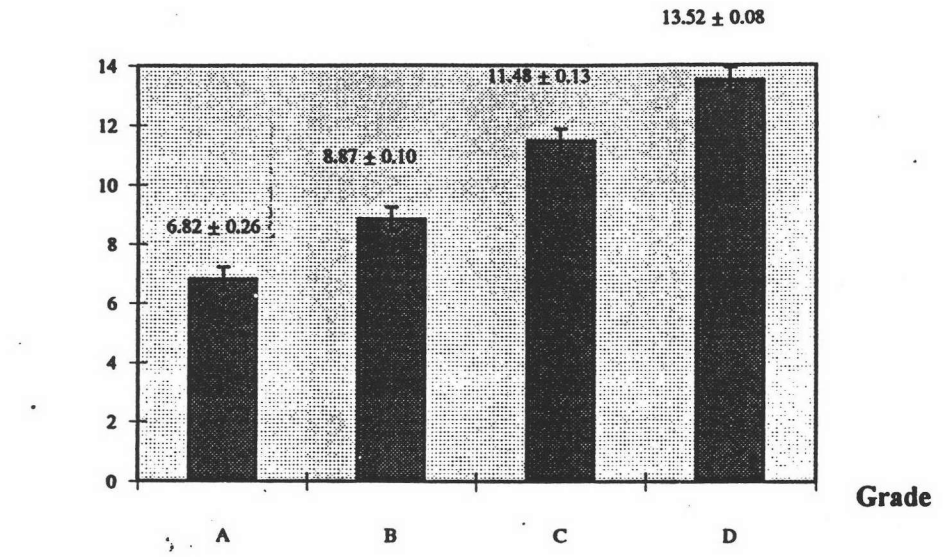
When acetone was used for oleoresin extraction from various grades (A, B, C, D) of Thai black pepper, it was found that the black pepper grade D gave the highest yield of oleoresin and volatile oil and piperine (Figure 10 and Table 6).

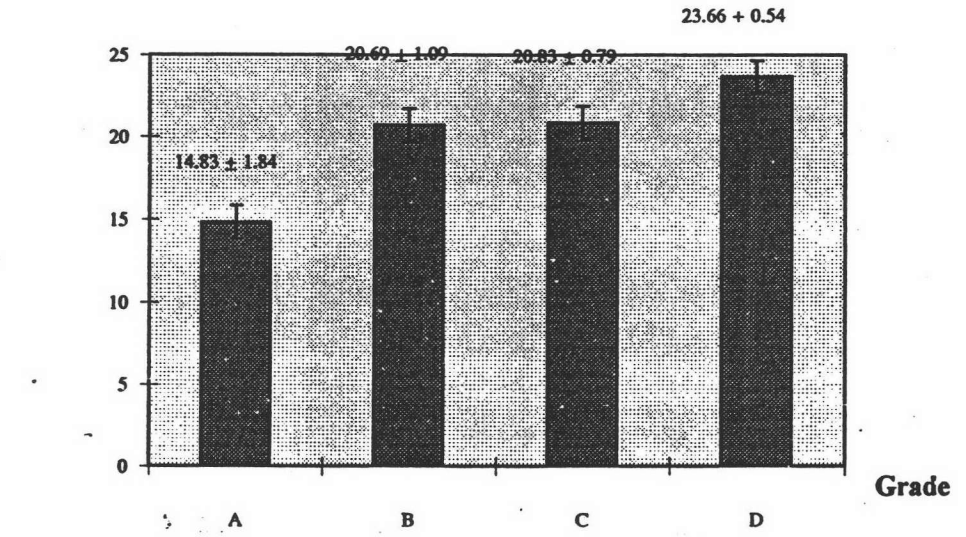
Table 6 The effect of different grades of pepper on oleoresin preparation.

Grade	Oleoresin (% w/w)	Volatile oil (% v/w)	Piperine (% w/w)
A	6.82 ± 0.26	14.83 ± 1.84	39.15 ± 1.97
B	8.87 ± 0.10	20.69 ± 1.09	35.08 ± 2.73
C	11.48 ± 0.13	20.83 ± 0.79	30.25 ± 1.78
D	13.52 ± 0.08	23.66 ± 0.54	31.41 ± 0.89

(each value represents the mean ± SD for 3 separated preparations)

Furthermore, the GC chromatograms of grade D black pepper had higher content of various oil component than the other grades (Figure 10).





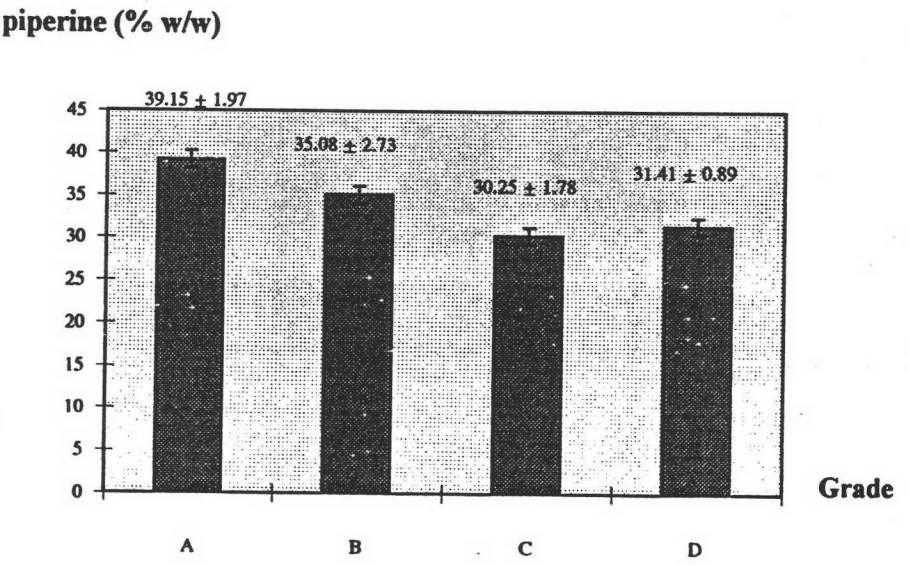


Figure 10 The effect of different grades of pepper on oleoresin preparation.

grade

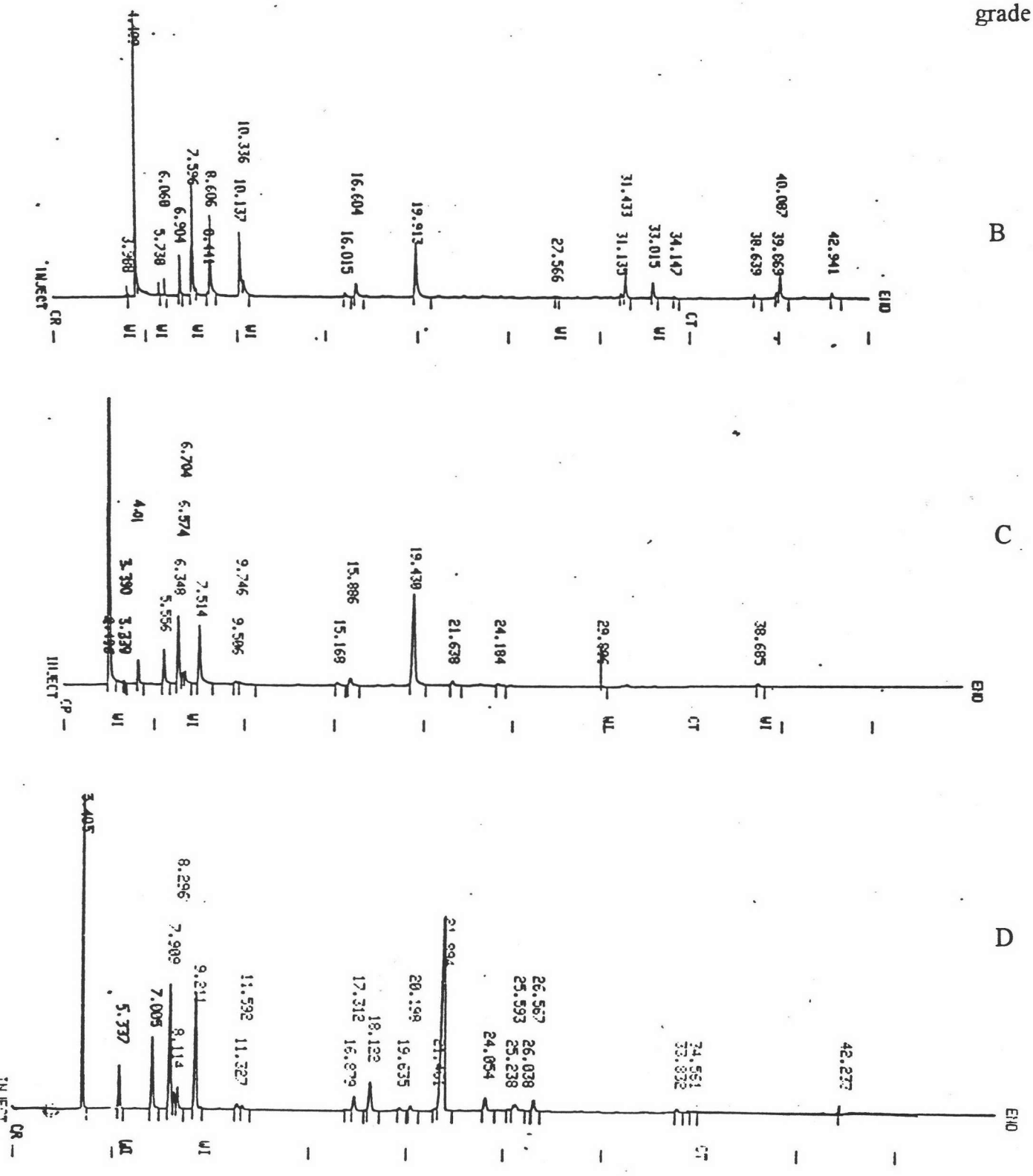


Figure 11 GC chromatograms of volatile oil components on the effect of different grades of pepper on oleoresin preparation.

3. Optimization of the maceration time

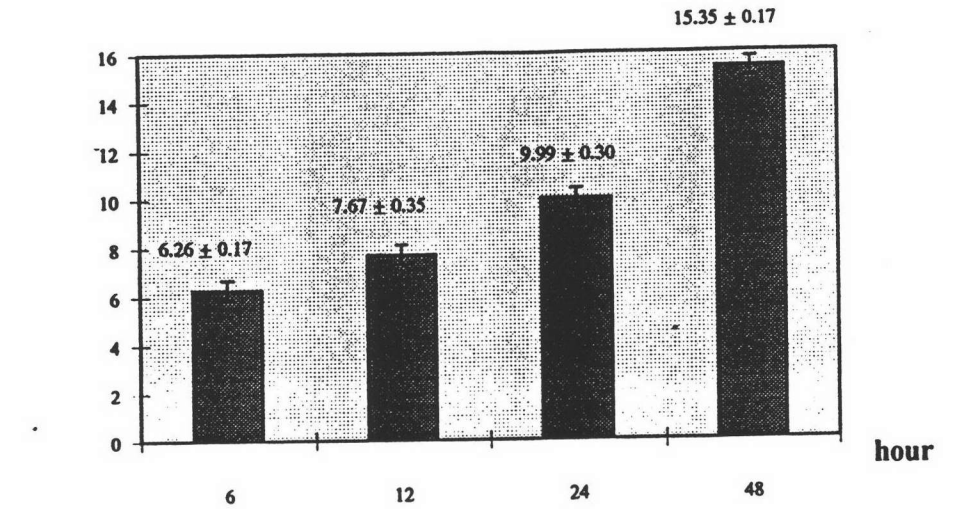
When Thai black pepper grade D (330 grams) was macerated in 1,000 ml of acetone for various periods of time : 6,12,24 and 48 hours, it was found that the maceration time of 48 hours was optimized. This is shown in Table 7 and Figure 12.

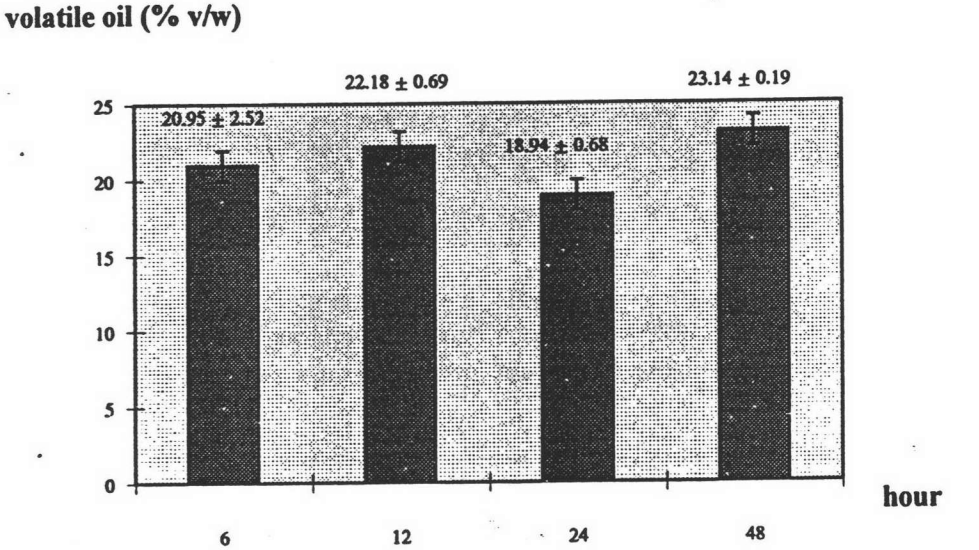
Table 7 Optimization of the maceration time

Hours	Oleoresin (% w/w)	Volatile oil (% v/w)	Piperine (% w/w)
6	6.26 ± 0.17	20.95 ± 2.52	27.40 ± 0.18
12	7.67 ± 0.35	22.18 ± 0.69	28.57 ± 1.31
24	9.99 ± 0.30	18.94 ± 0.68	32.54 ± 0.69
48	15.35 ± 0.17	23.14 ± 0.19	28.82 ± 0.91

(each value represents the mean ± SD for 3 separated preparations)

It can be seen that the 3 parameters of oleoresin weight, volatile oil and piperine content increased continuously with time to 48 hours. Therefore, this maceration time was used throughout this study.





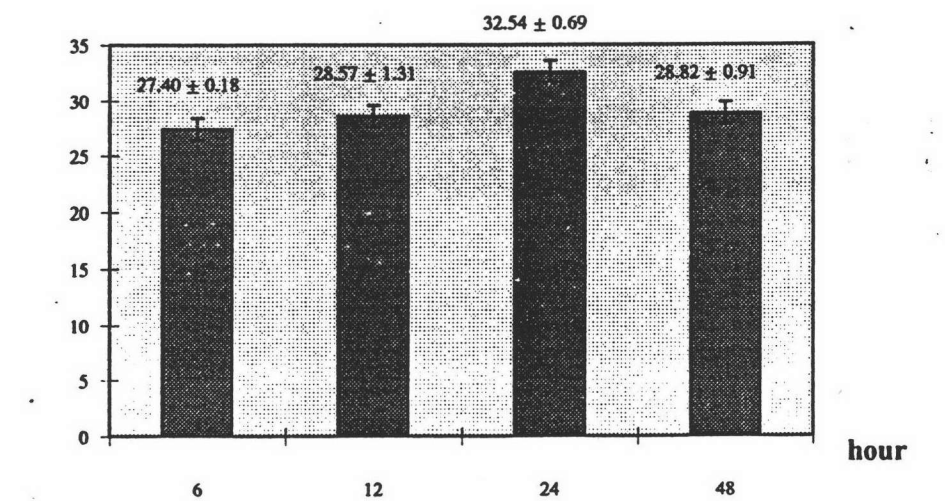
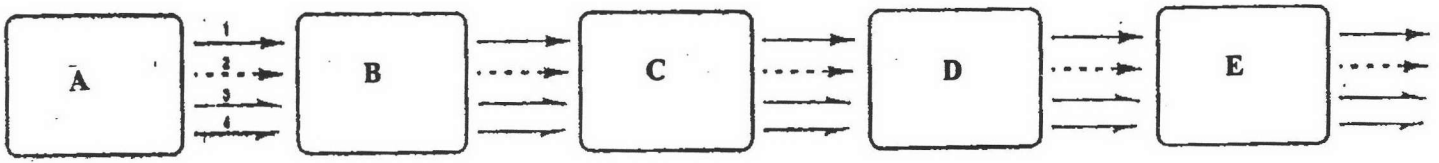


Figure 12 : Optimization of the maceration time

4. Study on a step-wise maceration of black pepper for oleoresin extraction.

Oleoresin preparation by step-wise maceration was carried out by macerating the grade D black pepper in 5 containers with acetone in a consecutive manner. The objective of this study was to find an effective method of using acetone in the extraction of oleoresin from Thai black pepper. Experimentally, the efficiency of acetone in the extraction of oleoresin was first evaluated by macerating the black pepper powder in a sequential manner (Method 1) in order to observe the saturation level of the solvent. The results of using Method 1 (as shown below) showed that acetone staeted to be saturated with oleoresin after passing 2 containers of black pepper.

Method 1 ;



The almost saturation was all demonstrated wiyh the oleoresin weight, volatile oil and piperine content (Table 8 and Figure 13,14,15). When the second portion of pure acetone was again sequentially passed the same 5 containers, it was found that the acetone still had an ability to extract olroresin until became saturated until passing container 4 (D). However, with the third round of pure acetone , very little olroresin or volatile oil was detected in the first 2 containers but gradually incresed until passing container 5 (E). After the third portion of acetone was passed, very small amount of oleoresin left in the black pepper especially in yhe first three containers. Therefore, the forth round of acetone showed very low levels of oleoresin, volatile oil and piperine content

although there were some in containers 4 and 5 (D and E). Analysis of the oil quality by GC , it was found that all fractions gave very similar patterns of GC chromatograms (Figure 16). Therefore, the step-wise maceration appeared to have no effect on the volatile oil quality of pepper oleoresin.

From these results of oleoresin extraction from grade D black pepper using Method 1, it could be concluded as follows:

1. The total yield of oleoresin was 224.75 g / 1,650 g black pepper
2. The total volatile oil content was 59.42 ml / 1,650 g black pepper
3. The total piperine content was 77.95 g / 1,650 g black pepper

Table 8 Method 1 step-wise maceration of black pepper for oleoresin extraction.

Container/no. of maceration	oleoresin wt. (g.)	volatile oil (ml.)	piperine (g.)
A/1	44.33 ± 4.04	9.00 ± 2.60	17.26 ± 0.26
A/2	9.33 ± 2.18	1.58 ± 0.38	3.03 ± 0.26
A/3	5.08 ± 0.88	0.29 ± 0.07	0.55 ± 0.05
A/4	2.50 ±0.75	0.25 ±0.00	0.12 ± 0.09
B/1	78.92 ±4.50	20.08 ±1.38	23.26 ± 1.54
B/2	37.83 ± 2.67	7.75 ± 1.15	11.20 ±1.38
B/3	8.92 ± 1.76	1.17 ±0.29	2.97 ±0.40
B/4	4.67 ± 1.42	0.29 ±0.07	0.30 ± 0.08
C/1	86.08 ± 1.27	22.92 ±1.44	26.96 ± 2.84
C/2	42.92 ± 3.26	14.17 ± 1.44	14.42 ±1.32
C/3	23.33 ± 1.58	3.83 ± 0.63	4.57 ± 0.76
C/4	7.25 ±0.90	1.03 ± 0.04	1.49 ± 0.15
D/1	81.17 ± 1.26	23.33 ± 1.91	28.89 ±0.18
D/2	70.08 ± 1.01	15.58 ± 0.63	20.24 ± 1.71
D/3	38.50 ± 1.39	5.17 ± 0.76	11.93 ± 1.32
D/4	12.42 ± 1.76	3.33 ±0.72	6.18 ±0.71
E/1	88.17 ±3.92	22.08 ± 2.60	28.04 ± 0.13
E/2	71.33 ± 3.21	18.33 ±1.44	25.45 ± 0.14
E/3	44.50 ± 2.65	15.05 ±1.13	16.96 ± 1.28
E/4	20.75 ± 3.04	3.92 ±0.29	7.50 ± 0.50

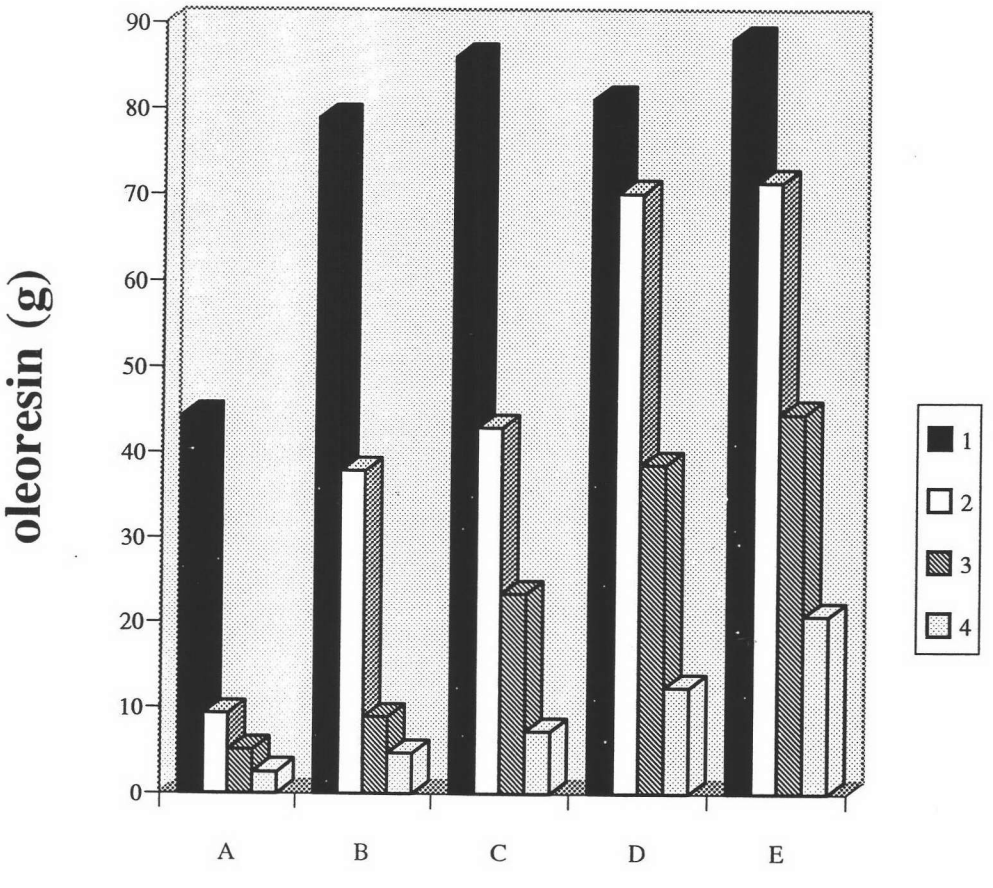


Figure 13 The average weight of oleoresin in each container in method 1 step-wise maceration.

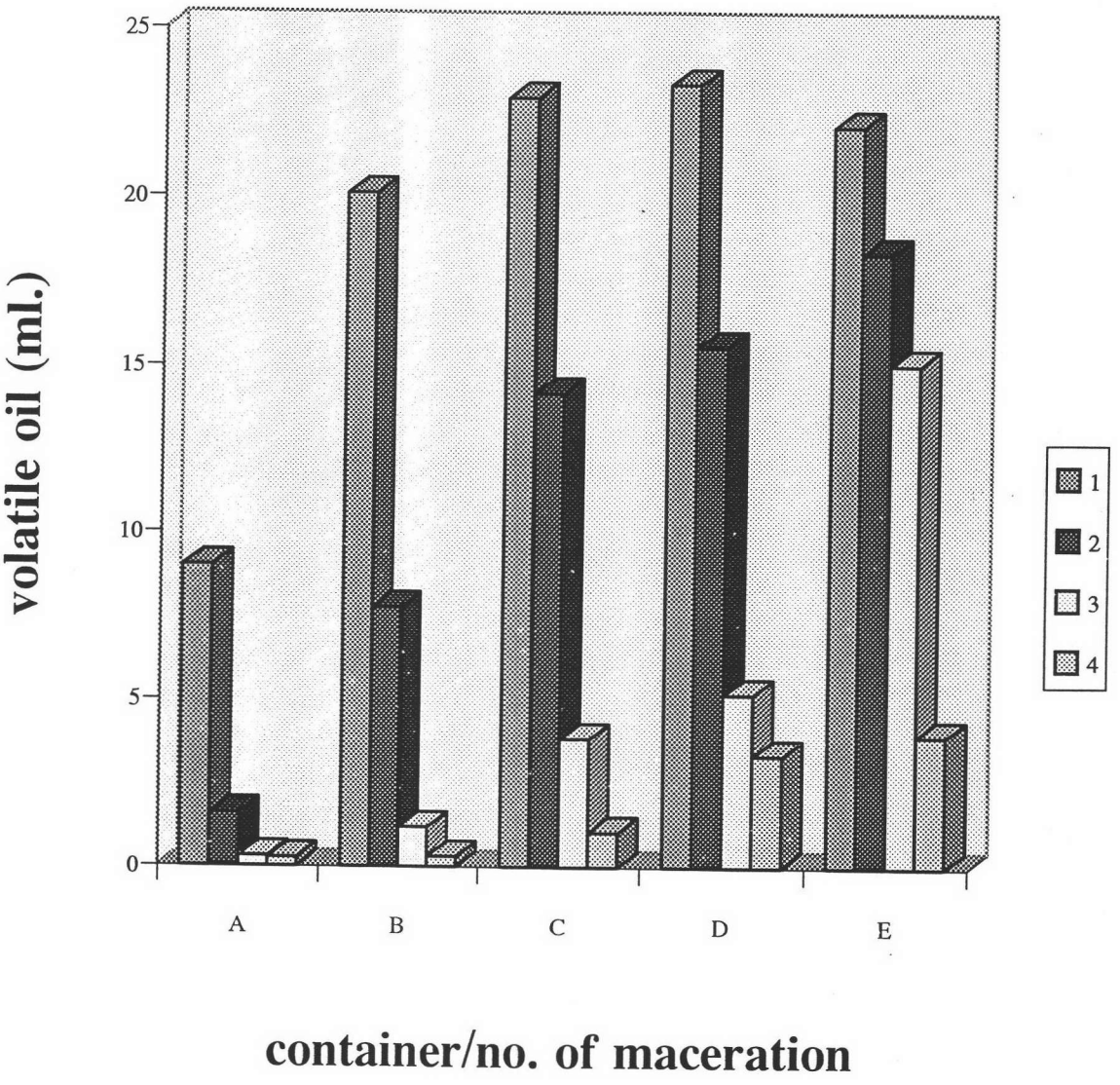


Figure 14 The average content of volatile oil in each container in method 1 step-wise maceration.

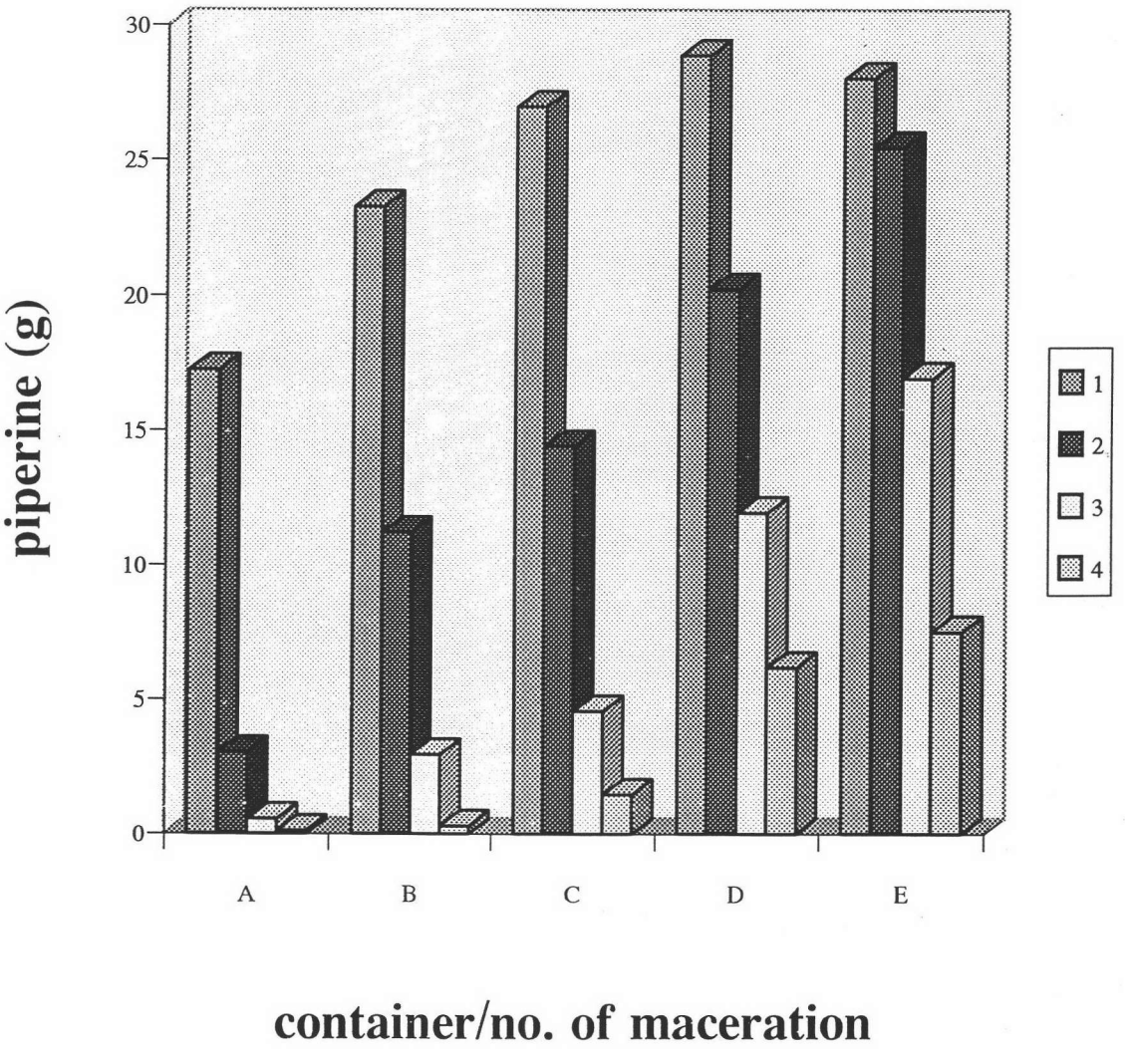


Figure 15 The average content of piperine in each container in method 1 step-wise maceration.

container/no.of maceration

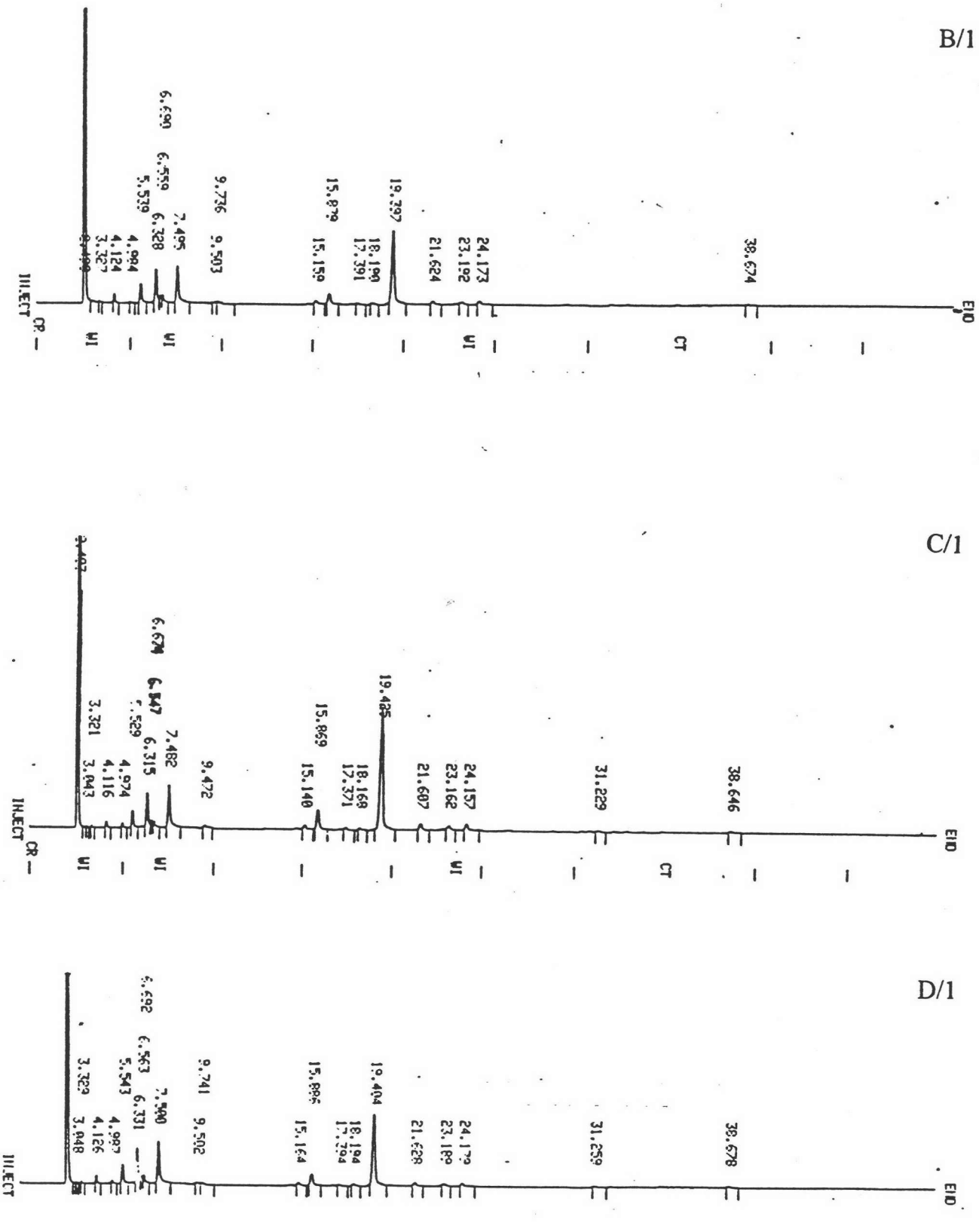


Figure 16 GC chromatograms of volatile oil components on method 1 step-wise maceration.

container/no.of maceration

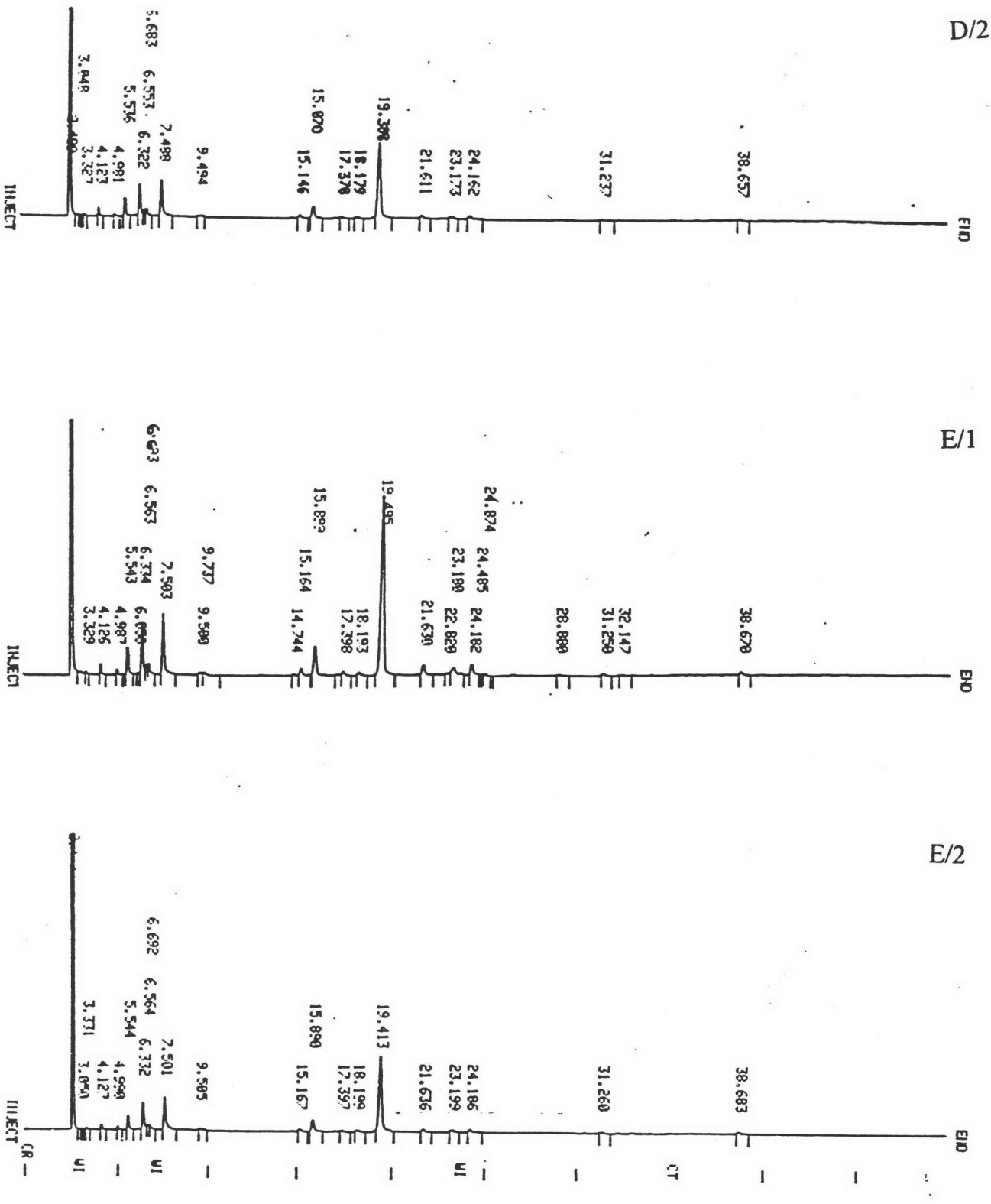


Figure 16 GC chromatograms of volatile oil components on method 1 step-wise maceration.

By expressed as the unit of percent of the original black pepper weight, the value of each parameter could be summarized as Table 9.

By comparison the specification of the black pepper and the specification of oleoresin with the results from the experiment shown in table 9 and 10.

Table 9 Comparison the specification of black pepper with method 1 step-wise maceration.

Parameter	Yield (% in black pepper)	Specification (%)
oleoresin content (w/w)	13.62 $\pm$ 0.58	10 - 15
volatile oil content (v/w)	3.60 $\pm$ 0.10	2 - 4
piperine content (w/w)	4.73 $\pm$ 0.11	5 - 9

If expressed the unit based on the weight of oleoresin, the resulted values of the oil and piperine content as shown in Table 10.

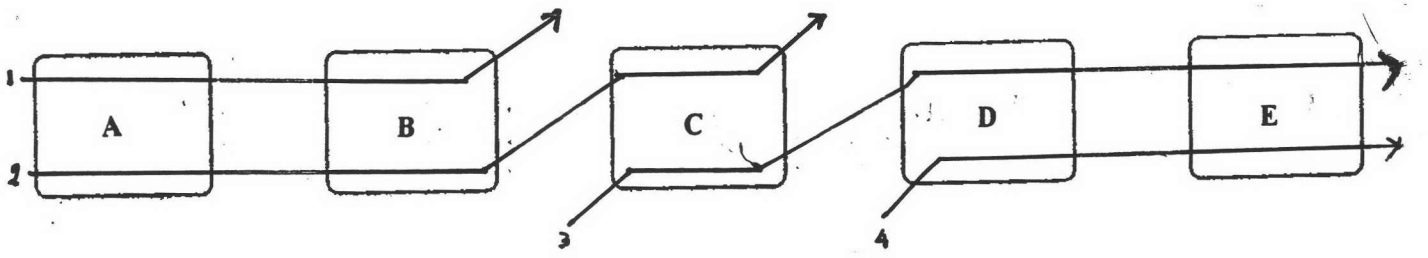
Table 10 Comparison the specification of oleoresin with method 1 step-wise maceration.

Parameter	Yield (% in oleoresin)	Specificatin (%)
volatile oil	26.43 $\pm$ 1.07	18 - 20
piperine (by UV-spectrometer)	34.67 $\pm$ 2.09	34 - 40

When the value of each parameter was compared with that of the generally accepted specification (Tables 9 and 10), it was found that the oleoresin obtained by Method 1 had most characteristics fell in the range of the standard values.

Based on the study of oleoresin extraction by using Method 1 , it was concluded that the first portion of acetone was quite saturated with oleoresin after passing the first two containers (A and B). Therefore, there was no need to pass the three containers. Instead , the second acetone portion could be used by passing A , B and C containers. This should have brought the second acetone portion the saturated level and at the same time caused very little oleoresin left in A and B containers. Therefore, the third acetone portion could start with container C and continue to container D and E. Finally, the last portion of acetone should have passed container D and E one move time to extract all the oleoresin still present in the last two containers. The diagram of the system called Method 2 is shown below.

Method 2 ;



The results obtained from this study is shown in Table 7 and Figures 17,18,19. As the Method 1, the analysis of volatile oil composition by GC also showed that the volatile oil extracted by each portion of acetone was all composed of similar composition (Figure 20).

From these results of oleoresin extraction from grade D black pepper using Method 2 , it could be concluded as follows :

1. The total yield of oleoresin was 253 g / 1,650 g black pepper
2. The total volatile oil content was 60.69 ml / 1,650 g black pepper
3. The total piperine content was 81.73 g / 1,650 g black pepper

Table 11 Method 2 step-wise maceration of black pepper for oleoresin extraction.

Container/portion of acetone	oleoresin wt. (g)	volatile oil (ml)	piperine (g)
A/1	48.25 ± 0.35	11.25 ± 0.35	14.98 ±0.07
A/2	7.50 ±0.00	1.63 ± 0.18	1.48 ±0.06
B/1	57.00 ± 0.35	13.98 ± 0.02	22.080 ± 0.14
B/2	17.13 ±0.18	3.38 ± 0.18	3.21 ±0.04
C/1	58.13 ±0.18	14.21 ± 0.41	20.88 ± 0.45
C/2	16.75 ± 0.00	3.50 ± 0.00	2.74 ±0.07
D/1	61.88 ± 1.24	15.34 ±0.34	21.44 ± 0.65
D/2	17.56 ±0.80	4.13 ±0.53	3.14 ±0.04
E/1	69.63 ± 1.24	16.92 ± 0.11	24.80 ± 0.45
E/2	68.50 ±1.41	15.58 ±0.03	21.25 ±0.14

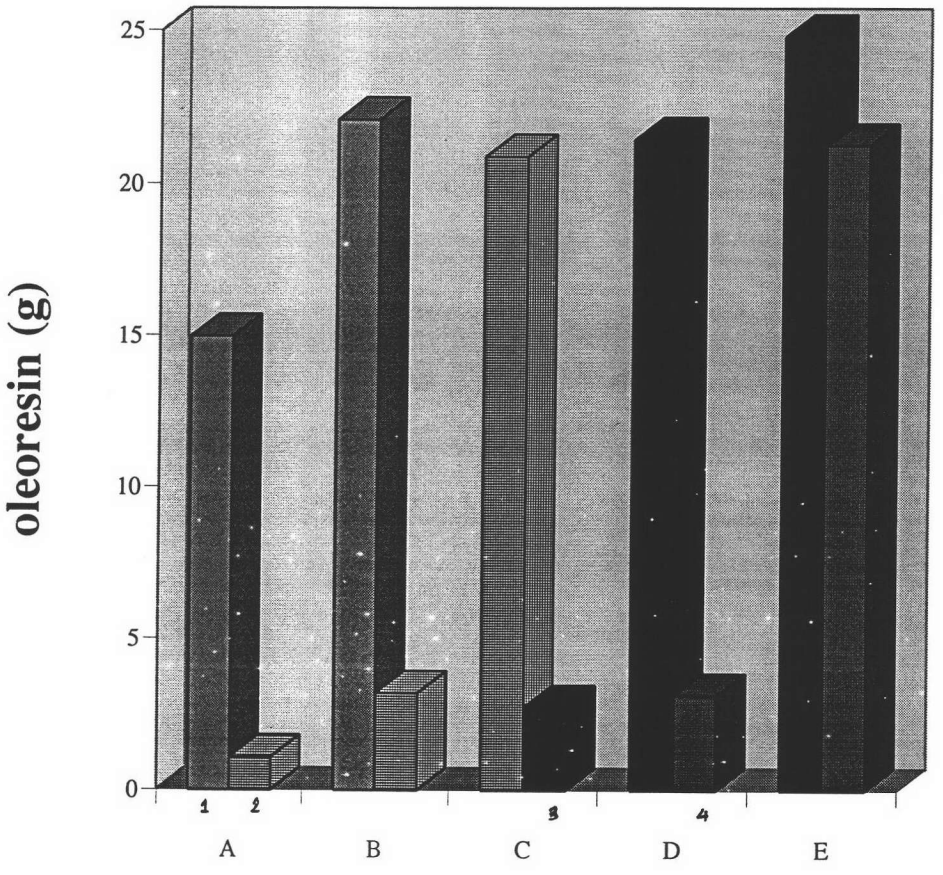


Figure 17 The average weight of oleoresin in each container in method 2 step-wise maceration.

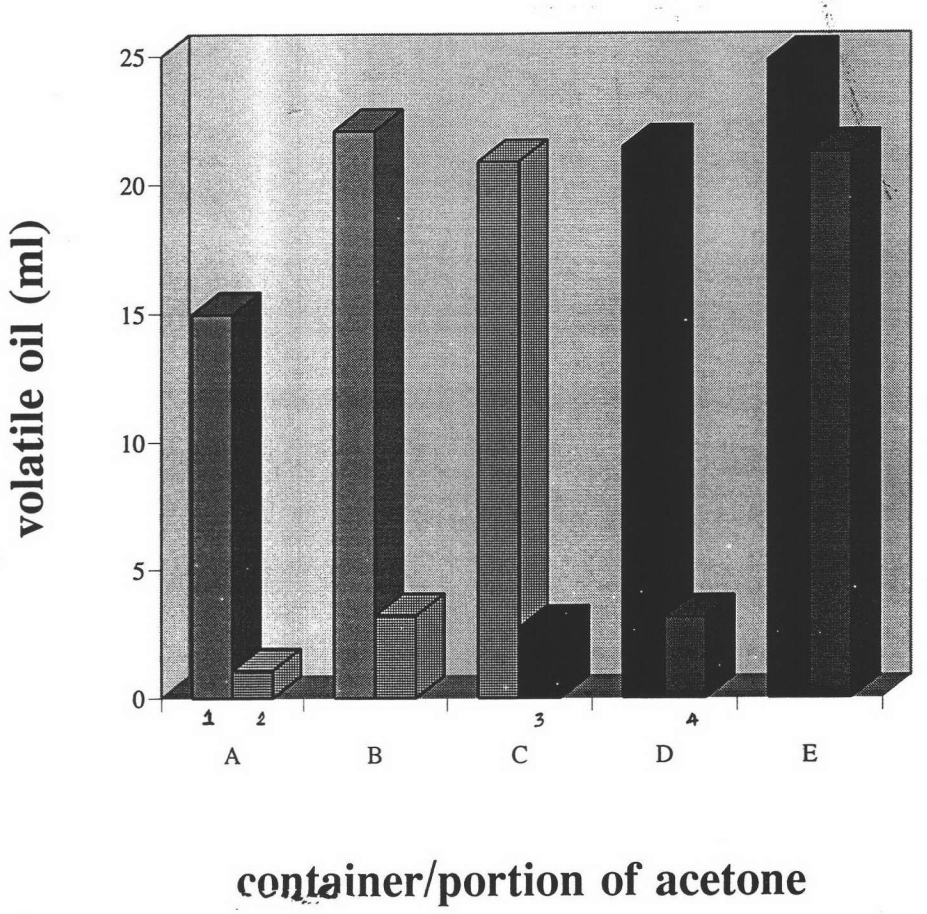


Figure 18 The average content of volatile oil in each container in method 2 step-wise maceration.

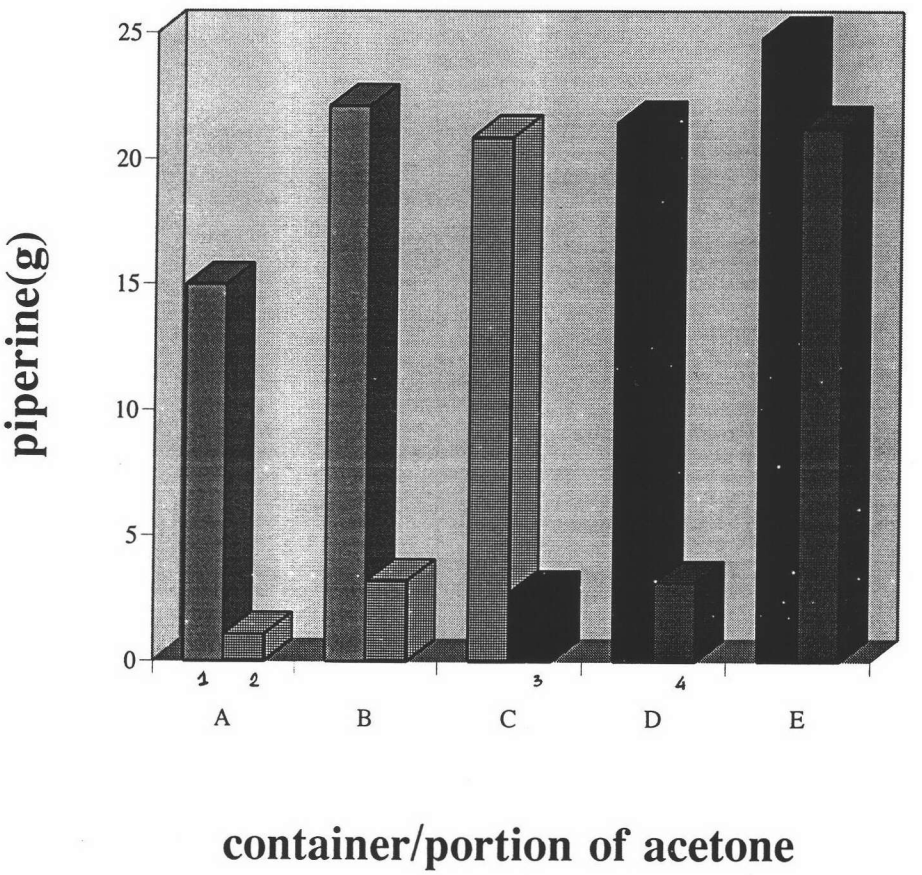


Figure 19 The average content of piperine in each container in method 2 step-wise maceration.

container/portion of acetone

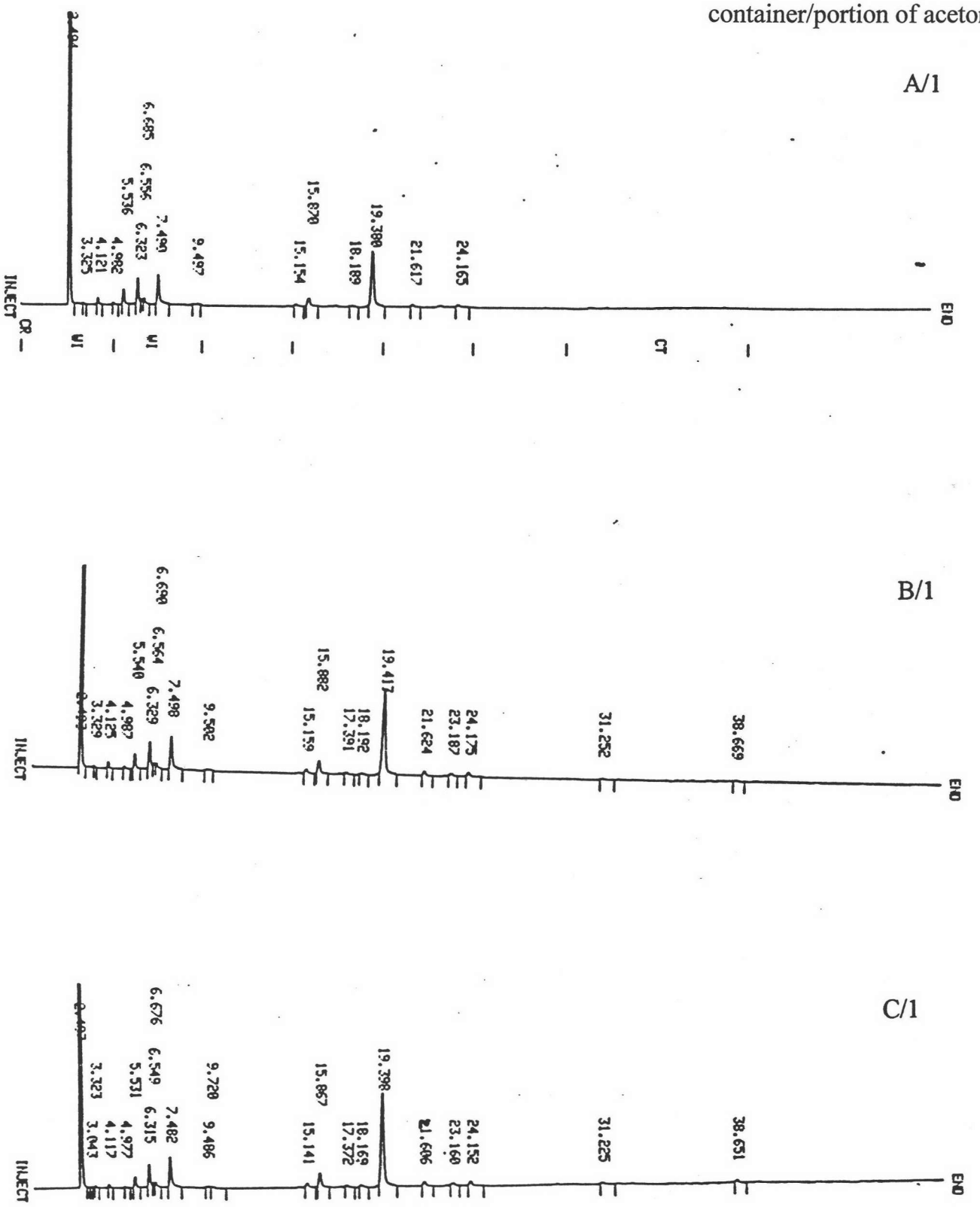


Figure 20 GC Chromatograms of volatile oil components on method 2 step-wise maceration.

container/portion of acetone

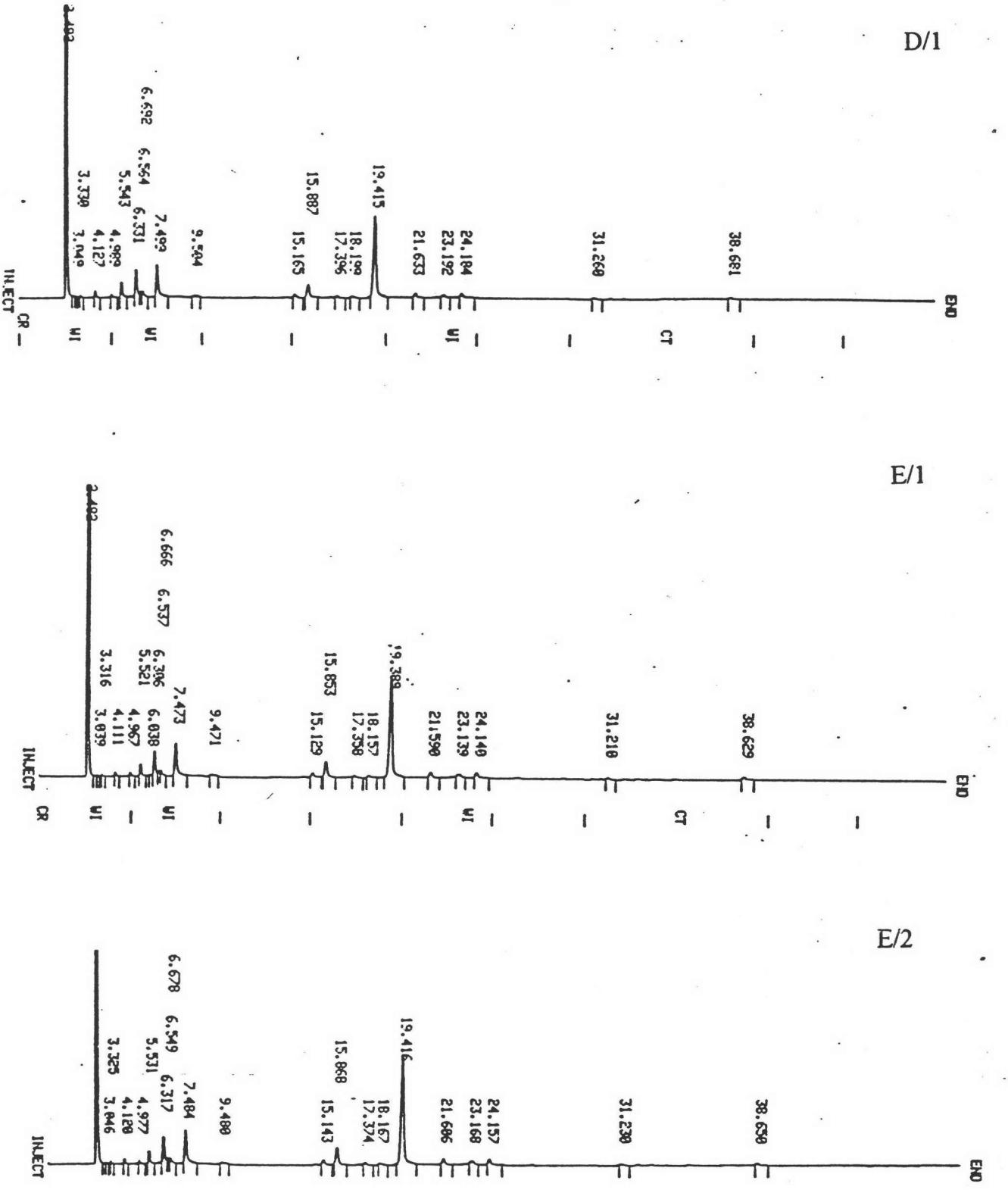


Figure 20 GC Chromatograms of volatile oil components on method 2 step-wise maceration.

By comparison the specification of the black pepper and the specification of oleoresin with the results from the experiment shown in Table 12 and 13.

Table 12 Comparison the specification of black pepper with method 2 step-wise maceration.

Parameter	Yield (% in black pepper)	Specification (%)
oleoresin content (w/w)	15.35 ± 0.15	10 - 15
volatile oil content (v/w)	3.68 ± 0.04	2 - 4
piperine content (w/w)	5.44 ± 0.00	5 - 9

If expressed the unit based on the weight of oleoresin, the resulted values of the oil and piperine content as shown in Table 13.

Table 13 Comparison the specification of oleoresin with method 2 step-wise maceration.

Parameter	Yield (% in oleoresin)	Specification (%)
volatile oil	23.96 ± 0.00	18 - 20
piperine (by UV-spectrometer)	35.43 ± 0.34	34 - 40

When the value of each parameter was compared with that of the generally accepted specification (Tables 12 and 13), it was found that the oleoresin obtained by Method 2 had most characteristics fell in the range of the standard values.

5. Study on residual solvent present in prepared oleoresin.

Calibration curve of acetone

The calibration curve of acetone figure 21 showed linearity of the relationship in range 0.25-100% concentration and the correlation coefficient which was found to be 0.990 for acetone.

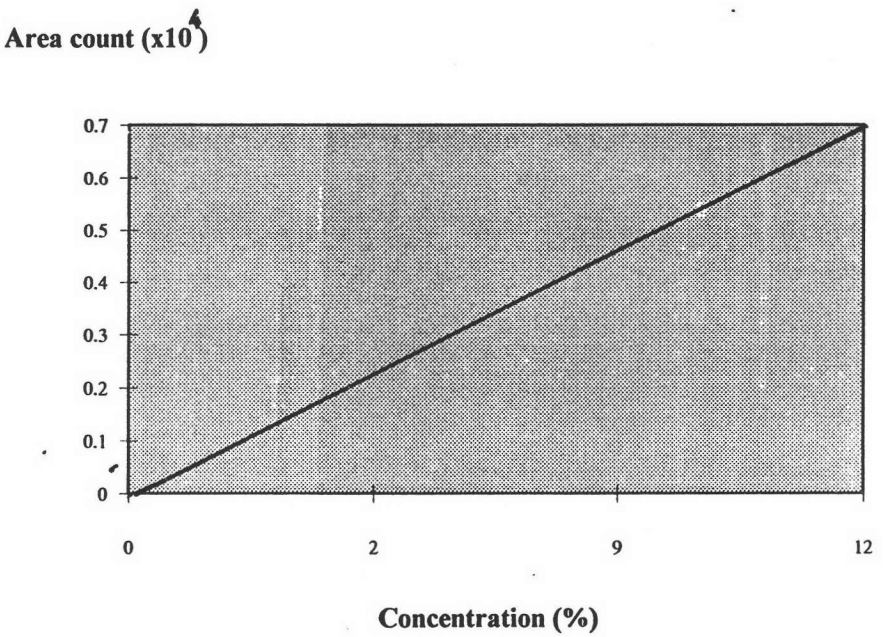


Figure 21 The calibration curve of acetone

In order to find out the amount of residual acetone which was used as a solvent in oleoresin preparation from grade D black pepper by step-wise maceration technique , the technique of GC used.

Figure 22, 23 and 24 showed GC chromatograms of residual acetone in prepared oleoresin. The acetone peak was orderly shown, included Retention time 3.180, 3.249 and 3.243 min and the area count were 54559, 56628 and 56855 respectively.

The amount of residual acetone was calculated from these figure of area count. Therefore, the amount of residual acetone were 28.80, 24.60 and 29.60 ppm and 29.33 ± 0.46 ppm by average.

The specification of oleoresin mentioned that, the residual solvent from black pepper oleoresin must less than 30 ppm. Therefore, the amount of residual acetone from this experiment is well accepted.

VARIAN 3400 GAS CHROMATOGRAPH
METHOD 1 RUN 15
TIME 11:03 27 APR 95
SAMPLE: PEPPER
RUN MODE: ANALYSIS
CALCULATION TYPE: PERCENT

PEAK NO.	PEAK NAME	TIME MIN	RESULT PERCENT	AREA COUNTS
1		3.180	0.9563	54559
2		3.544	98.5281	5620723
3		3.907	0.5155	29408
TOTALS:			100.0000	5704690

DETECTED PEAKS: 3 REJECTED PEAKS: 0
AMOUNT STANDARD: 1.00000000
MULTIPLIER: 1.00000000 DIVISOR: 1.00000000
NOISE: 27.0 OFFSET: -33

RACK 1 VIAL 6 INJ 1

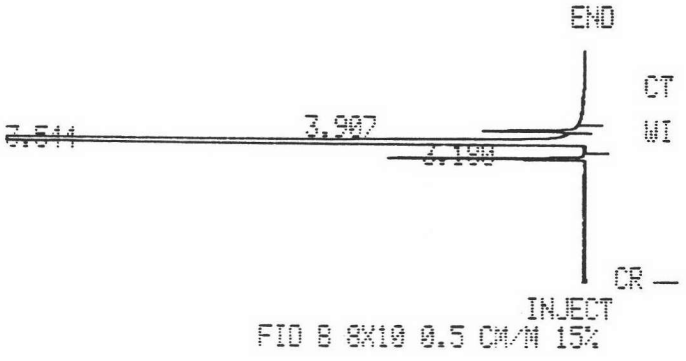


Figure 22 GC Chromatogram of residual acetone.

VARIAN 3400 GAS CHROMATOGRAPH
METHOD 1 RUN 10
TIME 15:28 08 FEB 95
SAMPLE: PEPPER
RUN MODE: ANALYSIS
CALCULATION TYPE: PERCENT

PEAK NO.	PEAK NAME	TIME MIN	RESULT PERCENT	AREA COUNTS
1		3.249	0.7445	56628
2		3.644	89.4084	6800250
3		4.948	0.0823	6260
4		6.453	0.2587	19682
5		7.283	0.5184	39430
6		7.501	0.0782	5950
7		7.666	0.0855	6509
8		8.501	0.6309	47986
9		16.386	0.1073	8166
10		17.171	0.4126	31386
11		18.674	0.0546	4155
12		19.242	0.0319	2427
13		20.707	3.3241	252832
14		22.977	0.1630	12404
15		24.508	0.1252	9526
16		25.477	0.2118	16112
17		25.723	0.0990	7530
18		26.270	0.0637	4850
19		28.505	0.0811	6171
20		29.380	0.0889	6763
21		32.682	0.0620	4722
22		33.429	0.2960	22515
23		33.750	0.3322	25273
24		34.173	0.1621	12334
25		34.363	0.2107	16025
26		34.656	0.0595	4532
27		37.731	0.0640	4872
28		40.168	1.7604	133895
29		40.750	0.2997	22795
30		42.848	0.1818	13834
TOTALS:			100.0000	7605829

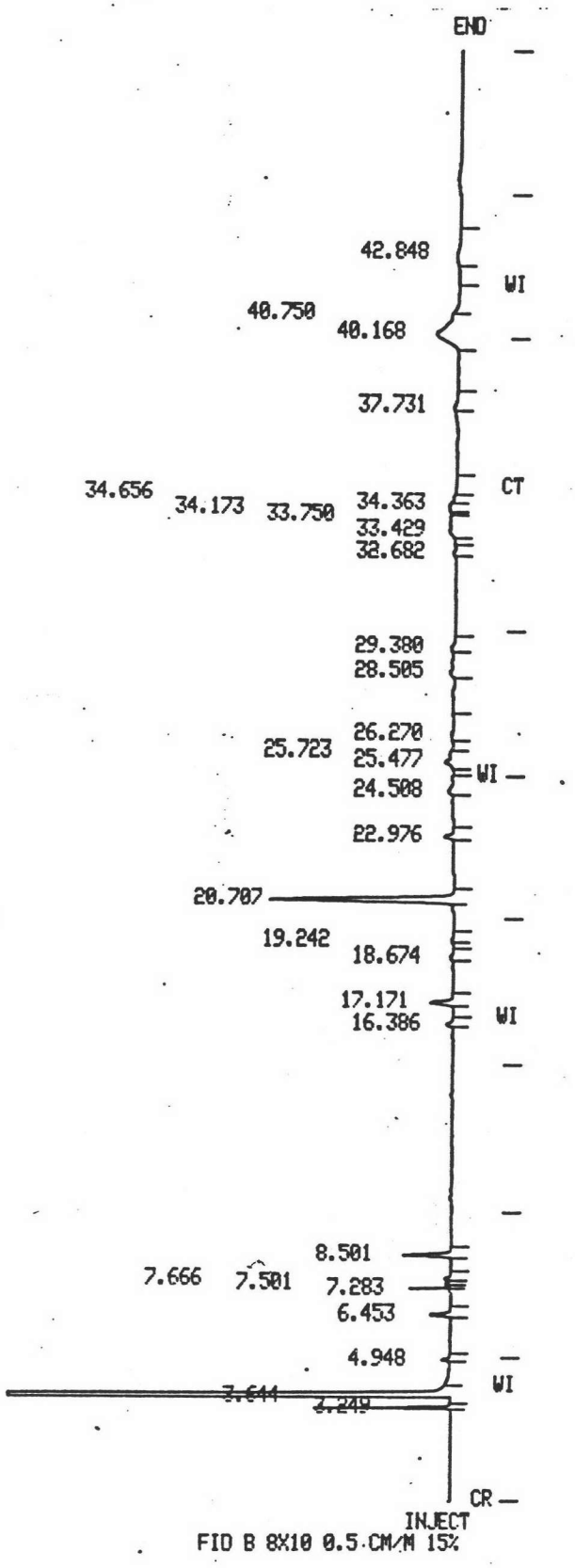


Figure 23 GC Chromatogram of residual acetone.

VARIAN 3400 GAS CHROMATOGRAPH
METHOD 1 RUN 11
TIME 16:26 08 FEB 95
SAMPLE: PEPPER
RUN MODE: ANALYSIS
CALCULATION TYPE: PERCENT

PEAK NO.	PEAK NAME	TIME MIN	RESULT PERCENT	AREA COUNTS
1		3.243	0.8888	56855
2		3.645	93.8629	6003638
3		4.938	0.0637	4077
4		6.439	0.2134	13654
5		7.269	0.4365	27920
6		7.486	0.0661	4231
7		7.651	0.0671	4293
8		8.485	0.5275	33741
9		13.971	0.0580	3712
10		16.368	0.0886	5668
11		17.117	0.3561	22395
12		18.529	0.0449	2873
13		20.373	2.8940	185107
14		22.176	0.1428	9134
15		23.254	0.1119	7157
16		23.859	0.1831	11715
TOTALS:			100.0000	6396176

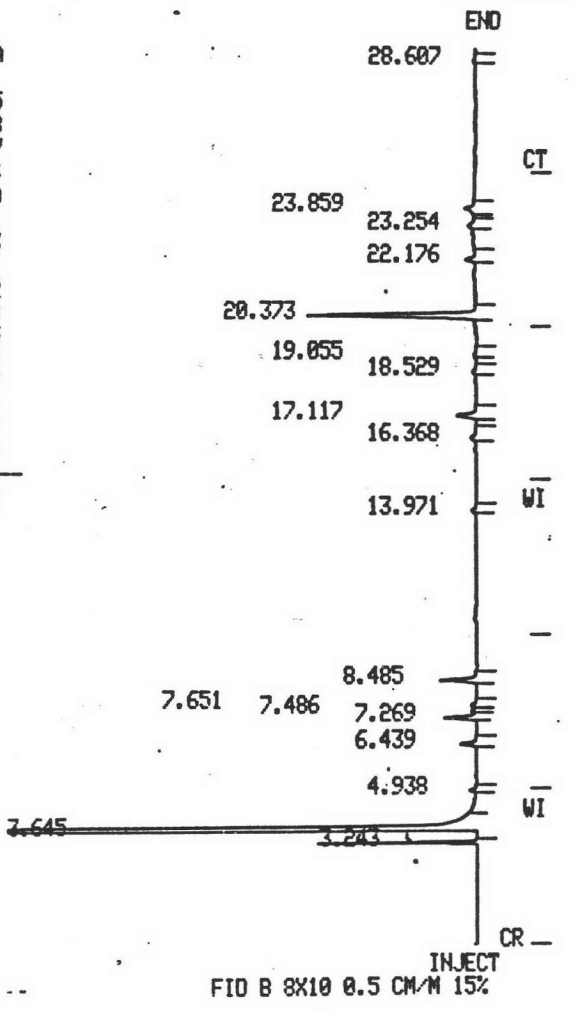


Figure 24 GC Chromatogram of residual acetone.