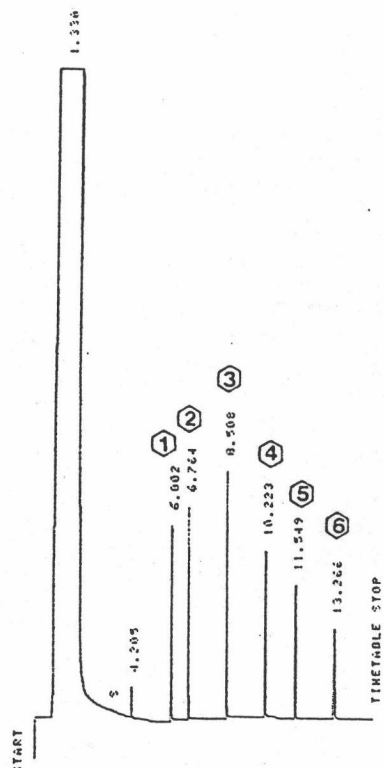
CHAPTER IV

RESULTS AND DISCUSSION

1. The Study of Gas Chromatographic Separation of PEs on Two Detectors

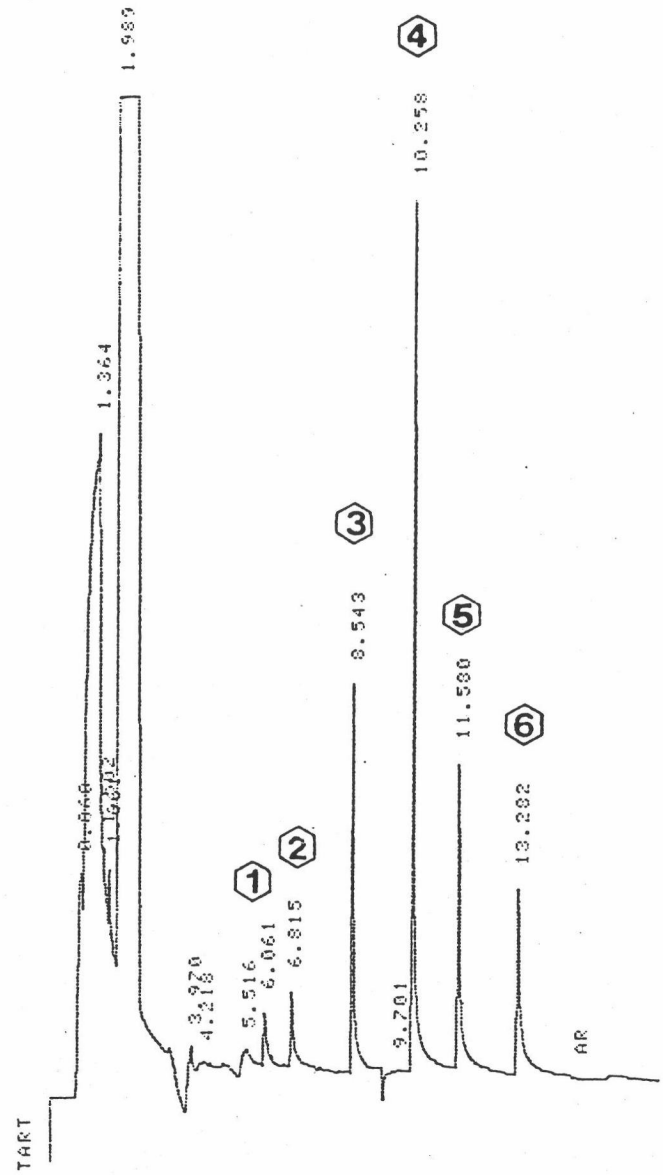


(1) Dimethyl phthalate 3471.00 ppb (2) Diethyl phthalate 3207.00 ppb

(3) Dibuthyl phthalate 3237.00 ppb (4) Butylbenzyl phthalate 3096.00 ppb

(5) Di(2-ethylhexyl) phthalate 3030.00 ppb (6) Di-n-octyl phthalate 3414.00 ppb

Figure 4.1 The gas chromatogram of some phthalate esters in ethyl acetate on Ultra-1(Cross linked Methyl Silicone Gum) capillary column. The GC conditions were described in Table 3.1(GC-FID).



(1) Dimethyl phthalate 462.80 ppb

(2) Diethyl phthalate 427.60 ppb

(3) Dibuthyl phthalate 431.60 ppb

(4) Butylbenzyl phthalate 412.80 ppb

(5) Di(2-ethylhexyl) phthalate 404.40 ppb

(6) Di-n-octyl phthalate 455.20.ppb

Figure 4.2 The gas chromatogram of some phthalate esters in ethyl acetate on Ultra-1(Cross linked Methyl Silicone Gum) capillary column. The GC conditions were described in Table 3.2(GC-ECD).

It was shown that the gas chromatograms detected by ECD detector demonstrated better sensitivity than those detected by FID detector. In this study both FID and ECD were used as detector.

2. The Study of Linearity of GC-ECD Detector

The calibration curves of standard mixture of six phthalate esters was shown in Figure 4.3 to 4.8. The mixture of six phthalate esters were used in the linearity study with the conditon in Table 3.2, the results of linearity of PEs were shown in Figure 4.9 to 4.14.

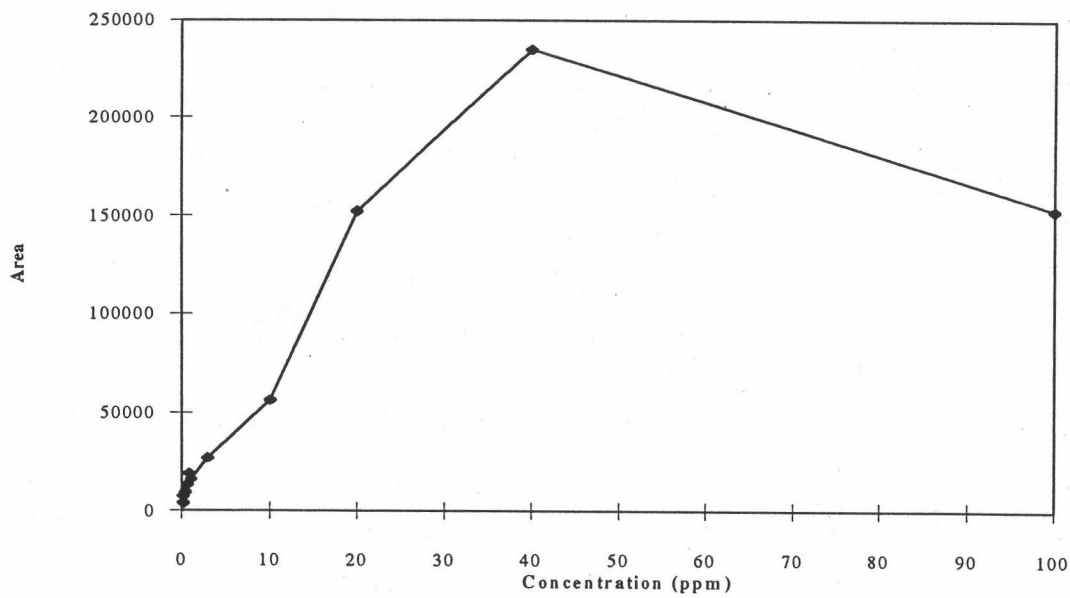


Figure 4.3 The calibration curve of DMP in standard mixture PEs.

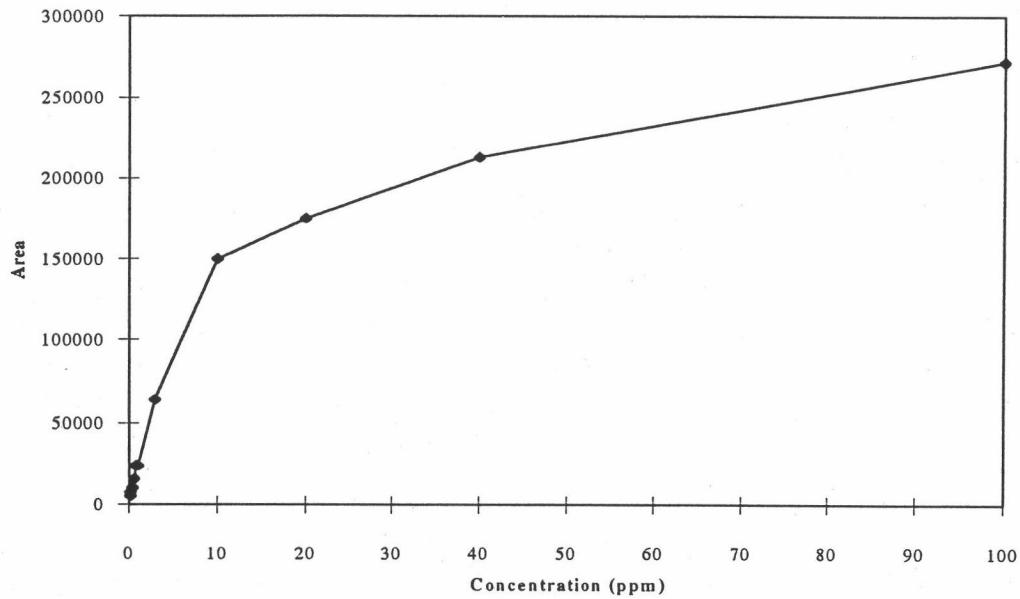


Figure 4.4 The calibration curve of DEP in standard mixture PEs.

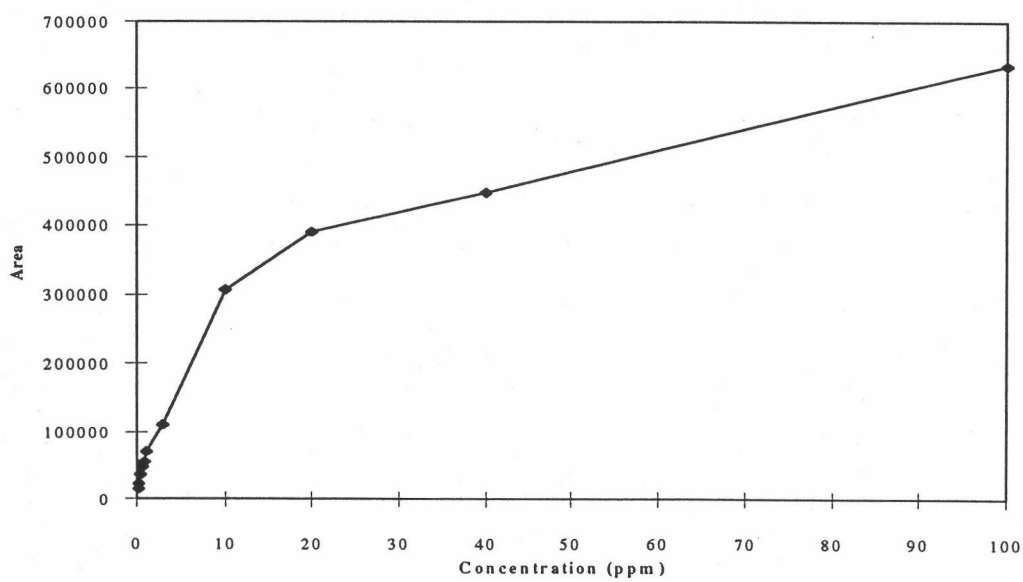


Figure 4.5 The calibraion curve DBP in standard mixture PEs

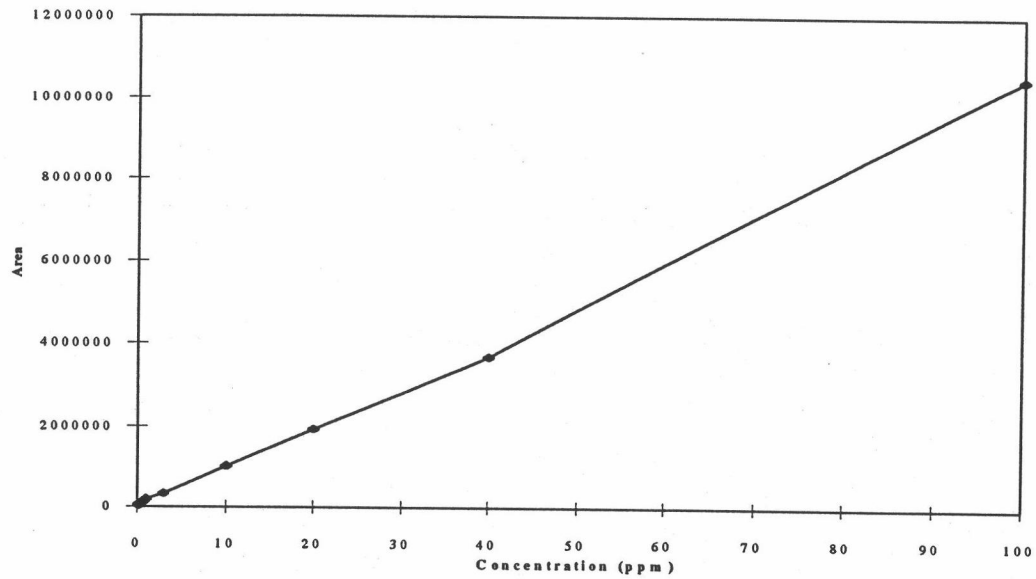


Figure 4.6 The calibration curve of BBP in standard mixture PEs.

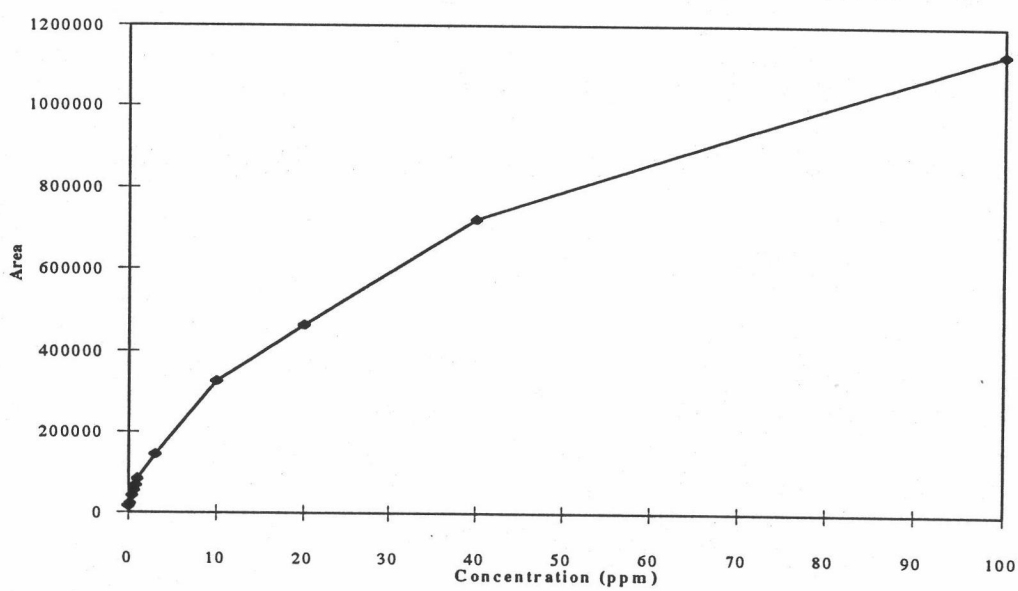


Figure 4.7 The calibration curve of DEHP in standard mixture PEs.

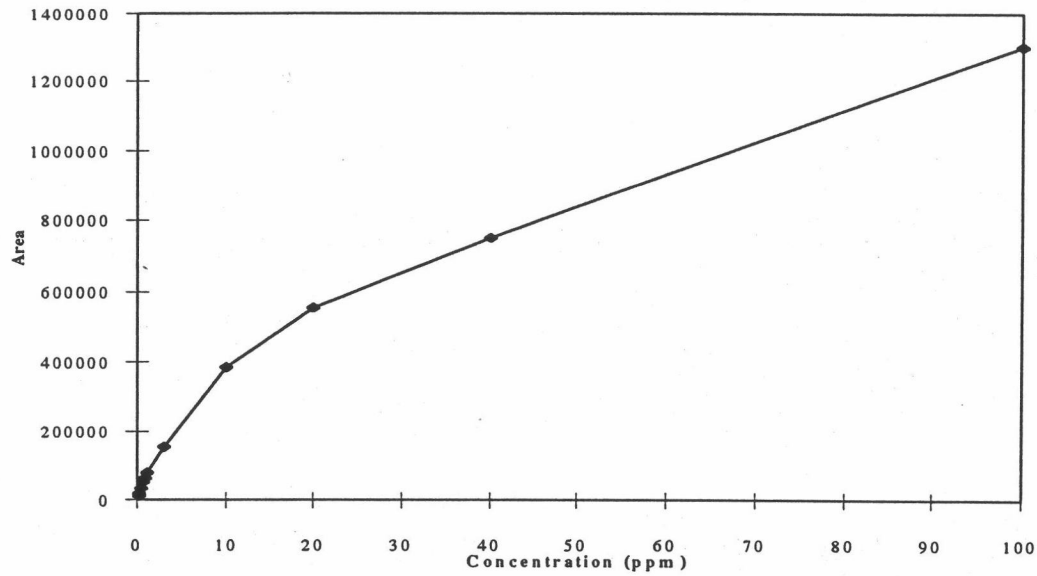


Figure 4.8 The calibration curve of DOP in standard mixture PEs.

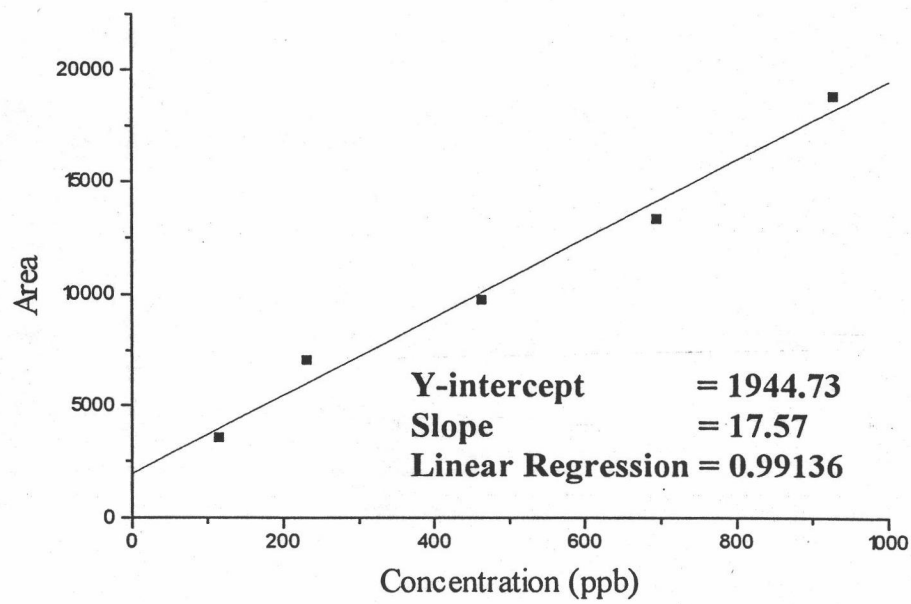


Figure 4.9 The Linearity of DMP in standard mixture PEs.

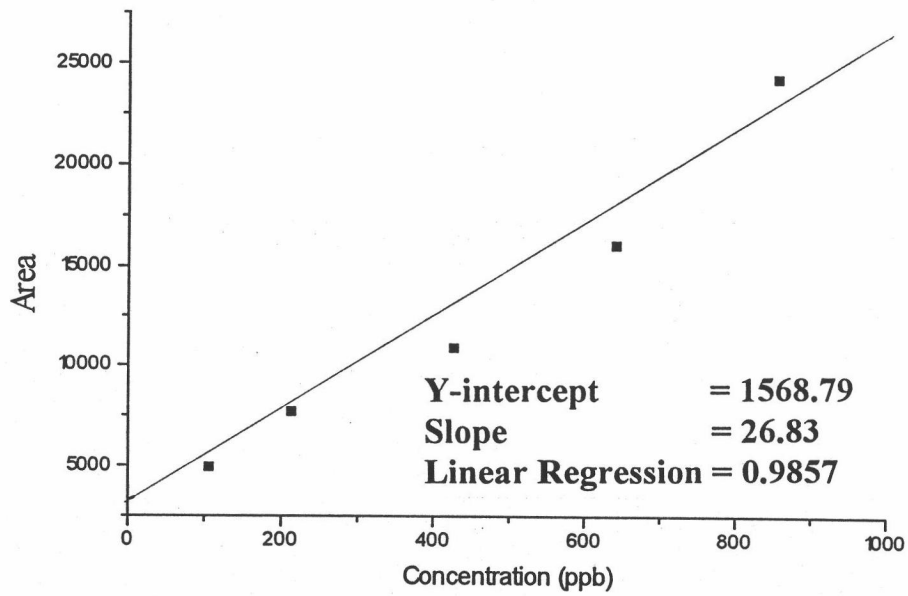


Figure 4.10 The Linearity of DEP in standard mixture PEs.

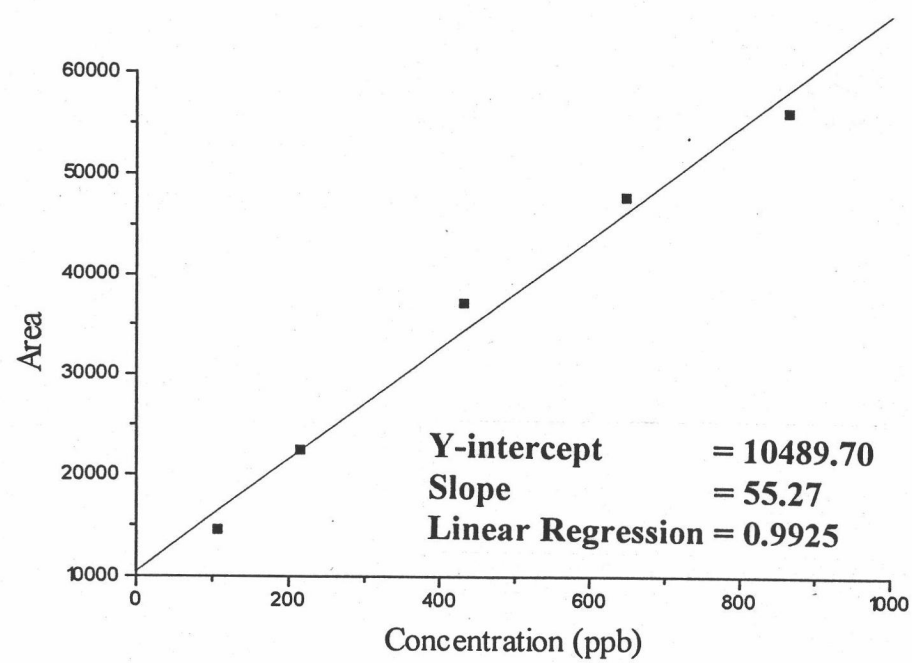


Figure 4.11 The Linearity of DBP in standard mixture PEs.

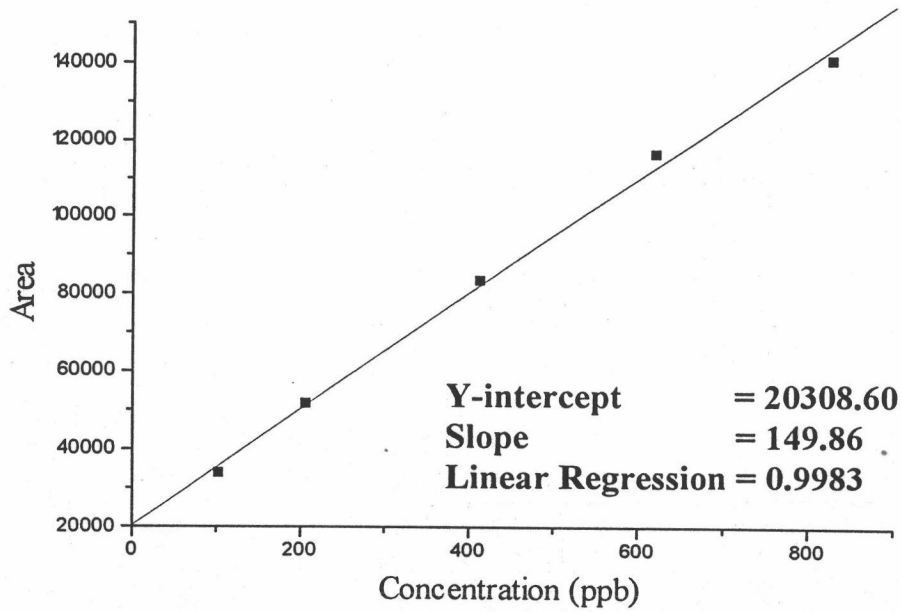


Figure 4.12 The Linearity of BBP in standard mixture PEs.

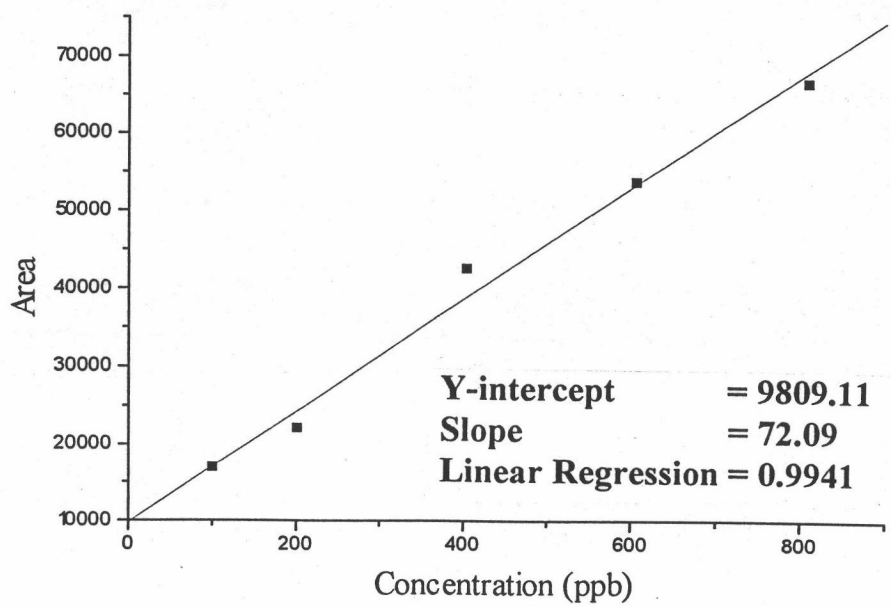


Figure 4.13 The Linearity of DEHP in standard mixture PEs.

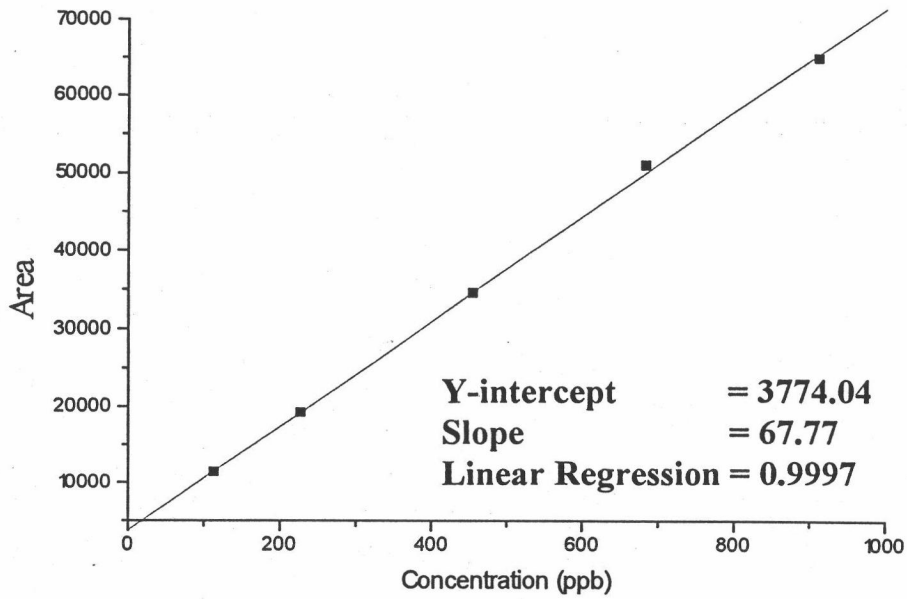


Figure 4.14 The Linearity of DOP in standard mixture PEs.

The relationship of peak area and concentration of PEs showed that the linear range of PEs was between 100 - 1000 ppb. Thus, PEs could not be detected as linear in concentrations higher than 1000 ppb. For this reason, this study detected only low concentration in spiked standard milk. The sensitivity to hydrocarbons with no electronegative elements to capture electrons was never good but, in contrast it was extremely good for hydrocarbons with highly electronegative elements. Phthalate esters, molecular structures of which were shown in Appendix A, have two main parts, one was the benzene ring with two carbonyl groups and the other was a hydrocarbon. From the theory of the electron capture detector, we could expect that the carbonyl groups might obtain electrons from the carrier gas and produce the signal. Unfortunately, the hydrocarbon part, especially short-chain hydrocarbons, also gave electrons to

carbonyl groups. Thus, phthalate esters were not good substances to be detected by ECD, unlike halogenated compounds. Eventhough they could also be detected in better sensitivity than FID due to specific characterstics. The result was showed that BBP was the best compound that provided highest response signal due to its lower ability to give electrons to carbonyl groups. This related to the stability of benzene ring. DMP was the worst compound for ECD detection because methyl groups was most likely to give electrons to carbonyl groups. Similar to DMP, the hydrocarbon chains of DEP, DBP and DOP could give electrons to carbonyl groups. However they were farther apart from the carbonyl group than DMP and this resulted in higher response signals. BBP was the only substance which had a linear range in both low and high concentration.

3. The Result of Time Requirement for Various Methods

The following methods: direct sample through disk, dilute sample before pass through disk, combine filter aid with disk, dilute sample before use with filter aid and disk, extract sample before pass through disk, adjust acidity (or pH) before disk step, centrifuge and follow Schuffenburg's method, were defined as methods 1 through 8, respectively.

The time consumed for each sample preparation method was studied. Each method could be classified into there preparation steps :

1. Sample pretreatment, i.e., centrifugation, shaking, pH adjustment and precipitation.
2. Disk step.
3. Cleanup and evaporation step.

In this study, the methods of preparation were divided into two major methods.

1. Methods 1-4, the sample was directed through the Empore™ extraction disk.

2. Each of the four remaining methods, specific times. The volume of sample and its characteristics significantly resulted in its time requirement. Method 5 consumed the longest time requirement, in contrast to method 1, even though the same volumes of sample were used.

Three steps of preparation procedure were introduced:

1. The step of preparation before disk usage
2. The step of disk, this step was also divided into three steps.
   2.1 Conditioning disk, necessary for every method.
   2.2 Sample application, time requirement for each method was varied.
   2.3 Elution step
3. The step of clean up and evaporation which used the same time requirement for each method.

Table 4.1 Time requirement of various method

method	Volume	Time before Disk	Time in Disk Step			Time Clean up and Evaporation Step	Total Requirement
			Prepare Disk	Sample Applying	Elution		
	(mL)	(min)	(min)	(min/25 mL)	(min)	(min)	(min)
1	25	-	6	9.83 ±0.68	4	28±5	47.83 ± 5.68
2	25	-	6	22.64 ±1.81	4	28±5	60.64 ± 6.81
3	25	-	6	11.34 ±0.42	4	28±5	49.34 ± 5.42
4	25	-	6	26.62 ±1.01	4	28±5	64.62 ± 6.01
5	25	36	6	46.29 ±1.47	4	-	92.29 ± 6.47
6	50	10	6	15.60 ±1.47	14	28±5	63.60 ± 6.47
7	100	30	6	5.16 ±0.37	4	28±5	74.16 ± 5.37
8	100	35	6	7.90 ±1.57	4	28±5	80.90 ± 6.57

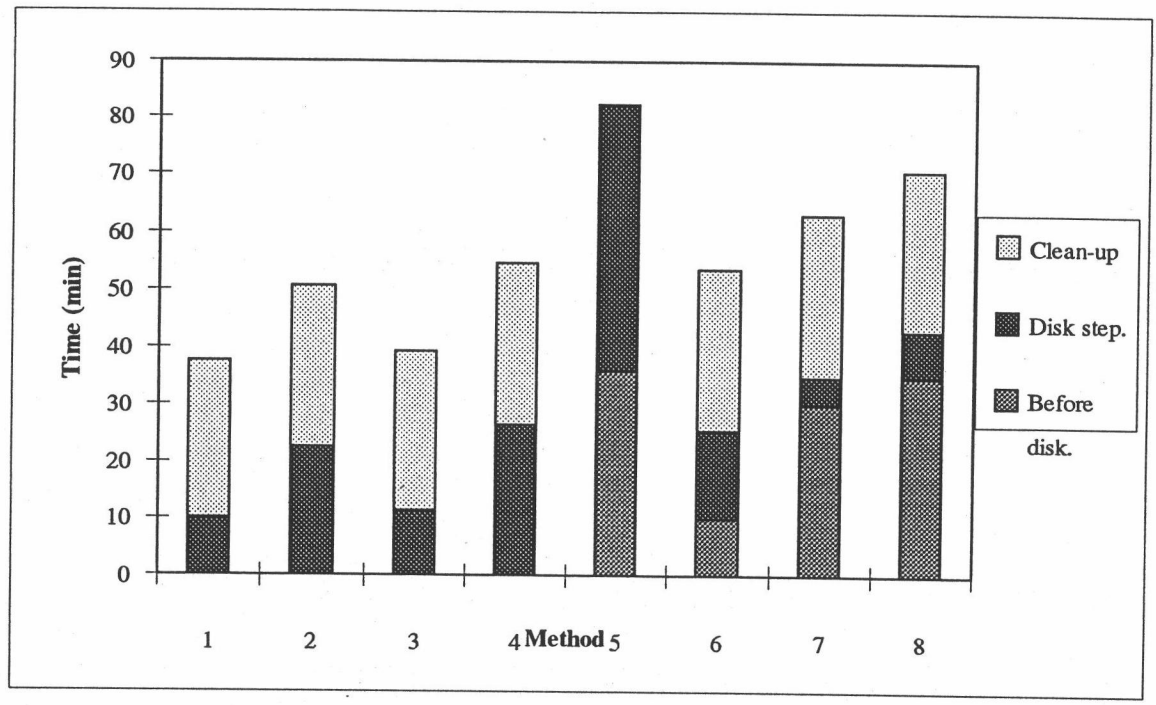


Figure 4.15 Schemetic of relationship between time requirement and methods

The filter aid method (method 3) was introduced so as to help increase the flow rate. This method had less uncertainty and took longer time when compared with method 1. Method 5 was the one that consumed the longest time because of the shaking. Methods 6, 7 and 8 were the methods that utilized highest volumes and separated milk into two phases; liquid and solid.

4. The Result of Percent Recovery and Precision of Various Methods

The preparation of samples were used in determining percent recovery and precision was described in chapter 3. Three low and three high concentrations of spiked standard mixture PEs in non-package standard milk were used. Both low and high concentration samples were detected by GC-FID and their external standard calibration curves were shown in chapter 3.

Following the linearity concept of ECD detector, only the low concentration samples were detected by this detector.

4.1 The study of percent recovery and precision of low concentration level standard mixture PEs by GC-FID

The result of percent recoveries of various method were shown in table 4.2-4.9.

Table 4.2 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 1 by GC-FID

Substance	Area	Blank Conc. (μg/kg)	Initial Conc. (μg/kg)	Final Conc. (μg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	907		59.2	247.71			
	819	-	59.2	221.64	215.62	72.84	±16.46
	670		59.2	177.49			
DEP	1075		55.8	211.12			
	705	-	55.8	116.35	160.06	57.37	±29.87
	847		55.8	152.72			
DBP	1441		53.6	277.98			
	1621	119.14	53.6	312.02	307.29	70.21	±8.87
	1726		53.6	331.88			
BBP	1526		54.7	278.68			
	1638	-	54.7	299.03	285.46	104.37	±4.12
	1526		54.7	278.68			
DEHP	2818		52.5	382.20			
	2589	74.71	52.5	346.83	353.37	106.15	±7.41
	2487		52.5	331.08			
DOP	1579		51.0	261.26			
	1338	-	51.0	218.21	229.76	90.10	±12.01
	1291		51.0	209.81			

Tripicate analysis

Table 4.3 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 2 by GC-FID

Substance	Area	Blank Conc. (μg/kg)	Initial Conc. (μg/kg)	Final Conc. (μg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	937	-	59.2	256.60	256.41	86.62	±4.97
	893		59.2	243.57			
	979		59.2	269.05			
DEP	1188	-	55.8	240.06	259.27	92.93	±9.76
	1375		55.8	287.95			
	1226		55.8	249.79			
DBP	2314	142.78	53.6	443.06	370.39	84.93	±17.00
	1729		53.6	332.44			
	1746		53.6	335.66			
BBP	1813	-	54.7	330.83	288.25	105.39	±15.11
	1589		54.7	290.13			
	1334		54.7	243.79			
DEHP	3562	94.94	52.5	497.10	452.26	136.12	±18.34
	2652		52.5	356.56			
	3601		52.5	503.12			
DOP	1986	-	51.0	333.97	280.32	109.93	±25.81
	1225		51.0	198.02			
	1846		51.0	308.96			

Tripicate analysis

Table 4.4 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 3 by GC-FID

Substance	Area	Blank Conc. (µg/kg)	Initial Conc. (µg/kg)	Final Conc. (µg/kg)	Mean	Percent Recovery Substracted with Blank	% RSD
DMP	615		59.2	161.20			
	647	-	59.2	170.68	167.22	56.49	±3.13
	644		59.2	169.79			
DEP	892		55.8	164.25			
	966	-	55.8	183.20	181.66	65.11	±9.19
	1022		55.8	197.54			
DBP	1974		53.6	378.77			
	2076	114.60	53.6	398.06	387.03	101.65	±2.57
	2003		53.6	384.26			
BBP	1637		54.7	298.85			
	1636	-	54.7	298.67	293.46	107.30	±3.13
	1549		54.7	282.86			
DEHP	3143		52.5	432.39			
	2881	61.43	52.5	391.93	399.24	128.69	±7.56
	2761		52.5	373.39			
DOP	1351		51.0	220.53			
	1208	-	51.0	194.99	212.25	83.24	±7.05
	1355		51.0	221.25			

Tripicate analysis

Table 4.5 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 4 by GC-FID

Substance	Area	Blank Conc. (µg/kg)	Initial Conc. (µg/kg)	Final Conc. (µg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	1051	-	59.2	290.38	305.19	103.11	±8.16
	1054		59.2	291.27			
	1198		59.2	333.94			
DEP	1156	-	55.8	231.86	260.63	93.42	±15.22
	1204		55.8	244.16			
	1445		55.8	305.88			
DBP	1864	124.06	53.6	357.97	362.57	89.00	±2.95
	1848		53.6	354.95			
	1953		53.6	374.80			
BBP	1590	-	54.7	290.31	267.35	97.75	±8.70
	1334		54.7	243.79			
	1467		54.7	267.96			
DEHP	2428	84.91	52.5	321.97	308.68	85.25	±4.08
	2332		52.5	307.14			
	2266		52.5	296.95			
DOP	1598	-	51.0	264.66	245.66	96.34	±7.15
	1473		51.0	242.33			
	1404		51.0	230.00			

Tripicate analysis

Table 4.6 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 5 by GC-FID

Substance	Area	Blank Conc. (μg/kg)	Initial Conc. (μg/kg)	Final Conc. (μg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	1946		59.2	555.56			
	2050	-	59.2	586.38	560.20	189.26	±3.89
	1889		59.2	538.67			
DEP	3906		55.8	936.20			
	3610	-	55.8	860.39	897.70	321.76	±5.97
	3751		55.8	896.51			
DBP	2610		53.6	499.04			
	2192	-	53.6	419.99	488.32	182.21	±11.45
	2858		53.6	545.93			
BBP	-		54.7	-			
	-	-	54.7	-	-	-	-
	-		54.7	-			
DEHP	4772		52.5	683.97			
	5677	381.42	52.2	823.73	799.07	159.10	±12.37
	6103		52.5	889.52			
DOP	-		51.0	-			
	1529	-	51.0	252.33	206.15	80.84	±31.68
	1012		51.0	159.97			

Tripicate analysis

Table 4.7 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 6 by GC-FID

Substance	Area	Blank Conc. (µg/kg)	Initial Conc. (µg/kg)	Final Conc. (µg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	1883	-	59.2	536.90	537.88	90.86	±4.91
	1977		59.2	564.75			
	1799		59.2	512.01			
DEP	2786	-	55.8	649.35	640.55	114.79	±4.11
	2636		55.8	610.93			
	2833		55.8	661.38			
DBP	3304	220.31	53.6	630.27	642.37	78.74	±3.06
	3488		53.6	665.06			
	3312		53.6	631.78			
BBP	2577	-	54.7	469.65	476.98	87.20	±1.34
	2633		54.7	479.82			
	2642		54.7	481.46			
DEHP	4156	103.28	52.5	588.83	626.36	99.63	±5.64
	4610		52.5	658.95			
	4431		52.5	631.30			
DOP	3682	-	51.0	636.94	661.24	129.65	±4.24
	3990		51.0	691.97			
	3782		51.0	654.81			

Tripicate analysis

Table 4.8 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 7 by GC-FID

Substance	Area	Blank Conc. (µg/kg)	Initial Conc. (µg/kg)	Final Conc. (µg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	2779	-	59.2	802.37	921.87	77.86	±11.69
	3283		59.2	951.70			
	3485		59.2	1011.55			
DEP	3856	-	55.8	923.40	1115.83	99.99	±15.35
	4828		55.8	1172.35			
	5138		55.8	1251.75			
DBP	4151	242.62	53.6	790.44	827.81	77.22	±5.97
	4250		53.6	809.16			
	4645		53.6	883.85			
BBP	1726	-	54.7	315.02	368.20	33.66	±15.17
	1991		54.7	363.17			
	2339		54.7	426.40			
DEHP	3264	121.20	52.5	451.08	465.44	32.78	±11.29
	3073		52.5	421.58			
	3734		52.5	523.66			
DOP	1646	-	51.0	273.23	336.23	32.96	±22.09
	1893		51.0	317.35			
	2457		51.0	418.11			

Tripicate analysis

Table 4.9 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 8 by GC-FID

Substance	Area	Blank Conc. (µg/kg)	Initial Conc. (µg/kg)	Final Conc. (µg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	2615		59.2	753.78			
	2982	-	59.2	862.52	770.08	65.04	±11.10
	2413		59.2	693.93			
DEP	4834		55.8	1173.89			
	3126	-	55.8	736.43	987.09	88.45	±22.86
	4354		55.8	1050.95			
DBP	2188		53.6	419.24			
	2672	220.31	53.6	510.76	489.01	45.62	±12.65
	2811		53.6	537.05			
BBP	4826		54.7	878.30			
	4561	-	54.7	830.15	867.52	79.30	±3.84
	4913		54.7	894.11			

Tripicate analysis

4.2 The study of percent recovery and precision of low concentration level standard mixture PEs by GC-ECD

Table 4.10 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 1 by GC-ECD

Substance	Area	Blank Conc. (μg/kg)	Initial Conc. (μg/kg)	Final Conc. (μg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	6854		59.2	279.40			
	6429	-	59.2	255.21	271.24	91.63	±5.12
	6849		59.2	279.11			
DEP	6710		55.8	191.60			
	7983	-	55.8	239.05	211.39	75.77	±11.67
	7030		55.8	203.53			
DBP	25538		53.6	272.26			
	24472	119.14	53.6	252.97	296.98	66.36	±20.30
	30703		53.6	365.71			
BBP	73728		54.7	356.36			
	73875	-	54.7	357.34	360.42	131.78	±1.72
	75404		54.7	367.55			
DEHP	39476		52.5	411.53			
	36251	74.71	52.5	366.79	386.95	118.95	±5.86
	37386		52.5	382.54			
DOP	18824		51.0	222.07			
	17798	-	51.0	206.94	211.39	82.90	±4.40
	17678		51.0	205.16			

Tripicate analysis

Table 4.11 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 2 by GC-ECD

Substance	Area	Blank Conc. (µg/kg)	Initial Conc. (µg/kg)	Final Conc. (µg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	7106	-	59.2	293.74	276.99	93.58	±6.73
	6459		59.2	256.92			
	6870		59.2	280.31			
DEP	9222	-	55.8	285.22	304.91	109.29	±5.88
	10164		55.8	320.33			
	9865		55.8	309.19			
DBP	30665	142.78	53.6	365.02	395.43	94.27	±8.12
	34201		53.6	429.00			
	32171		53.6	392.27			
BBP	90196	-	54.7	466.22	467.15	170.80	±0.20
	90327		54.7	467.10			
	90482		54.7	468.13			
DEHP	51290	94.94	52.5	575.41	563.19	178.38	±2.27
	49450		52.5	549.88			
	50487		52.5	564.27			
DOP	22889	-	51.0	282.06	275.44	108.01	±2.41
	22443		51.0	275.48			
	21989		51.0	268.78			

Tripicate analysis

Table 4.12 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 3 by GC-ECD

Substance	Area	Blank Conc. (μg/kg)	Initial Conc. (μg/kg)	Final Conc. (μg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	5716 5832 6356	-	59.2 59.2 59.2	214.63 221.23 251.05	228.97	77.35	±8.48
DEP	7110 6540 7220	-	55.8 55.8 55.8	206.51 185.27 210.61	200.80	71.97	±6.77
DBP	25978 32608 29476	114.60	53.6 53.6 53.6	280.22 400.18 343.51	341.31	84.59	±17.58
BBP	75972 78117 81345	-	54.7 54.7 54.7	371.33 385.64 407.18	388.05	141.88	±4.65
DEHP	36234 39779 41964	61.43	52.5 52.5 52.5	366.56 415.73 446.04	409.44	132.58	±9.80
DOP	14795 15945 16534	-	51.0 51.0 51.0	162.62 179.59 188.28	176.83	176.83	±7.38

Tripicate analysis

Table 4.13 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 4 by GC-ECD

Substance	Area	Blank Conc. (μg/kg)	Initial Conc. (μg/kg)	Final Conc. (μg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	8450	-	59.2	370.23	373.59	126.21	±4.72
	8233		59.2	357.88			
	8844		59.2	392.66			
DEP	9581	-	55.8	298.60	282.33	101.19	±5.11
	8842		55.8	271.06			
	9010		55.8	277.32			
DBP	28140	124.06	53.6	319.34	322.62	74.09	±1.30
	28582		53.6	327.34			
	28241		53.6	321.17			
BBP	76380	-	54.7	374.06	382.08	139.70	±2.02
	78687		54.7	398.45			
	77680		54.7	382.73			
DEHP	34002	84.91	52.5	335.59	324.56	91.30	±3.05
	32625		52.5	316.49			
	32992		52.5	321.58			
DOP	22073	-	51.0	270.02	263.77	103.44	±2.12
	21338		51.0	259.17			
	21539		51.0	262.14			

Tripicate analysis

Table 4.14 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 5 by GC-ECD

Substance	Area	Blank Conc. (μg/kg)	Initial Conc. (μg/kg)	Final Conc. (μg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	11147		59.2	523.73			
	7687	-	59.2	326.81	384.86	130.02	±31.39
	7287		59.2	304.04			
DEP	7572		55.8	223.73			
	9692	-	55.8	302.74	264.48	94.79	±14.96
	8732		55.8	266.96			
DBP	20196		53.6	175.61			
	29500	-	53.6	343.95	269.33	100.50	±31.85
	26432		53.6	288.44			
BBP	68050		54.7	318.49			
	60618	-	54.7	268.91	298.21	109.03	±8.72
	66362		54.7	307.22			
DEHP	19901		52.5	139.99			
	36741	381.42	52.5	373.59	304.15	-	±46.93
	38564		52.5	398.88			
DOP	8990		51.0	76.97			
	14666	-	51.0	160.72	125.64	49.27	±34.62
	13210		51.0	139.24			

Tripicate analysis

Table 4.15 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 6 by GC-ECD

Substance	Area	Blank Conc. (µg/kg)	Initial Conc. (µg/kg)	Final Conc. (µg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	10264	-	59.2	473.48	415.84	70.24	±12.08
	8846		59.2	392.77			
	8644		59.2	381.27			
DEP	11222	-	55.8	359.76	356.88	63.96	±2.95
	11380		55.8	365.65			
	10832		55.8	345.22			
DBP	44204	220.31	53.6	609.99	605.78	71.92	±1.83
	43277		53.6	593.22			
	44434		53.6	614.15			
BBP	117932	-	54.7	651.25	645.21	117.95	±1.73
	115091		54.7	632.30			
	118053		54.7	652.06			
DEHP	58794	103.28	52.5	679.50	667.05	107.38	±1.62
	57410		52.5	660.30			
	57486		52.5	661.35			
DOP	37624	-	51.0	499.48	498.27	97.70	±1.19
	37106		51.0	491.84			
	37895		51.0	503.48			

Tripicate analysis

Table 4.16 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 7 by GC-ECD

Substance	Area	Blank Conc. (µg/kg)	Initial Conc. (µg/kg)	Final Conc. (µg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	14550	-	59.2	717.42	794.14	67.07	±19.41
	19016		59.2	971.60			
	14128		59.2	693.40			
DEP	16913	-	55.8	571.85	600.80	53.84	±5.26
	18594		55.8	634.50			
	17562		55.8	596.04			
DBP	72117	242.62	53.6	1115.02	713.89	43.96	±55.94
	27973		53.6	316.32			
	49750		53.6	710.33			
BBP	114824	-	54.7	630.52	477.91	43.69	±49.93
	50729		54.7	202.94			
	110292		54.7	600.29			
DEHP	54029	121.20	52.5	613.40	478.19	34.00	±24.55
	38793		52.5	402.05			
	40023		52.5	419.12			
DOP	43679	-	51.0	588.83	314.6	30.85	±80.86
	9621		51.0	86.28			
	21988		51.0	268.76			

Tripicate analysis

Table 4.17 The result of percent recovery and precision of low concentration level standard mixture PEs in Method 8 by GC-ECD

Substance	Area	Blank Conc. (µg/kg)	Initial Conc. (µg/kg)	Final Conc. (µg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	24987		59.2	1311.44			
	29543	-	59.2	1570.75	1148.06	122.30	±8.99
	27632		59.2	1461.98			
DEP	23825		55.8	829.45			
	28798	-	55.8	1014.79	927.23	83.09	±10.04
	26723		55.8	937.46			
DBP	33444		53.6	415.31			
	44975	220.31	53.6	623.94	519.67	27.93	±20.07
	39217		53.6	519.76			
BBP	59806		54.7	263.49			
	60240	-	54.7	266.38	264.73	24.20	±0.56
	59928		54.7	264.30			
DEHP	19417		52.5	133.28			
	19869	-	52.5	139.55	136.54	13.00	±2.30
	19671		52.5	136.80			
DOP	7516		51.0	55.22			
	8856	-	51.0	74.99	66.96	6.56	±15.52
	8563		51.0	70.66			

Tripicate analysis

4.3 The study of percent recovery and precision of high concentration level standard mixture PEs by GC-FID

Table 4.18 The result of percent recovery and precision of high concentration level standard mixture PEs in Method 1 by GC-FID

Substance	Area	Blank Conc. (mg/kg)	InitialConc. (mg/kg)	Final Conc. (mg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	89466.5		5.12	17.88			
	87996		5.12	17.59	17.92	70.00	±1.95
	91560.5		5.12	18.29			
DEP	90603.5		5.09	16.41			
	107499		5.09	19.44	17.25	67.76	±11.11
	87698		5.09	15.89			
DBP	122732	0.12	5.13	20.12			
	129289		5.13	21.21	20.37	78.95	±3.68
	120642		5.13	19.78			
BBP	137355		5.54	22.66			
	144122		5.54	23.78	22.72	82.03	±4.52
	131780		5.54	21.73			
DEHP	20795	0.07	5.36	2.79			
	26191		5.36	3.64	3.03	11.03	±17.56
	20013		5.36	2.66			
DOP	16138		6.05	2.60			
	19210		6.05	3.13	2.74	9.04	±12.54
	15429		6.05	2.48			

Tripicate analysis

Table 4.19 The result of percent recovery and precision of high concentration level standard mixture PEs in Method 2 by GC-FID

Substance	Area	Blank Conc. (mg/kg)	Initial Conc. (mg/kg)	Final Conc. (mg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	96982		5.12	19.35			
	98513	-	5.12	19.65	19.03	74.34	±4.34
	90559		5.12	18.09			
DEP	90149		5.09	16.33			
	107029	-	5.09	19.35	17.24	67.72	±10.68
	88457		5.09	16.02			
DBP	146492		5.13	24.07			
	138337	0.14	5.13	22.71	23.16	89.75	±3.37
	138342		5.13	22.71			
BBP	150830		5.54	24.89			
	143972	-	5.54	23.76	23.93	86.39	±3.72
	140260		5.54	23.14			
DEHP	59363		5.36	8.90			
	56331	0.09	5.36	8.41	8.50	31.37	±4.14
	55026		5.36	8.20			
DOP	60113		6.05	10.11			
	58741	-	6.05	9.87	9.83	32.49	±3.10
	56473		6.05	9.50			

Tripicate analysis

Table 4.20 The result of percent recovery and precision of high concentration level standard mixture PEs in Method 3 by GC-FID

Substance	Area	Blank Conc. (mg/kg)	InitialC Conc. (mg/kg)	Final Conc. (mg/kg)	Mean	Percent Recovery Substracted with Blank	% RSD
DMP	89397	-	5.12	17.87	17.80	69.52	±0.59
	88415		5.12	17.67			
	89229		5.12	17.85			
DEP	86939	-	5.09	15.75	15.80	62.10	±0.34
	87543		5.09	15.86			
	87217		5.09	15.80			
DBP	126566	0.11	5.13	20.76	20.57	79.75	±0.91
	125422		5.13	20.57			
	124311		5.13	20.38			
BBP	132630	-	5.54	21.87	21.68	78.26	±0.95
	131620		5.54	21.70			
	130172		5.54	21.46			
DEHP	26016	0.06	5.36	3.61	3.46	12.69	±4.58
	24015		5.36	3.30			
	25130		5.36	3.47			
DOP	27924	-	6.05	4.61	4.42	14.60	±4.73
	25489		6.05	4.20			
	26931		6.05	4.44			

Tripicate analysis

Table 4.21 The result of percent recovery and precision of high concentration level standard mixture PEs in Method 4 by GC-FID

Substance	Area	Blank Conc. (mg/kg)	InitialC Conc. (mg/kg)	Final Conc. (mg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	115727	-	5.12	23.01	22.44	87.66	±2.36
	112284		5.12	22.34			
	110374		5.12	21.97			
DEP	124995	-	5.09	22.57	21.88	85.96	±3.33
	121412		5.09	21.93			
	116893		5.09	21.12			
DBP	129289	0.12	5.13	21.21	19.78	76.63	±6.28
	115863		5.13	18.98			
	116852		5.13	19.15			
BBP	139911	-	5.54	23.08	22.16	80.00	±4.33
	134789		5.54	22.23			
	128385		5.54	21.16			
DEHP	30509	0.08	5.36	4.32	4.59	16.80	±11.92
	29855		5.36	4.22			
	36140		5.36	5.22			
DOP	30318	-	6.05	5.02	4.95	16.36	±1.32
	29762		6.05	4.93			
	29586		6.05	4.90			

Tripicate analysis

Table 4.22 The result of percent recovery and precision of high concentration level standard mixture PEs in Method 5 by GC-FID

Substance	Area	Blank Conc. (mg/kg)	Initial Conc. (mg/kg)	Final Conc. (mg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	6910	-	5.12	1.74	1.86	7.27	±5.77
	7765		5.12	1.91			
	7935		5.12	1.94			
DEP	49671	-	5.09	9.07	9.34	36.70	±5.11
	49577		5.09	9.06			
	54235		5.09	9.89			
DBP	119487	-	5.13	19.58	20.23	78.88	±5.33
	119789		5.13	19.63			
	130896		5.13	21.48			
BBP	107054	-	5.54	17.62	18.74	67.64	±7.20
	111432		5.54	18.35			
	122791		5.54	20.24			
DEHP	114996	0.38	5.36	17.69	18.61	73.50	±6.93
	117229		5.36	18.04			
	130100		5.36	20.08			
DOP	107603	-	6.05	18.21	20.26	66.97	±9.96
	119904		6.05	20.31			
	131247		6.05	22.25			

Tripicate analysis

Table 4.23 The result of percent recovery and precision of high concentration level standard mixture PEs in Method 6 by GC-FID

Substance	Area	Blank Conc. (mg/kg)	Initial Conc. (mg/kg)	Final Conc. (mg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	181866		5.12	35.95			
	189454	-	5.12	37.43	36.54	71.38	±2.14
	183451		5.12	36.26			
DEP	244893		5.09	44.07			
	243617	-	5.09	43.84	43.57	85.59	±1.55
	237812		5.09	42.80			
DBP	218397		5.13	36.00			
	207126	0.22	5.13	34.13	35.45	68.68	±3.25
	219786		5.13	36.23			
BBP	211761		5.54	35.02			
	208812	-	5.54	34.53	35.40	63.89	±3.13
	221567		5.54	36.65			
DEHP	97269		5.36	14.89			
	94349	0.10	5.36	14.42	14.81	27.43	±2.34
	98642		5.36	15.10			
DOP	89452		6.05	15.11			
	87856	-	6.05	14.84	14.84	24.52	±1.90
	86143		6.05	14.55			

Tripicate analysis

Table 4.24 The result of percent recovery and precision of high concentration level standard mixture PEs in Method 7 by GC-FID

Substance	Area	Blank Conc. (mg/kg)	Initial Conc. (mg/kg)	Final Conc. (mg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	390510		5.12	76.74			
	400188	-	5.12	78.63	77.78	75.96	±1.24
	396831		5.12	77.98			
DEP	389969		5.09	70.07			
	400550	-	5.09	71.97	70.78	69.53	±1.46
	391320		5.09	70.31			
DBP	145818		5.13	23.95			
	150110	0.24	5.13	24.67	23.53	22.70	±5.93
	133891		5.13	21.97			
BBP	125117		5.54	20.62			
	131302	-	5.54	21.65	19.57	17.67	±14.08
	99990		5.54	16.45			
DEHP	61795		5.36	9.27			
	34279	0.12	5.36	4.92	7.02	6.44	±31.04
	46640		5.36	6.88			
DOP	67808		6.05	11.42			
	68724	-	6.05	11.58	10.38	8.58	±18.70
	48587		6.05	8.14			

Tripicate analysis

Table 4.25 The result of percent recovery and precision of high concentration level standard mixture PEs in Method 8 by GC-FID

Substance	Area	Blank Conc. (mg/kg)	Initial Conc. (mg/kg)	Final Conc. (mg/kg)	Mean	Percent Recovery Substracted with Blank	%RSD
DMP	398021	-	5.12	78.21	65.96	64.41	±30.81
	392717		5.12	77.17			
	215380		5.12	42.50			
DEP	414701	-	5.09	74.51	59.21	58.16	±43.89
	411379		5.09	73.91			
	161983		5.09	29.20			
DBP	126325	0.22	5.13	20.72	16.15	15.53	±45.77
	122641		5.13	20.11			
	47422		5.13	7.62			
BBP	132050	-	5.54	21.77	16.89	15.25	±43.71
	124431		5.54	20.51			
	51542		5.54	8.40			
DEHP	71803	-	5.36	10.86	8.48	7.91	±40.87
	66805		5.36	10.07			
	31626		5.36	4.50			
DOP	76711	-	6.05	12.94	9.91	8.19	±42.77
	69537		6.05	11.72			
	30577		6.05	5.07			

Tripicate analysis

Table 4.26 The comparison of percent recovery and precision of low concentration level standard mixture PEs in Methods by GC-FID

Method	DMP	DEP	DBP	BBP	DEHP	DOP
1	72.84 ±16.46	57.37 ±29.86	70.20 ±8.87	104.37 ±4.12	106.15 ±7.41	90.10 ±12.01
2	86.62 ±4.97	92.93 ±9.76	84.93 ±17.00	105.39 ±15.11	136.12 ±18.34	109.93 ±25.81
3	56.49 ±3.13	65.11 ±9.19	101.65 ±2.57	107.3 ±3.13	128.69 ±7.56	83.24 ±7.05
4	103.11 ±8.16	93.42 ±15.22	89.00 ±2.95	97.75 ±8.70	85.25 ±4.28	96.34 ±7.15
5	189.26 ±3.89	321.76 ±5.97	182.21 ±11.45	–	159.10 ±12.37	80.84 ±31.68
6	90.86 ±4.91	114.79 ±4.11	78.74 ±3.06	87.20 ±1.34	99.63 ±5.64	129.65 ±4.24
7	77.86 ±11.69	99.99 ±15.35	77.22 ±5.97	33.66 ±15.17	32.78 ±11.29	32.96 ±22.09
8	65.04 ±11.10	88.45 ±22.86	45.62 ±12.65	79.30 ±33.84	–	–

Table 4.27 The comparison of percent recovery and precision of low concentration level standard mixture PEs in Methods by GC-ECD

Method	DMP	DEP	DBP	BBP	DEHP	DOP
1	91.63 ±5.12	75.77 ±11.67	66.36 ±20.30	131.78 ±1.72	118.95 ±5.86	82.90 ±4.40
2	93.58 ±6.73	109.29 ±5.88	94.27 ±8.12	170.80 ±0.20	178.38 ±2.27	108.01 ±2.41
3	77.35 ±8.48	71.97 ±6.77	84.59 ±17.58	141.88 ±4.65	132.58 ±9.80	176.83 ±7.38
4	126.21 ±4.72	101.19 ±5.11	74.09 ±1.30	139.70 ±2.02	91.30 ±3.05	103.44 ±2.12
5	130.02 ±31.39	94.79 ±14.96	100.50 ±31.85	109.03 ±8.72	_	49.27 ±34.62
6	70.24 ±12.08	63.96 ±2.95	71.92 ±1.83	117.95 ±1.73	107.38 ±1.62	97.70 ±1.19
7	67.07 ±19.41	53.84 ±5.26	43.96 ±55.94	43.69 ±49.93	34.00 ±24.55	30.85 ±80.86
8	122.30 ±8.99	83.09 ±10.04	27.93 ±20.07	24.20 ±0.56	13.00 ±2.30	6.56 ±15.52

Table 4.28 The comparison of percent recovery and precision of high concentration level standard mixture PEs in Methods by GC-FID

Method	DMP	DEP	DBP	BBP	DEHP	DOP
1	70.00 ±1.95	67.76 ±11.11	78.95 ±3.68	82.03 ±4.52	11.03 ±17.56	9.04 ±12.54
2	74.34 ±4.34	67.72 ±10.68	89.75 ±3.37	86.39 ±3.72	31.37 ±4.14	32.49 ±3.10
3	69.52 ±0.59	62.10 ±0.34	79.75 ±0.91	78.26 ±0.95	12.69 ±4.58	14.60 ±4.73
4	87.66 ±2.36	85.96 ±3.33	76.63 ±6.28	80.00 ±4.33	16.80 ±11.92	16.36 ±1.32
5	7.27 ±5.77	36.70 ±5.11	78.88 ±5.33	67.64 ±7.20	73.50 ±6.93	66.97 ±9.96
6	71.38 ±2.14	85.59 ±1.55	68.68 ±3.25	63.89 ±3.13	27.43 ±2.34	24.52 ±1.90
7	75.96 ±1.24	69.53 ±1.46	22.70 ±5.93	17.67 ±14.08	6.44 ±31.04	8.58 ±18.70
8	64.41 ±30.81	58.16 ±43.89	15.53 ±45.77	15.25 ±43.71	7.91 ±40.87	8.19 ±42.77

The result of percent recovery of low and high concentrations of PEs were demonstrated that method 1 had lower percent recovery than method 2. It was obviously that some phthalate esters might be lose by attaching to the milky molecule (lipids or protein molecules) when passing through disk. Whereas the dilution sample before passing it through disk could increase percent recovery of phthalate esters. The dilution sample with water or buffer resulted in the change in ionic strength of matrix and analyte resulting in high percent recovery. Moreover, the higher dilution of sample the more analyte was adsorbed on sorbent. In water or non-matrix samples, filter aid helped the sample to slowly pass through the membrane disk, it would also improve the interaction of analyte with the sorbent. Moreover, milk and matrix samples which they slowly passed through disk, due to the lipid molecules trapped on the filter aid and obstructed the flow of the sample, therefore resulting in a lower percent recovery, but lower background. The main result of this was that the analyte was adsorbed on the lipid molecule on filter aid. Method 4 showed better results in percent recovery than method 3 but it still gave a lower percent recovery than method 2 as shown in Table 4.2-4.28. Method 5 was the method in use for a long time and it used selected solvent to extract the analyte from the matrix. Phthalate esters were slightly polar substance and the suitable solvents, i.e., methylene chloride, ethyl acetate, were used as a solvents for extraction. In this study ethyl acetate was used because of its lower toxicity and high stability with PEs. This method provided unsatisfactory results because milk lipids could interfere with PEs, since the polarity of lipid and PEs were alike. Methods 7 and 8 applied high volumes of sample through the disk because they passed only the liquid that precipitated and centrifuged the lipid molecule out from the sample through disk, but they had low percent recovery. It might be suggested that PEs which was slightly polar molecules were likely to adsorb on lipid molecule during the precipitation and centrifugration,

resulting in lower percent recovery than expected, especially for DEHP and DBP.

Unlike GC-FID, only GC-ECD could detect DEHP and DOP in method 8. This indicated the low background in ECD when compared to FID, because ECD was highly sensitive to compounds with electronegative atoms, but the interfering substances in milk were usually hydrocarbons compounds such as lipids and therefore, ECD would not response to these compounds.

The high concentration spiked levels of PEs was studied only by GC-FID and the trend of extraction efficiency for each method of high concentration would be low and however, they were high for the low concentration studied. From the result we could suggest that high concentrations of PEs might be lost with lipids while passing through the disk. Moreover, the results of studies yielded the low percent recovery for high concentration was that the capacity of membrane disk was low and it could not trapped all of analyte passing through it.

Precisions of each method were demonstrated in %RSD. The precision indicated the reproducibility of each method. The results of precision of each phthalate ester in milk by GC-FID and GC-ECD were shown in Table 4.2-4.28. Method had lower %RSD than method 2 and it could be implied that the higher volume of substance, resulted in the higher %RSD. It could be the same as in the case of filter aid (method 3) and filter aid with dilution sample (method 4). Filter aid gave better precision than the simple direct method because the sample was passed directly through the disk with constant flow rate. Method 6 gave satisfactory precision and recovery, therefore, it could be concluded from the results that sonication of the sample with solvent before elution had contributed to a better result. Method 7 rendered a rigid transparent yellow precipitate of milk sample and method 8, conducted at pH 4.2, rendered a clotted precipitate greater in size than that of method 7. It also brought about

low percent recovery and bad precision. According to result of time requirement, percent recovery and precision, it was clear that method 6 was chosen as the optimum method which should be used in the next study.

5.The Study of Method Detection Limit (MDL)

The method detection limit (MDL) was defined as the minimum concentration of substance that could be identified, measured and reported with 99% confidence. The analyte concentration was greater than zero and was determined from replicated analysis of a sample in a given matrix containing the analyte. MDL of each method was investigated except of the extraction method (method 5) because this method required a considerably high solvent volume and background. MDL method 5 had to be greater than 50 ppb. Thus, in the study of MDL only 7 methods were applied: which were directing (method 1), directing with dilution (method 2), filter aid (method 3), filter aid with dilution (method 4), pH adjustment (method 5), centrifugation (method 6), and Schufferburg's method (method 7). The results of the study were presented in Table 4.29

Table 4.29 The method detection limit of each phthalate ester in milk samples by GC-FID.

method	MDL of DMP (ppb)	MDL of DEP (ppb)	MDL of DBP (ppb)	MDL of BBP (ppb)	MDL of DEHP (ppb)	MDL of DOP (ppb)
1	41.44	33.48	26.80	21.88	15.75	25.50
2	35.52	27.90	21.44	16.41	10.50	20.40
3	41.44	33.48	26.80	21.88	15.75	25.50
4	35.52	27.90	21.44	16.41	10.50	20.40
6	17.76	16.74	10.72	10.94	5.25	10.20
7	23.84	16.74	10.72	16.41	10.50	15.30
8	59.20	55.80	53.60	54.70	-*	-*

* can not detected in concentration range

Table 4.30 The method detection limit of each phthalate ester in milk sample by GC-ECD.

method	MDL of DMP (ppb)	MDL of DEP (ppb)	MDL of DBP (ppb)	MDL of BBP (ppb)	MDL of DEHP (ppb)	MDL of DOP (ppb)
1	41.44	33.48	26.80	16.41	10.50	20.40
2	35.52	27.90	21.44	10.94	5.25	15.30
3	41.44	33.48	26.80	16.41	10.50	20.40
4	35.52	27.90	21.44	10.94	5.25	15.30
6	17.76	16.74	10.72	2.75	1.31	5.10
7	23.84	16.74	10.72	10.94	5.25	10.20
8	41.44	33.48	26.80	10.94	5.25	15.30

Table 4.29 and 4.30 demonstrated the method detection limit of each method in this study. The lowest concentrations of PEs abled to be detected by GC-FID and GC-ECD were shown in method 6. Normally in sample preparation procedures, the higher in volume the sample was, the more concentrated it would be. Method 7 and 8 utilized sample volumes four times greater than those of method 1-5, and also provided high MDL due to its high background. The higher sensitivity of ECD detector resulted in lower MDL than FID detector.

From the information in item 4; the result of percent recovery and precision of various methods and 5; the study of method detection limit and the time requirement information, it could be concluded they the optimum sample preparation procedure was method 6. Thus, this method was used to prepare sample in general.

6. The Determination of Phthalate Esters in Milk Samples from Several Markets.

Six samples were collected from supermarkets in Bangkok. Then the samples were undergone method as preparation procedure and deteced by GC-FID and GC-ECD as the previous study.

The retention times of unknown peaks obtained from gas chromatograms of unknown milk and yogurt samples were compared with those of the standard mixture of phthalate ester peaks as shown in Figures 4.1 and 4.2. From the retention data, in sample 1, a peak at retention time 6.007 min is found to be the DMP as in standard. The peak at retention time of 11.553 as which was DEHP was found in all samples using GC-FID and the gas chromatograms of all samples using GC-FID were shown in Figures 4.40-4.45. Similary, these peaks were also found and were detected by GC-ECD. Nevertheless, BBP and DBP peaks were not found in all samples when using

GC-FID and they could be detected by GC-ECD due to GC-ECD was higher sensitive than GC-FID. The gas chromatograms of all samples detected by GC-ECD were also shown in Figures 4.46-4.50.

To confirm the above result, the samples were spiked with the standard mixture solution of PEs and were detected under the same conditions. Furthermore, the identification of phthalate esters in all samples were confirmed by GC-MSD. The result of the peak which seemed to be DMP peak in sample 1 from GC-FID and GC-ECD was actually the ethyl vanilin peak with 90% of library matching quality and all peaks which seemed to be DEHP peaks in all samples were exactly DEHP peaks with 90 % of library matching quality. The mass patterns of samples and of those obtained from library searchs were compared. All of the total ion chromatograms of those samples were shown in Figures 4.47-4.64.

In further investigation by MSD, more types of phthalate ester could be detected in trace amount for each sample. MSD could identify all substances by its mass pattern (with high efficiency). Thus, the interfering substance which might appear in GC-FID with integrator would cause no problem in MSD due to the data processing capability. Therefore, more phthalate esters were detected as follows by GC-MSD.

sample	**other PEs being detected**
Yakult	BBP, DBP
Dutchmill	DBP, BBP
Thai-Danish	DBP
Yomost	DBP, BBP
Meiji	DEP

As shown in the chromatograms, it was obvious seen that milk samples had low background while yogurt samples had high background. From Tables 4.29 and 4.30, the concentrations of DEHP detected by GC-FID and GC-ECD were in the range of 23.57±2.05 to 32.60±1.86 for sample 1, 11.93±2.80 to 13.22±2.12 for sample 2, 14.75±5.67 to 22.25±5.60 for sample 3, 12.00±7.12 to 26.87±6.50 for sample 4, 16.81±6.75 to 35.93±4.50 for sample 5, and 22.31±5.40 to 32.07±6.79 for sample 6. These data significantly showed higher amount of DEHP found in milk samples (sample 1) than in yogurt samples (sample 3-6) when detected by GC-FID. But on the other hand, the amount of DEHP found in milk and yogurt samples differed only slightly when detected by GC-ECD. The results of GC-FID implied higher fat content in yogurt than in milk samples, which also resulted in high background of the chromatograms. In this study there was an attempt to sample the dairy products packed in plasticized plastic containers such as polystyrene, which was widely used in yogurt packaging. The Thai-Danish sample (sample 2) was the only sample packed in container other than polystyrene. When comparing the yakult sample with the Thai-Danish sample, the amount of DEHP in milk from the polystyrene container (such as yakult) seemed to be greater than that from the non-polystyrene one.

Table 4.31 The concentration of phthalate esters in six samples were detected by GC-FID

Sample	concentration of phthalate esters (μg/kg)					
	DMP	DEP	DBP	BBP	DEHP	DOP
1. Yakult(milk)	336.85+4.23	ND	ND	ND	32.60+1.86	ND
2. Thai-Dennish(milk)	ND	ND	ND	ND	13.22+2.12	ND
3. Yomost(yogurt)	ND	ND	ND	ND	14.75+5.67	ND
4. Meiji(yogurt)	ND	ND	ND	ND	12.00+7.12	ND
5. Yophalt(yogurt)	ND	ND	ND	ND	16.80+6.75	ND
6. Duchmill(yogurt)	ND	ND	ND	ND	32.07+6.79	ND

ND = non detectable

Table 4.32 The concentration of phthalate esters in six samples were detected by GC-ECD

Sample	concentration of phthalate esters (μg/kg)					
	DMP	DEP	DBP	BBP	DEHP	DOP
1. Yakult(milk)	137.87+5.62	ND	22.35±2.67	12.6+1.73	23.57+2.05	ND
2. Thai-Dennish(milk)	ND	ND	10.95±2.96	6.67+1.97	11.93+2.80	ND
3. Yomost(yogurt)	ND	ND	ND	4.89+5.12	22.25+5.60	ND
4. Meiji(yogurt)	ND	ND	9.15+6.60	5.76+6.32	26.87+6.50	ND
5. Yophalt(yogurt)	ND	ND	7.10±5.71	3.18+6.32	35.93+4.50	ND
6. Duchmill(yogurt)	ND	ND	6.20±7.00	18.6+6.75	22.31+5.40	ND

ND = non detectable

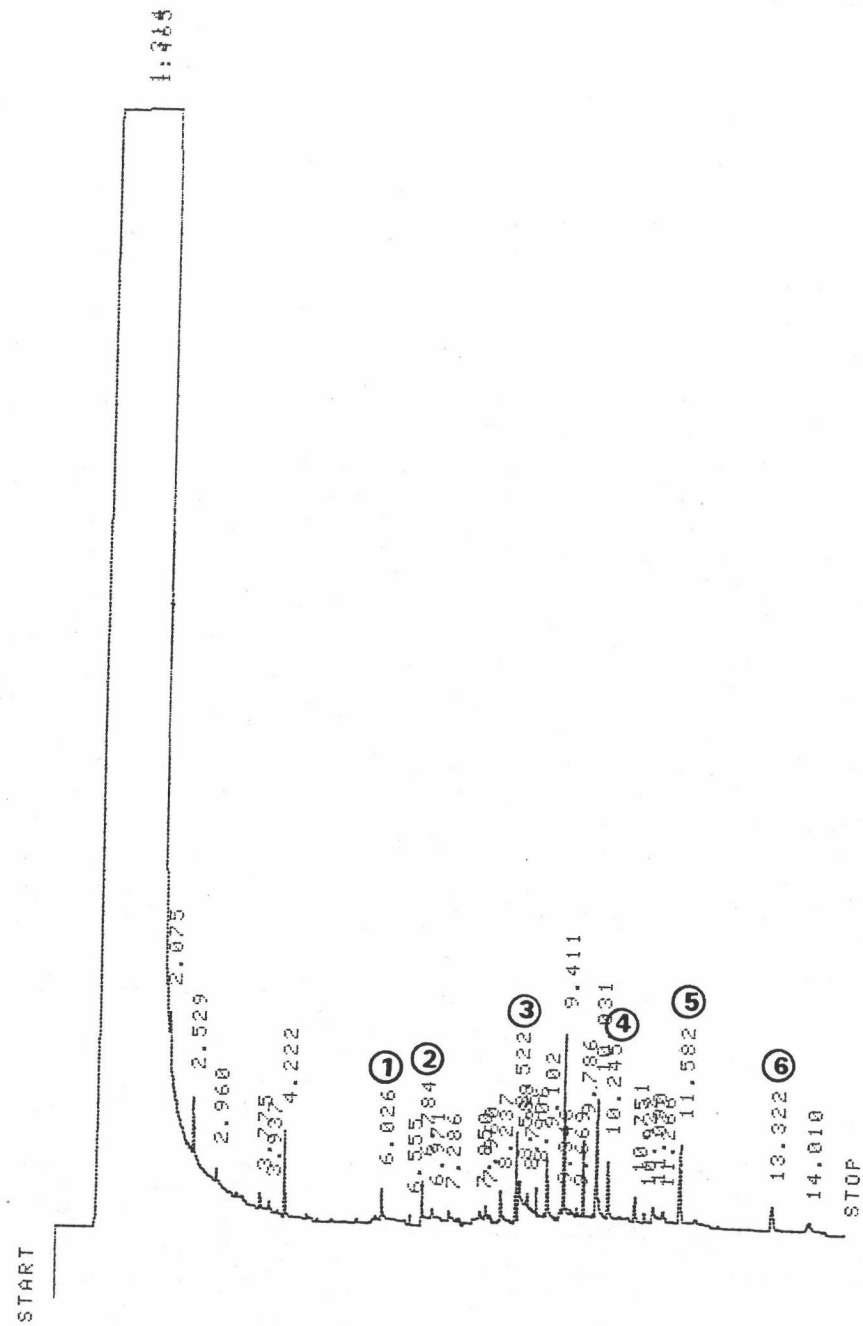


Figure 4.16 The gas chromatogram of standard mixture solution in milk by method 1

GC-condition: described in Table 3.1 Integrator Att.= 1

Concentration of DMP: 221.64 μg/kg DEP: 211.12 μg/kg

DBP : 312 μg/kg BBP: 278.67 μg/kg

DEHP: 331.08 μg/kg DOP: 209.81 μg/kg

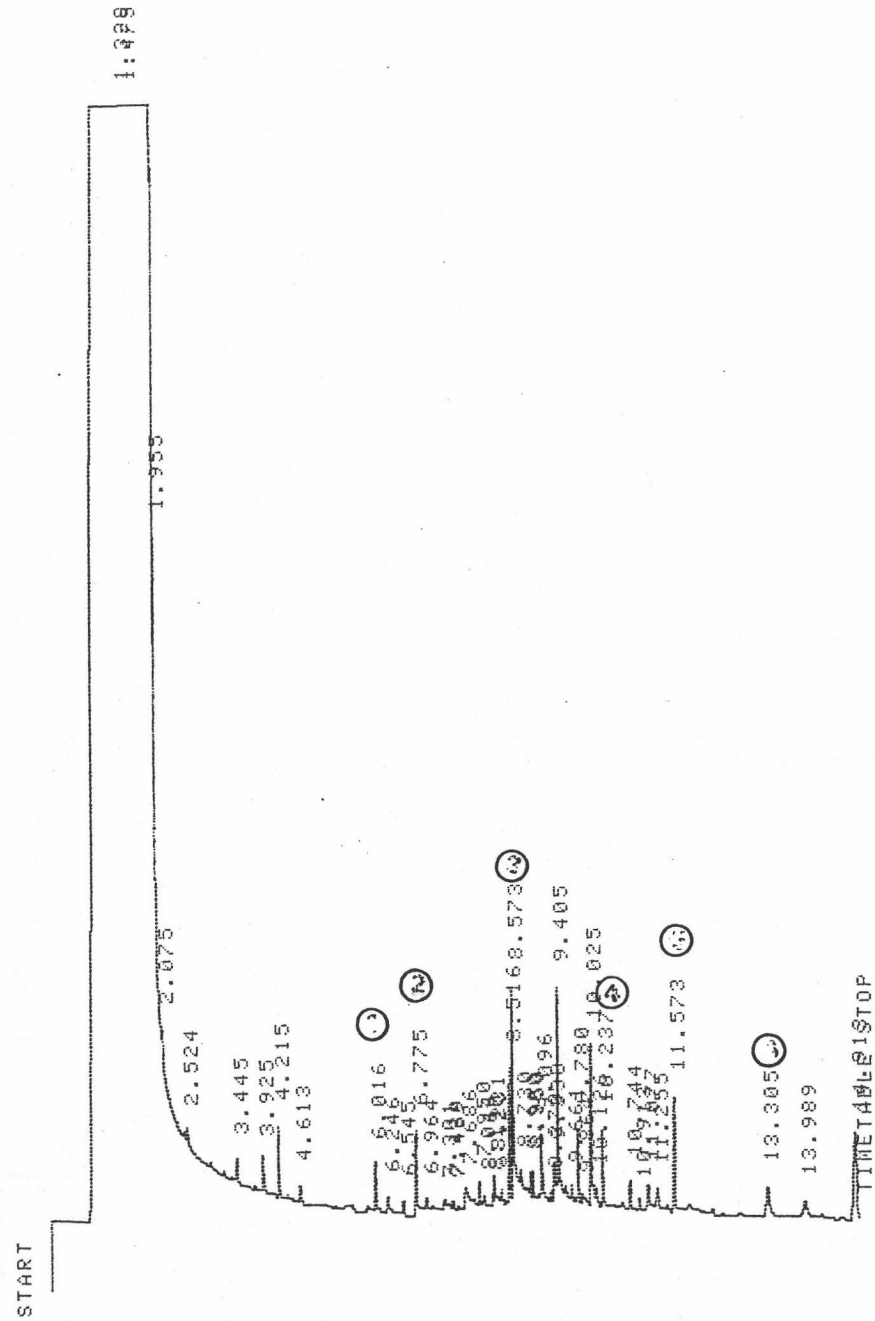


Figure 4.17 The gas chromatogram of standard mixture solution in milk by method 2

GC-condition: described in Table 3.1 Integrator Att. = 1

Concentration of DMP: 269.05 μg/kg DEP: 287.95 μg/kg

DBP : 335.66 μg/kg BBP: 290.13 μg/kg

DEHP: 497.10 μg/kg DOP: 308.96 μg/kg

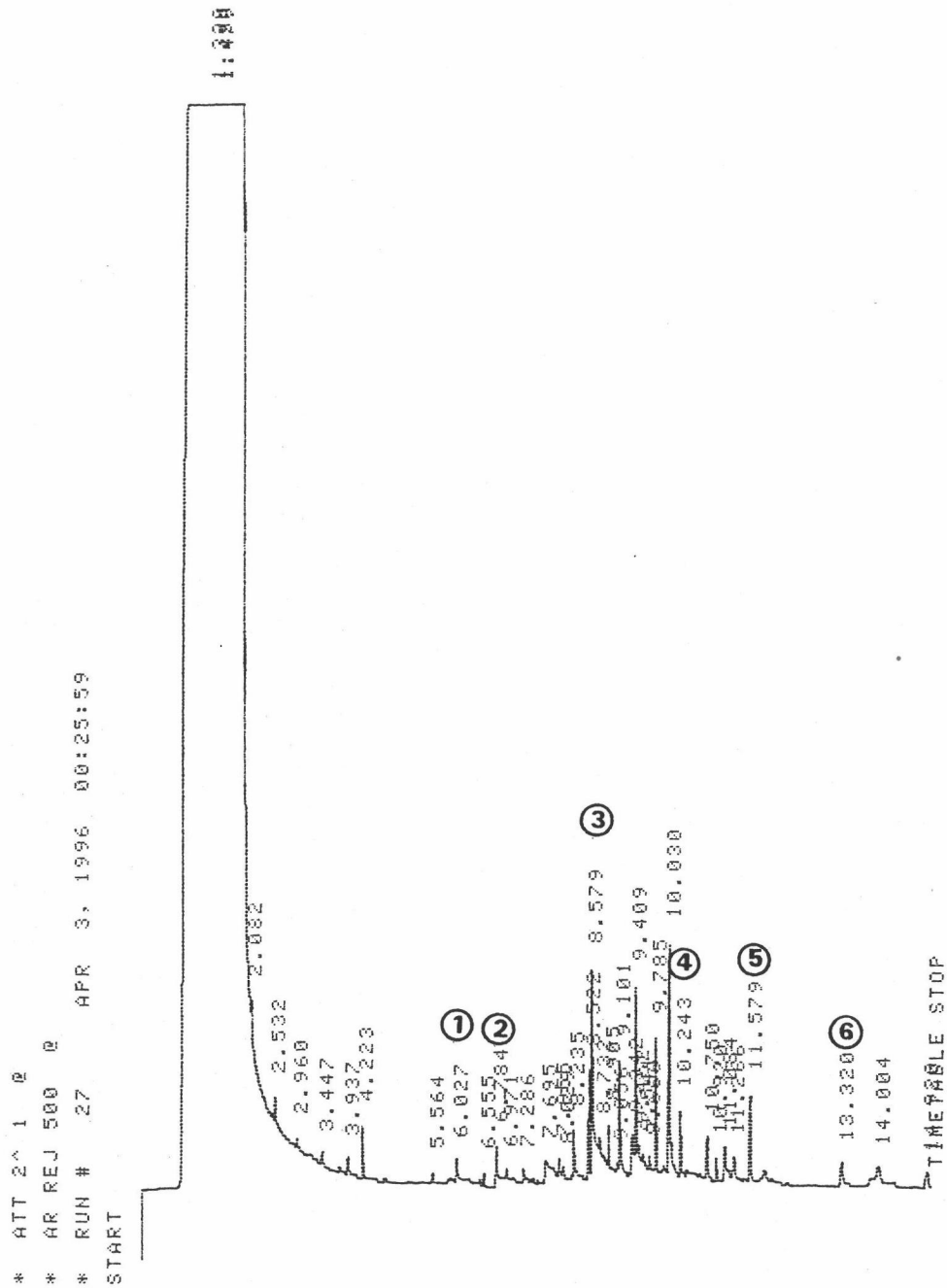


Figure 4.18 The gas chromatogram of standard mixture solution in milk by method 3

GC-condition: described in Table 3.1 Integrator Att. = 1

Concentration of DMP: 170.67 μg/kg DEP: 183.20 μg/kg

DBP : 378.77 μg/kg BBP: 282.86 μg/kg

DEHP: 373.39 μg/kg DOP: 220.53 μg/kg

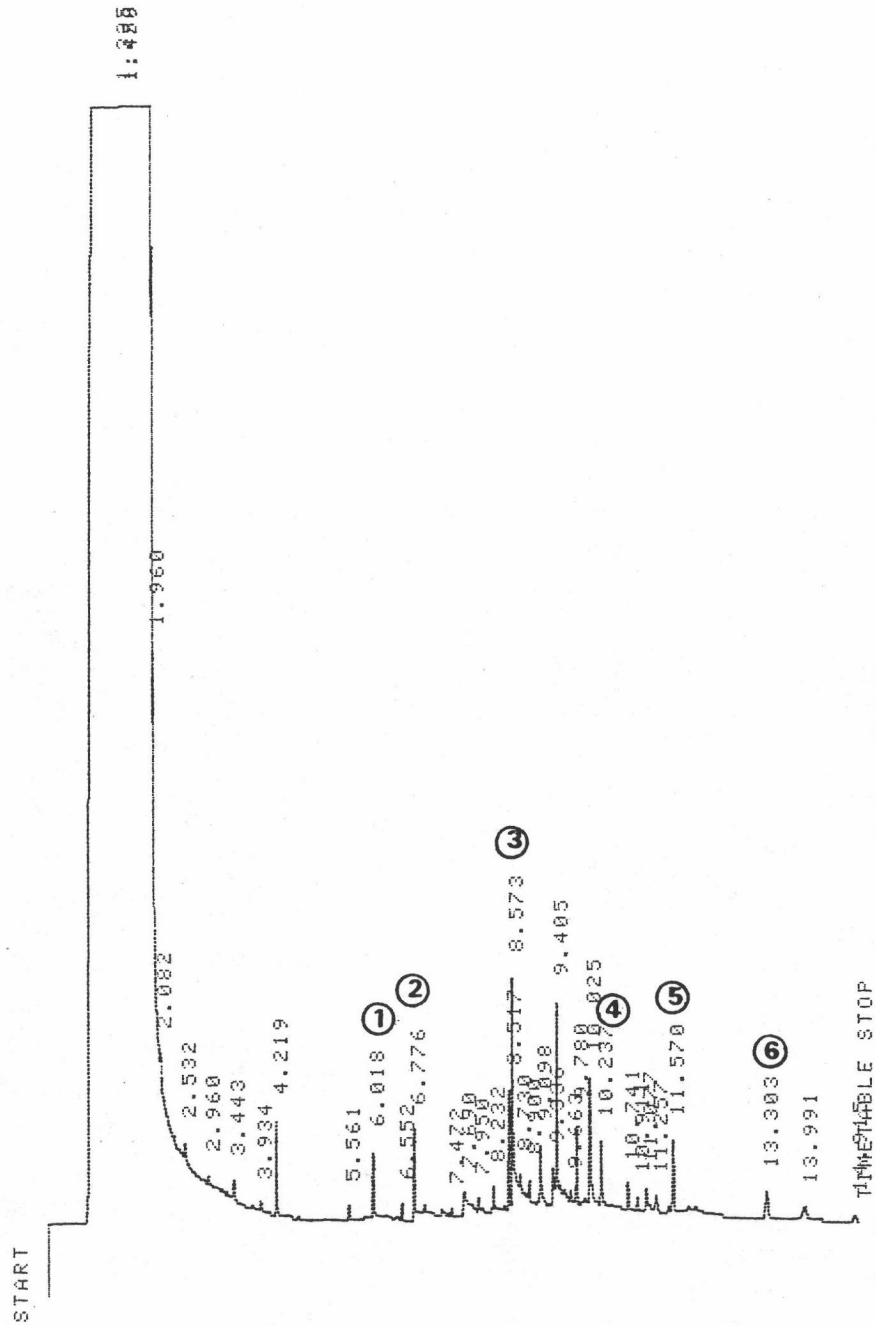


Figure 4.19 The gas chromatogram of standard mixture solution in milk by method 4

GC-condition: described in Table 3.1 Integrator Att.= 1

Concentration of DMP: 291.27 μg/kg DEP: 244.15 μg/kg

DBP : 357.97 μg/kg BBP: 267.35 μg/kg

DEHP: 321.96 μg/kg DOP: 264.65 μg/kg

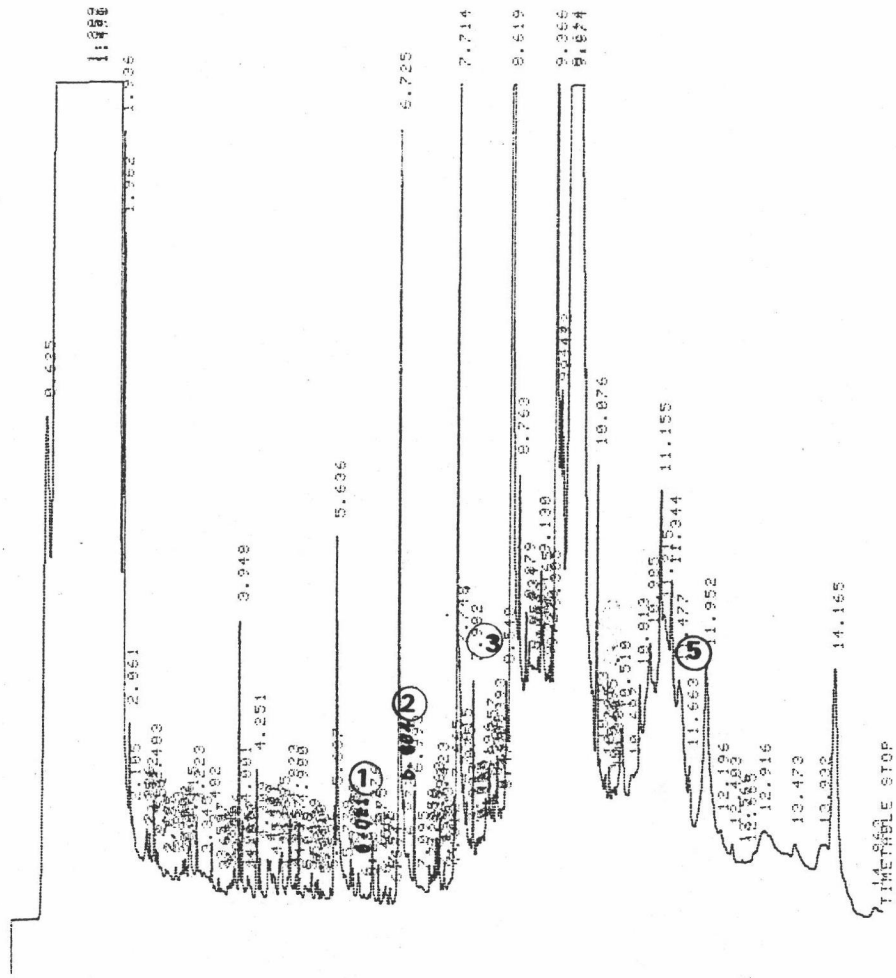


Figure 4.20 The gas chromatogram of standard mixture solution in milk by method 5

GC-condition: described in Table 3.1 IntegratorAtt. = 2

Concentration of DMP: 555.56 μg/kg DEP: 860.39 μg/kg

DBP : 419.99 μg/kg BBP: -

DEHP: 823.73 μg/kg DOP: 252.33 μg/kg

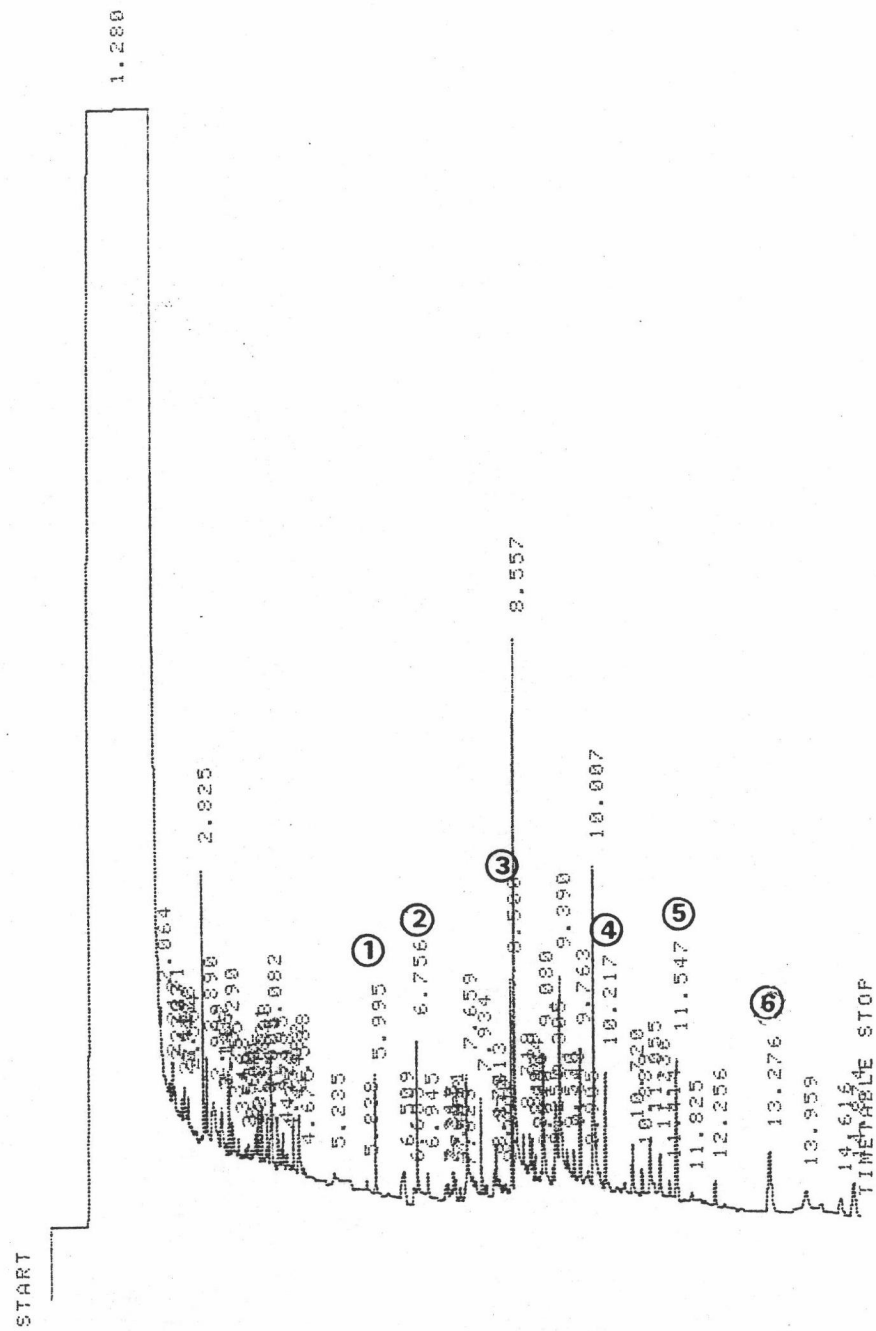


Figure 4.21 The gas chromatogram of standard mixture solution in milk by method 6

GC-condition: described in Table 3.1 Integrator Att. = 1

Concentration of DMP: 536.89 μg/kg DEP: 610.92 μg/kg

DBP : 630.27 μg/kg BBP: 479.82 μg/kg

DEHP: 631.30 μg/kg DOP: 636.94 μg/kg

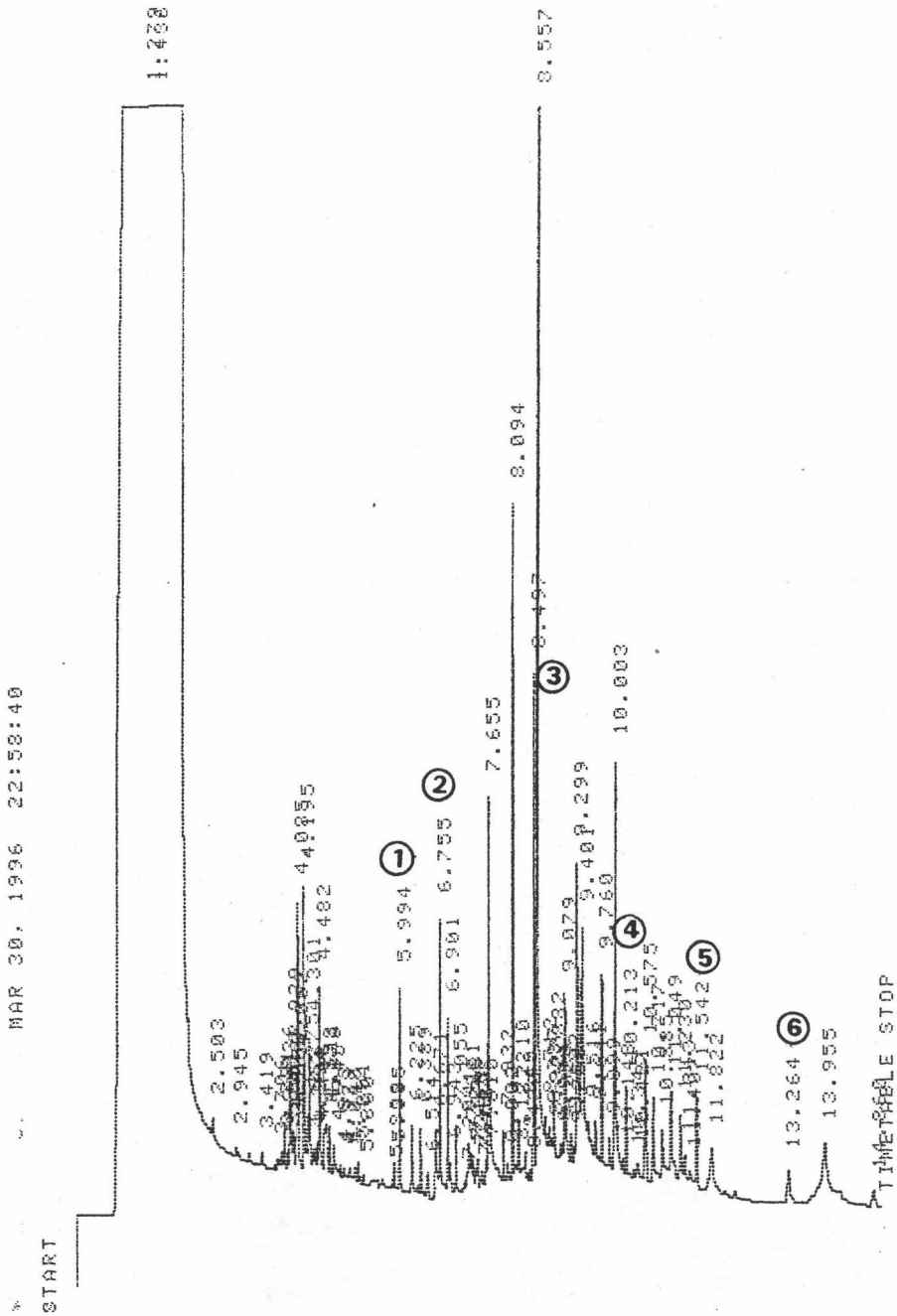


Figure 4.22 The gas chromatogram of standard mixture solution in milk by method 7

GC-condition: described in Table 3.1 Integrator Att. = 1

Concentration of DMP: 951.70 μg/kg DEP: 1172.35 μg/kg

DBP : 883.85 μg/kg BBP: 363.17 μg/kg

DEHP: 451.07 μg/kg DOP: 317.35 μg/kg

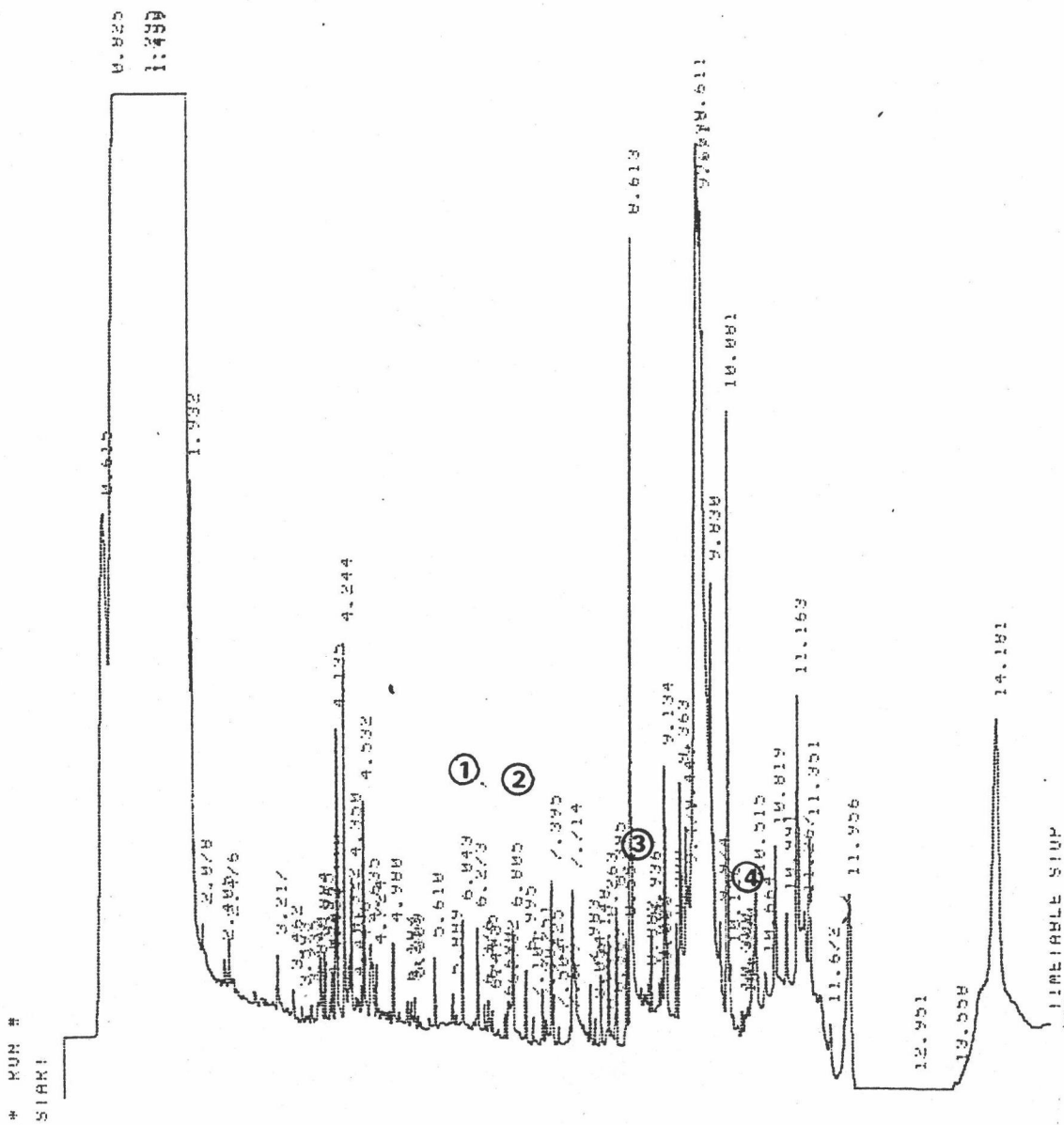


Figure 4.23 The gas chromatogram of standard mixture solution in milk by method 8

GC-condition: described in Table 3.1 Integrator Att. = 2

Concentration of DMP: 753.78 μg/kg DEP: 736.42 μg/kg

DBP : 419.24 μg/kg BBP: 830.15 μg/kg

DEHP: - DOP: -

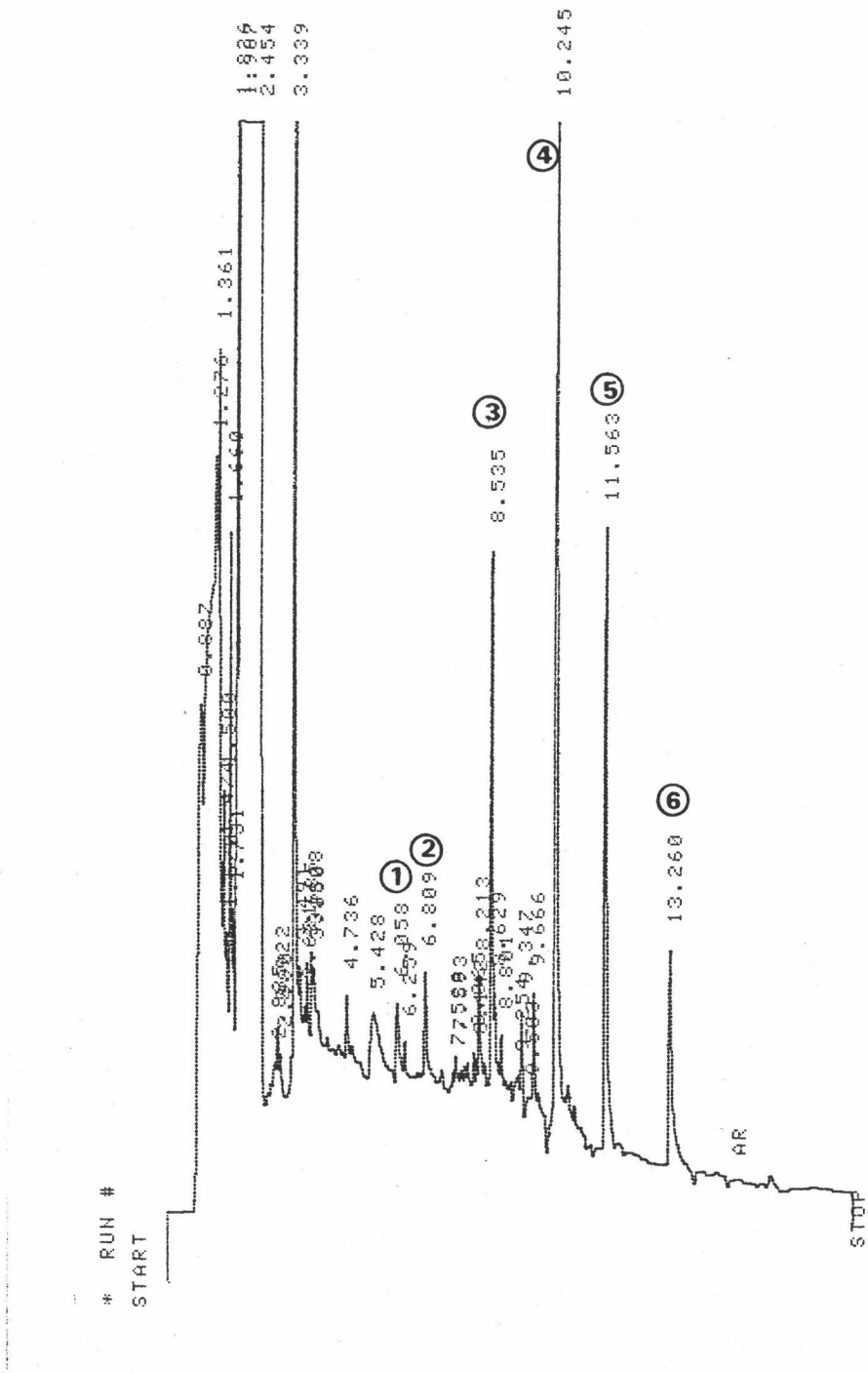


Figure 4.24 The gas chromatogram of standard mixture solution in milk by method 1

GC-condition: described in Table 3.2 Integrator Att. = 1

Concentration of DMP: 255.20 μg/kg DEP: 203.53 μg/kg

DBP : 272.26 μg/kg BBP: 356.36 μg/kg

DEHP: 382.53 μg/kg DOP: 222.07 μg/kg

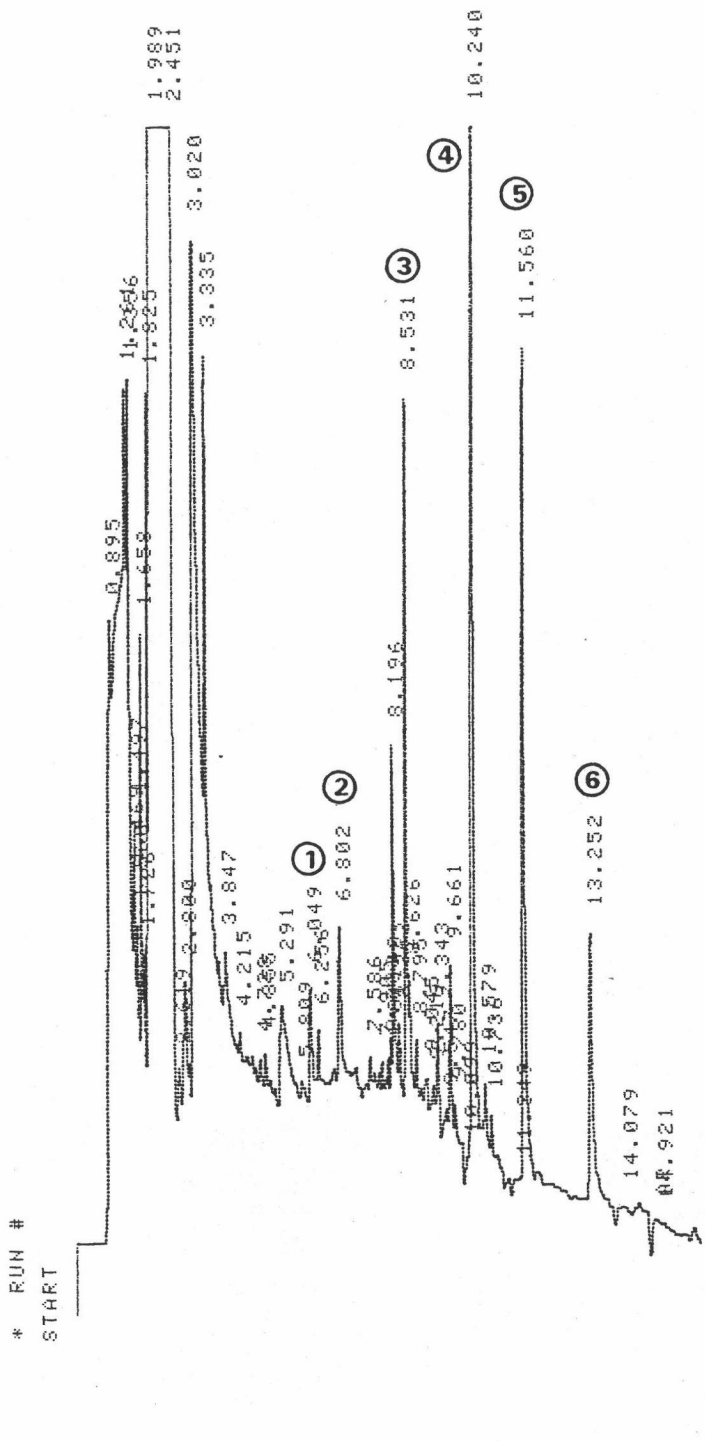


Figure 4.25 The gas chromatogram of standard mixture solution in milk by method 2

GC-condition: described in Table 3.2 Integrator Att. = 1

Concentration of DMP: 280.30 μg/kg DEP: 309.91 μg/kg

DBP : 365.02 μg/kg BBP: 469.09 μg/kg

DEHP: 564.27 μg/kg DOP: 218.77 μg/kg

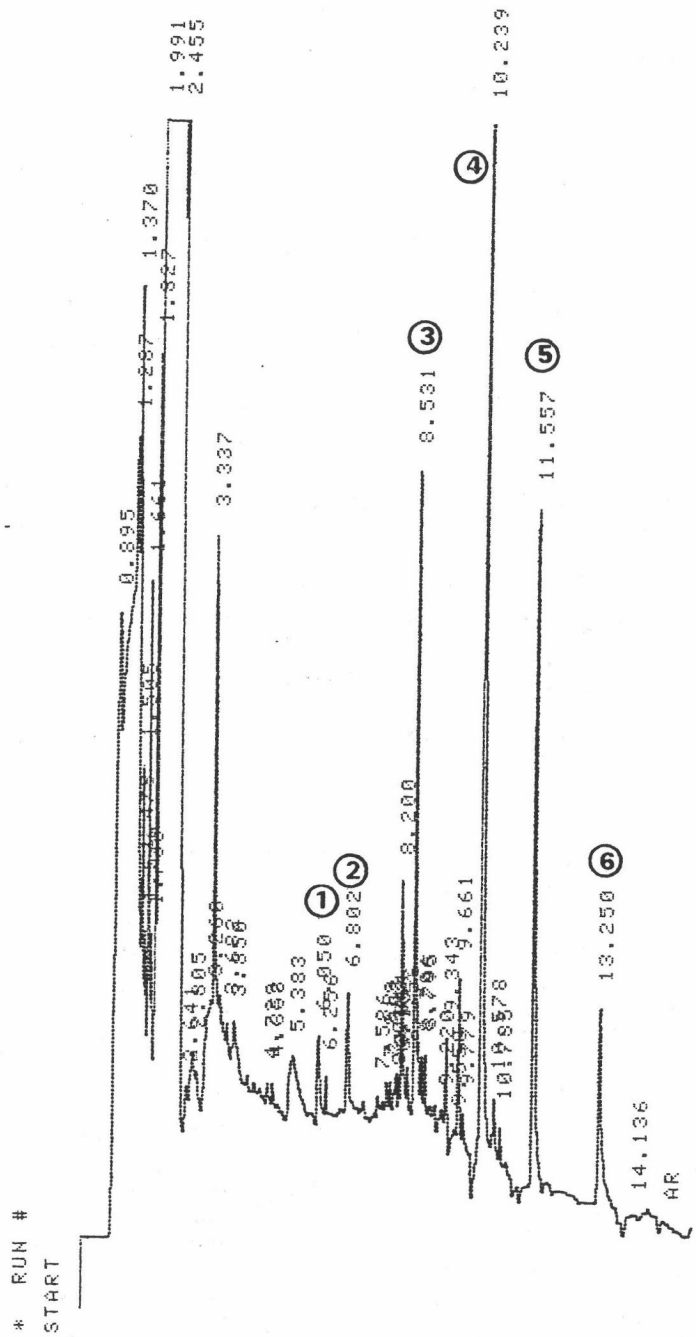


Figure 4.26 The gas chromatogram of standard mixture solution in milk by method 3

GC-condition: described in Table 3.2 Integrator Att. = 1

Concentration of DMP: 22122 μg/kg DEP: 206.51 μg/kg

DBP : 286.22 μg/kg BBP: 385.64 μg/kg

DEHP: 366.56 μg/kg DOP: 188.28 μg/kg

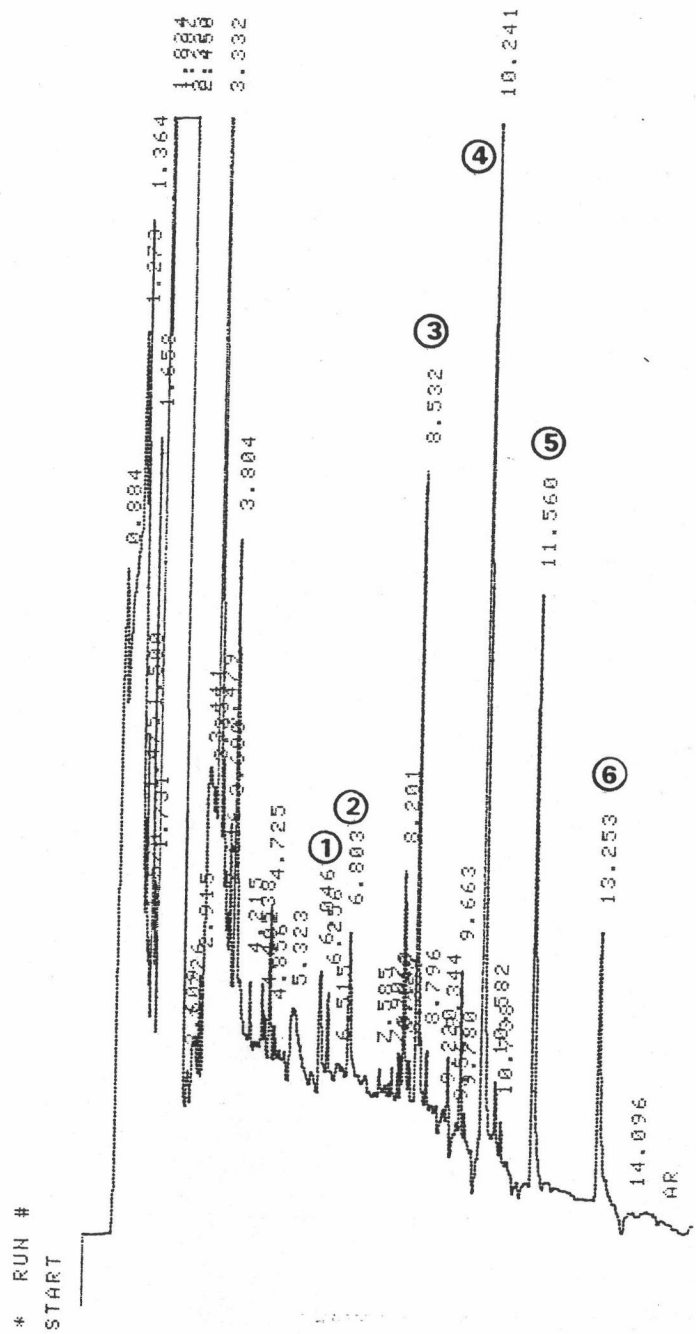


Figure 4.27 The gas chromatogram of standard mixture solution in milk by method 4

GC-condition: described in Table 3.2 Integrator Att. = 1

Concentration of DMP: 357.88 μg/kg DEP: 277.32 μg/kg

DBP: 319.34 μg/kg BBP: 382.72 μg/kg

DEHP: 316.49 μg/kg DOP: 262.13 μg/kg

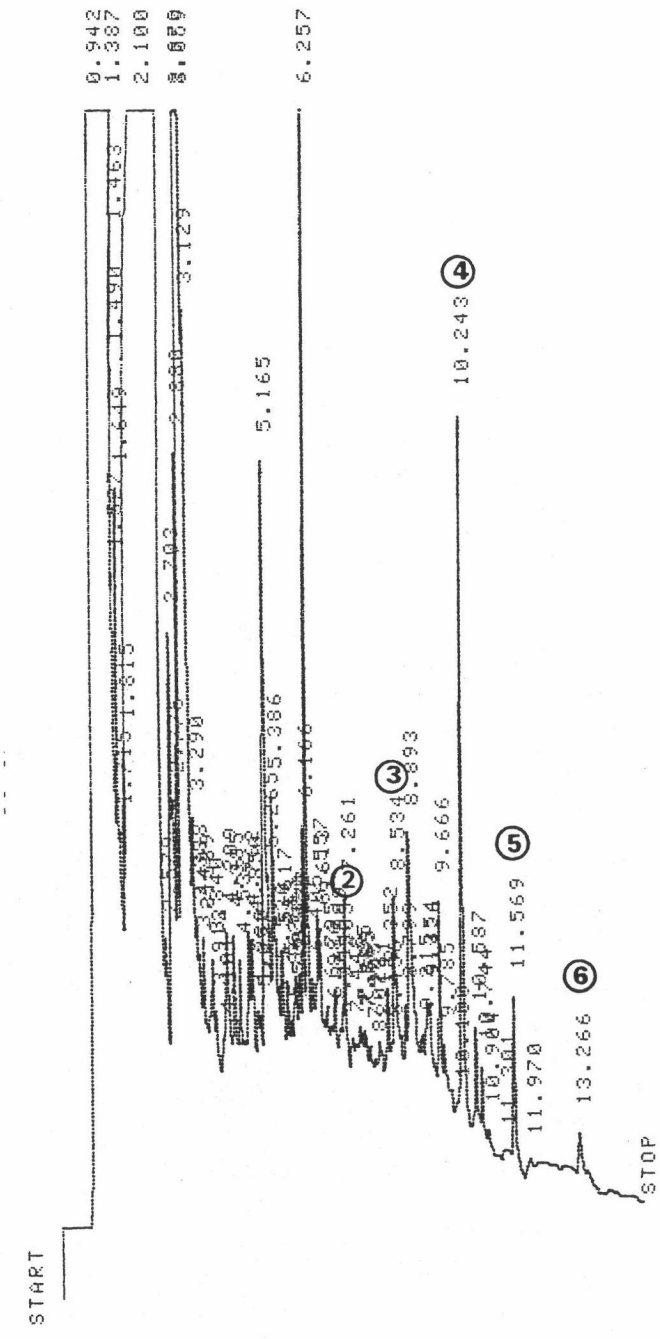


Figure 4.28 The gas chromatogram of standard mixture solution in milk by method 5

GC-condition: described in Table 3.2 Integrator Att. = 1

Concentration of DMP: 326.80 μg/kg DEP: 266.96 μg/kg

DBP: 288.44 μg/kg BBP: 307.22 μg/kg

DEHP: 373.55 μg/kg DOP: 76.96 μg/kg

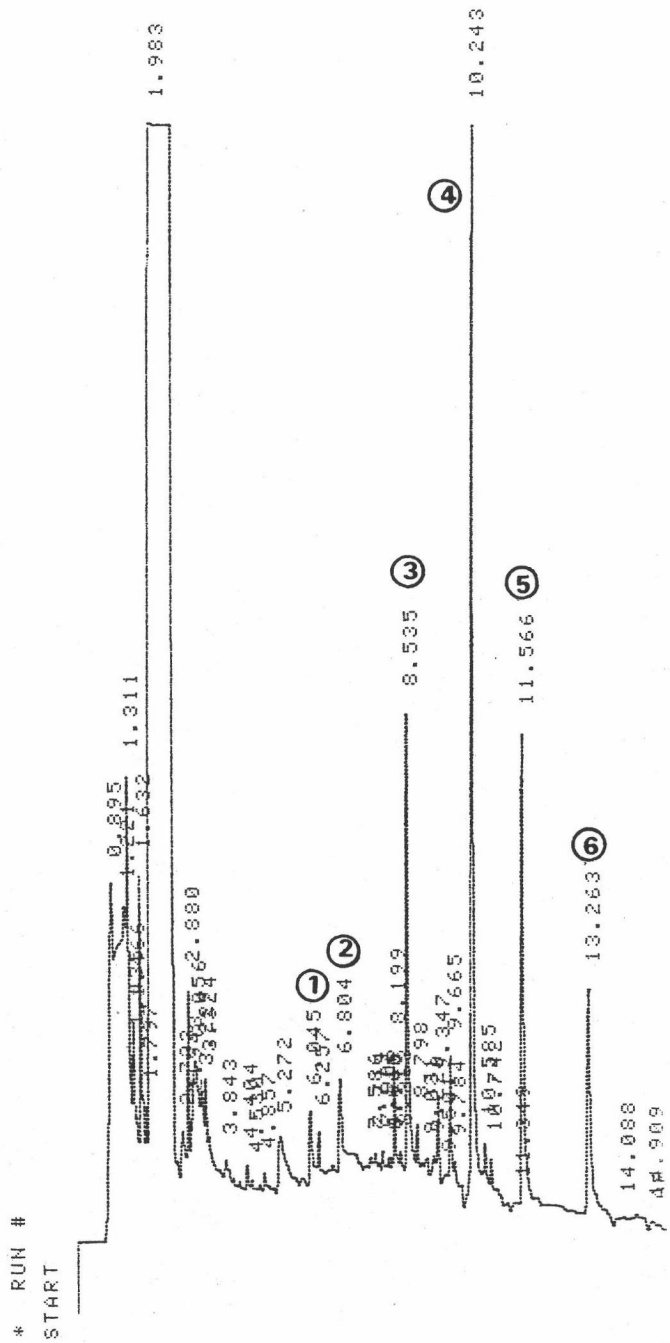


Figure 4.29 The gas chromatogram of standard mixture solution in milk by method 6

GC-condition: described in Table 3.2 Integrator Att. = 2

Concentration of DMP: 392.77 μg/kg DEP: 345.22 μg/kg

DBP : 593.21 μg/kg BBP: 632.30 μg/kg

DEHP: 660.30 μg/kg DOP: 499.48 μg/kg

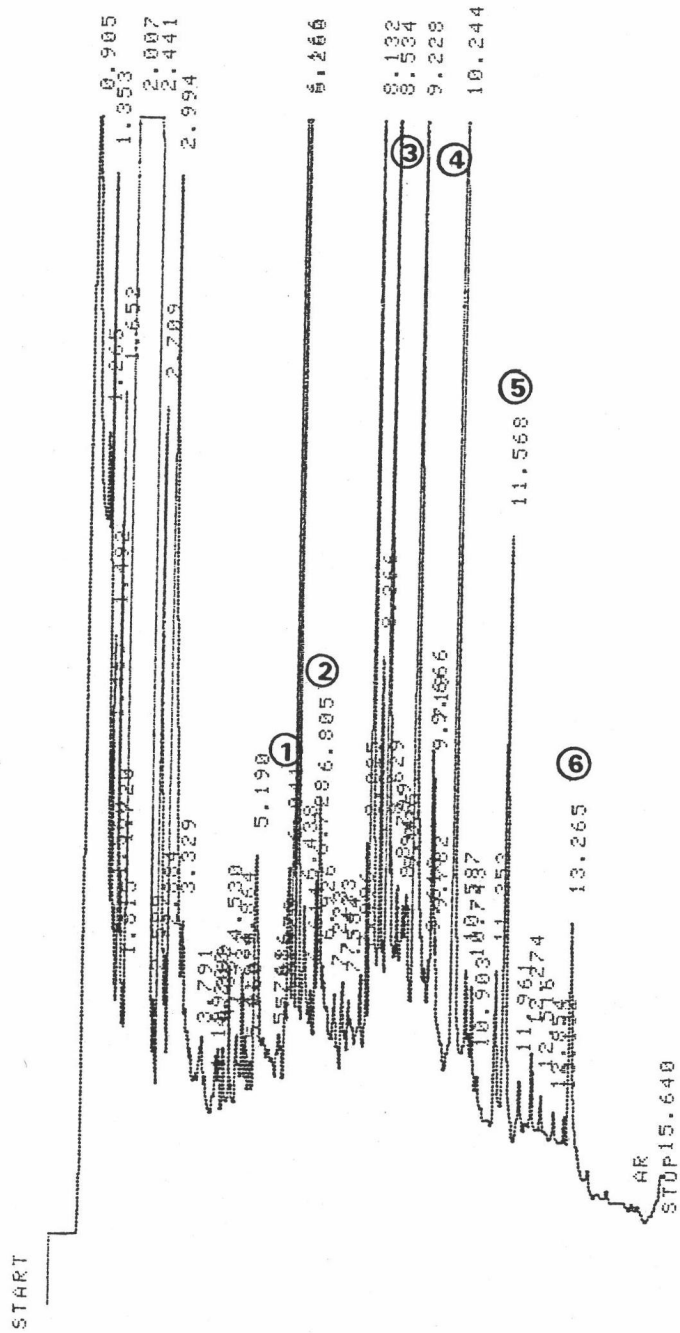


Figure 4.30 The gas chromatogram of standard mixture solution in milk by method 7

GC-condition: described in Table 3.2 Integrator Att. = 1

Concentration of DMP: 1311.44 μg/kg DEP: 829.45 μg/kg

DBP : 519.76 μg/kg BBP: 263.49 μg/kg

DEHP: 139.54 μg/kg DOP: 70.66 μg/kg

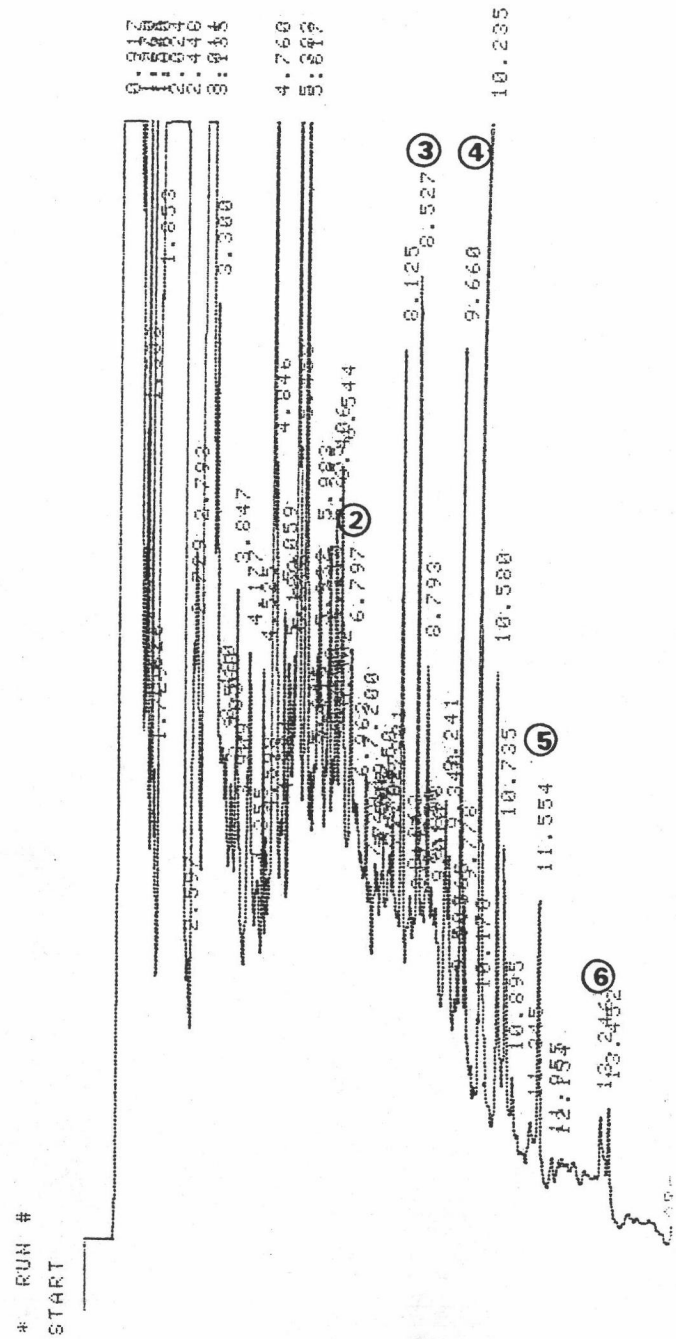


Figure 4.31 The gas chromatogram of standard mixture solution in milk by method 8

GC-condition: described in Table 3.2 Integrator Att. = 1

Concentration of DMP: 717.41 μg/kg DEP: 634.50 μg/kg

DBP : 710.33 μg/kg BBP: 600.28 μg/kg

DEHP: 613.40 μg/kg DOP: 314.62 μg/kg

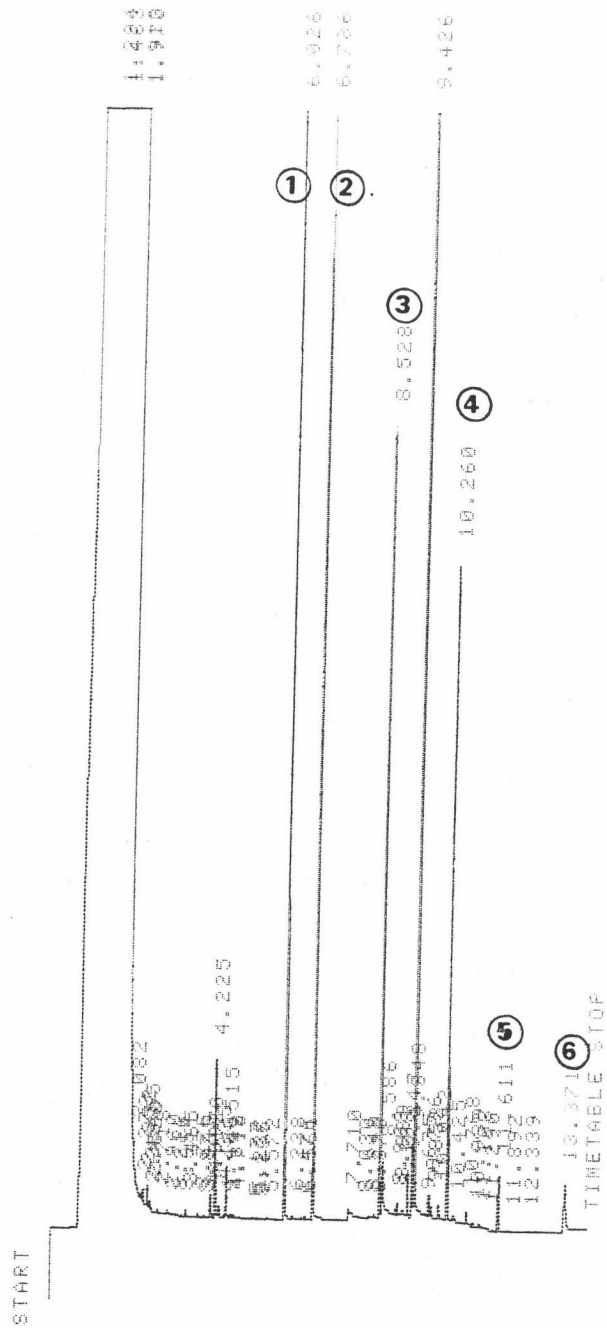


Figure 4.32 The gas chromatogram of standard mixture solution in milk by method 1

GC-condition : described in Table 3.1. Integrator Att = 5

concentration of DMP: 17.88 mg/kg DEP: 16.41 mg/kg

DBP : 20.12 mg/kg BBP: 22.66 mg/kg

DEHP: 2.79 mg/kg DOP: 2.60 mg/kg

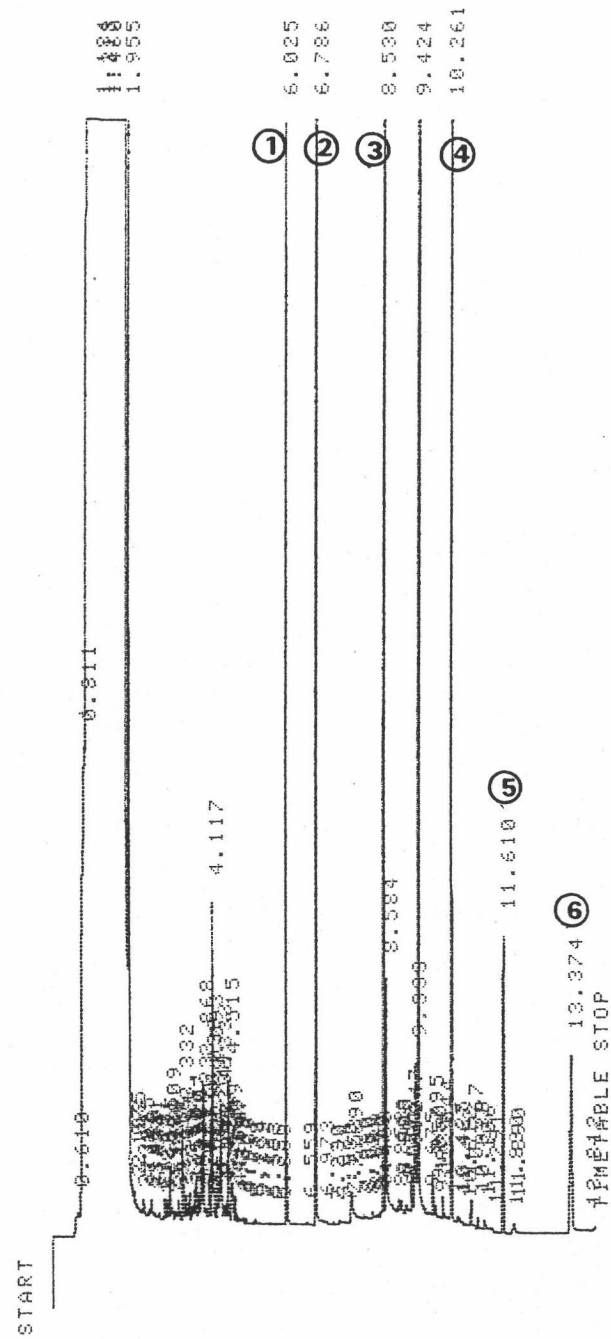


Figure 4.33 The gas chromatogram of standard mixture solution in milk by method 2

GC-condition : described in Table 3.1. Integrator Att = 5

concentration of DMP: 19.65 mg/kg DEP: 19.35 mg/kg

DBP : 22.71 mg/kg BBP: 23.76 mg/kg

DEHP: 8.41 mg/kg DOP: 9.87 mg/kg

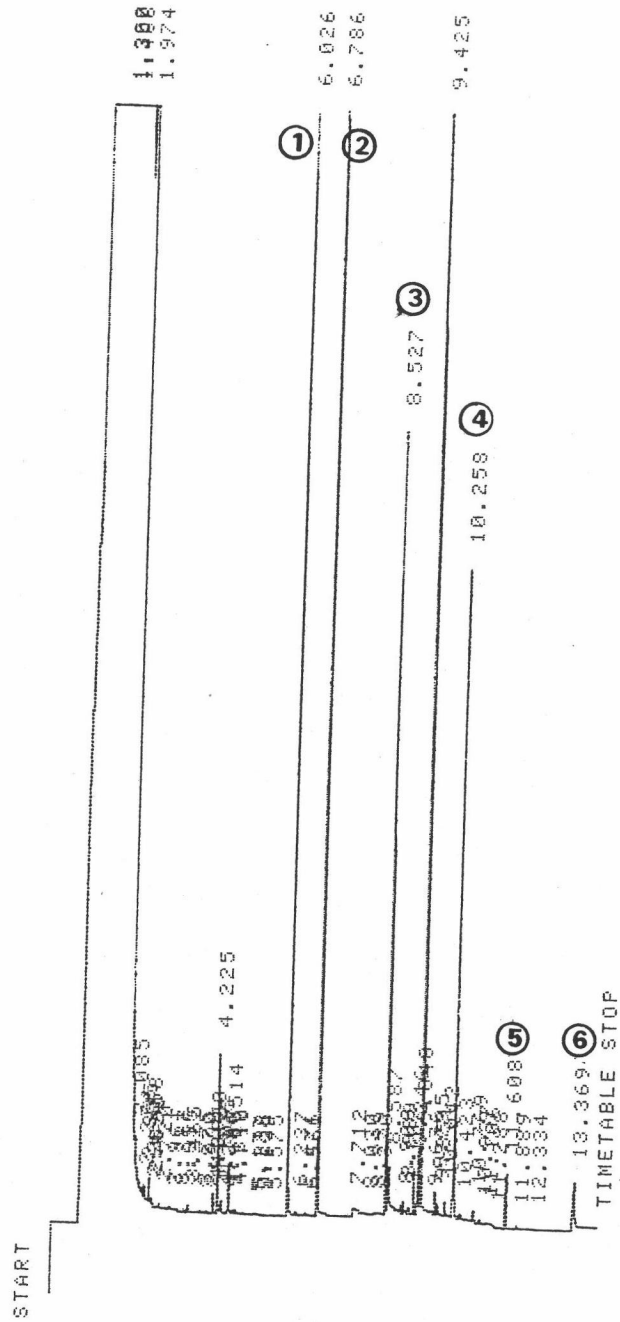


Figure 4.34 The gas chromatogram of standard mixture solution in milk by method 3

GC-condition : described in Table 3.1. Integrator Att = 5

concentration of DMP: 17.88 mg/kg DEP: 15.80 mg/kg

DBP : 20.38 mg/kg BBP: 21.46 mg/kg

DEHP: 3.47 mg/kg DOP: 4.44 mg/kg

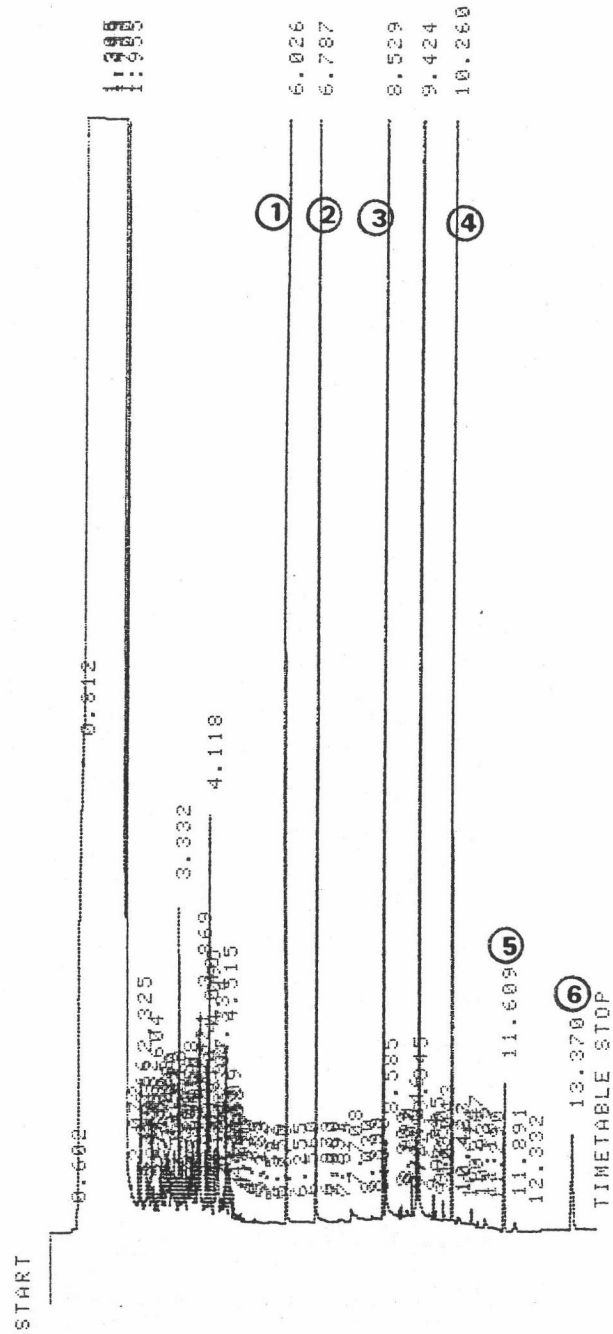


Figure 4.35 The gas chromatogram of standard mixture solution in milk by method 1

GC-condition : described in Table 3.1. Integrator Att = 5

concentration of DMP: 23.01 mg/kg DEP: 22.57 mg/kg

DBP : 21.21 mg/kg BBP: 23.08 mg/kg

DEHP: 4.32 mg/kg DOP: 4.44 mg/kg

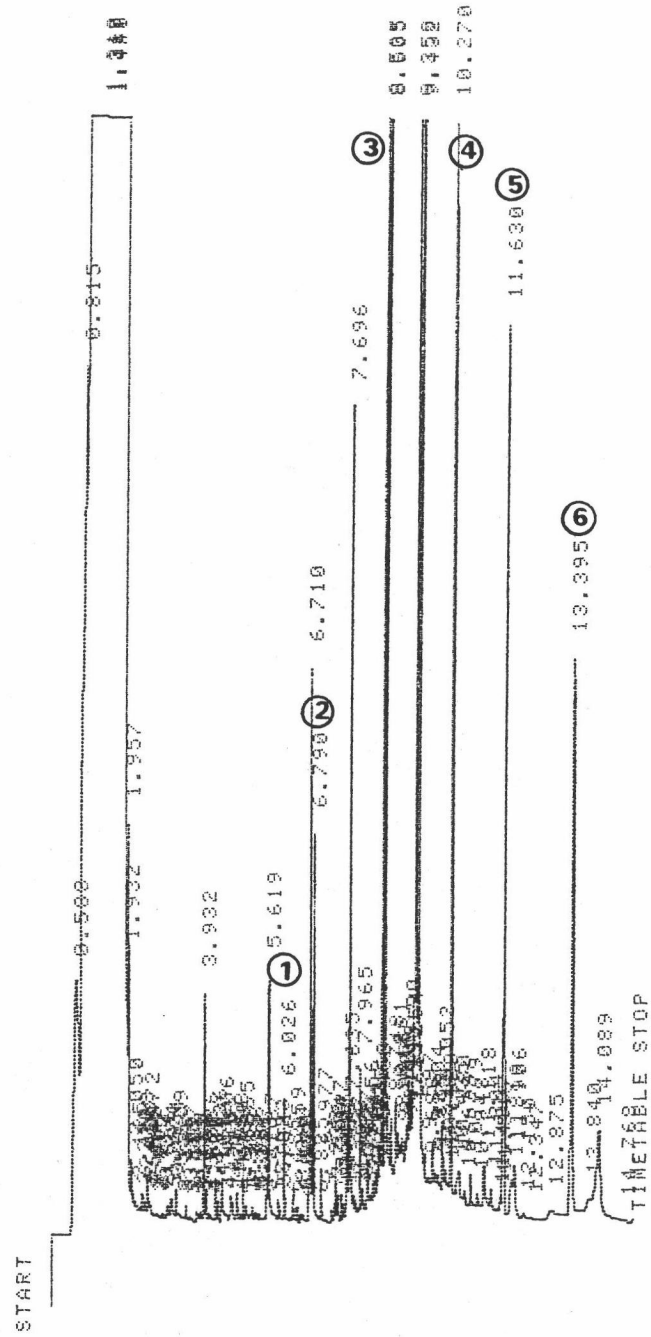


Figure 4.36 The gas chromatogram of standard mixture solution in milk by method 1

GC-condition : described in Table 3.1. Integrator Att = 5

concentration of DMP: 1.74 mg/kg DEP: 9.07 mg/kg

DBP : 19.58 mg/kg BBP: 17.62 mg/kg

DEHP: 17.69 mg/kg DOP: 18.21 mg/kg

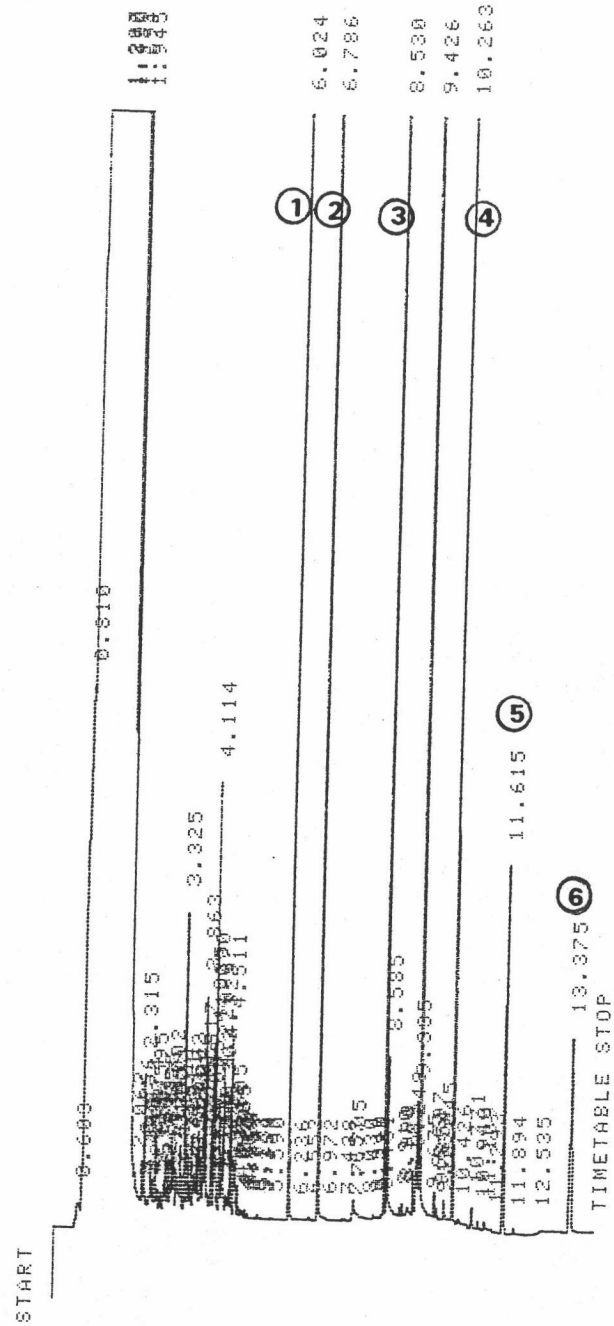


Figure 4.37 The gas chromatogram of standard mixture solution in milk by method 1

GC-condition : described in Table 3.1. Integrator Att = 5

concentration of DMP: 35.95 mg/kg DEP: 44.07 mg/kg

DBP : 36.00 mg/kg BBP: 35.02 mg/kg

DEHP: 14.89 mg/kg DOP: 15.11 mg/kg

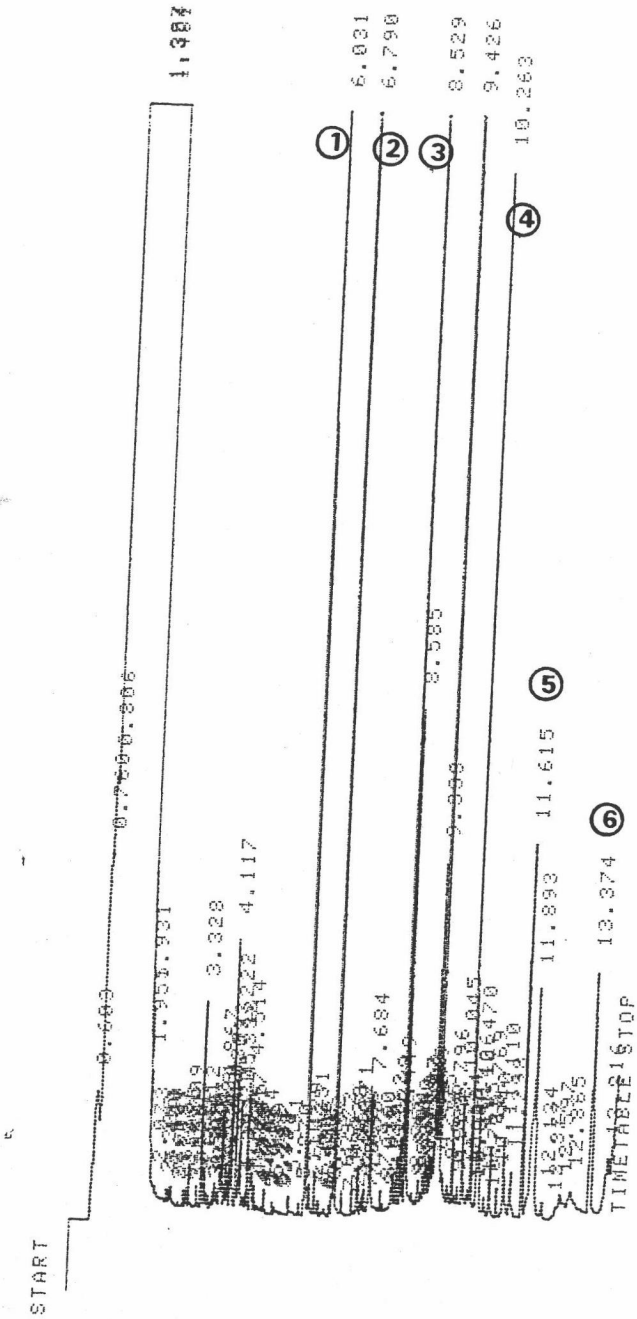


Figure 4.38 The gas chromatogram of standard mixture solution in milk by method 1

GC-condition : described in Table 3.1. Integrator Att = 5

concentration of DMP: 76.74 mg/kg DEP: 70.07 mg/kg

DBP : 23.95 mg/kg BBP: 20.62 mg/kg

DEHP: 9.27 mg/kg DOP: 11.42 mg/kg

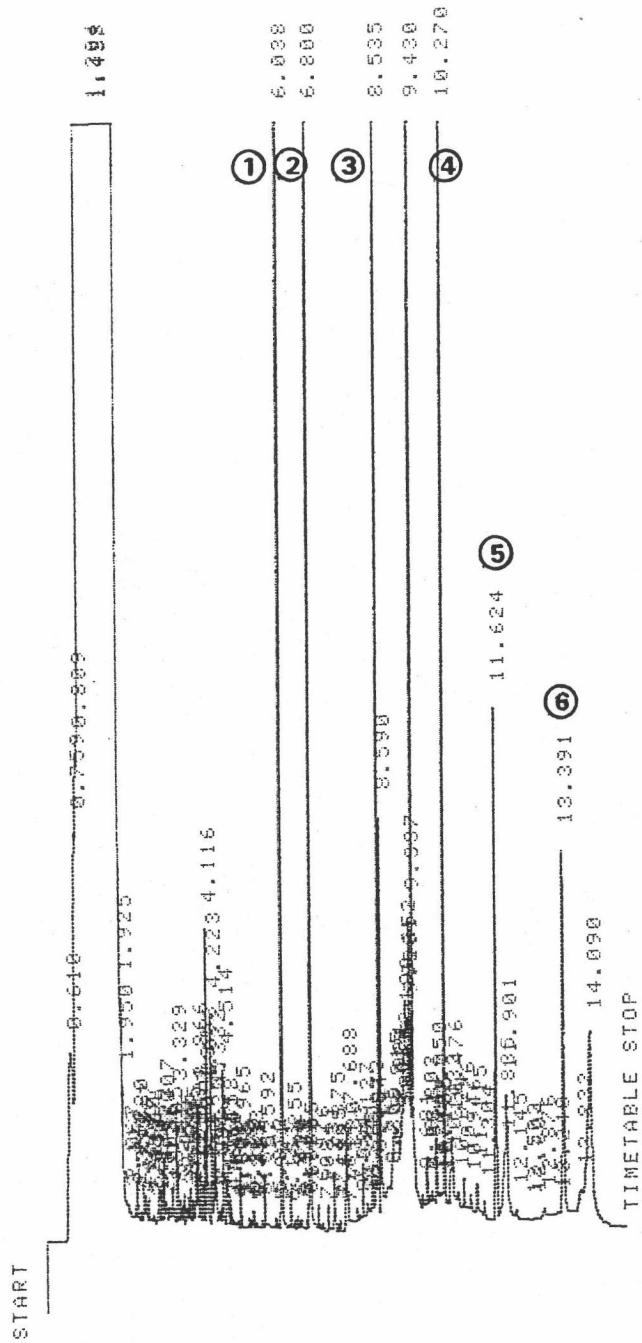


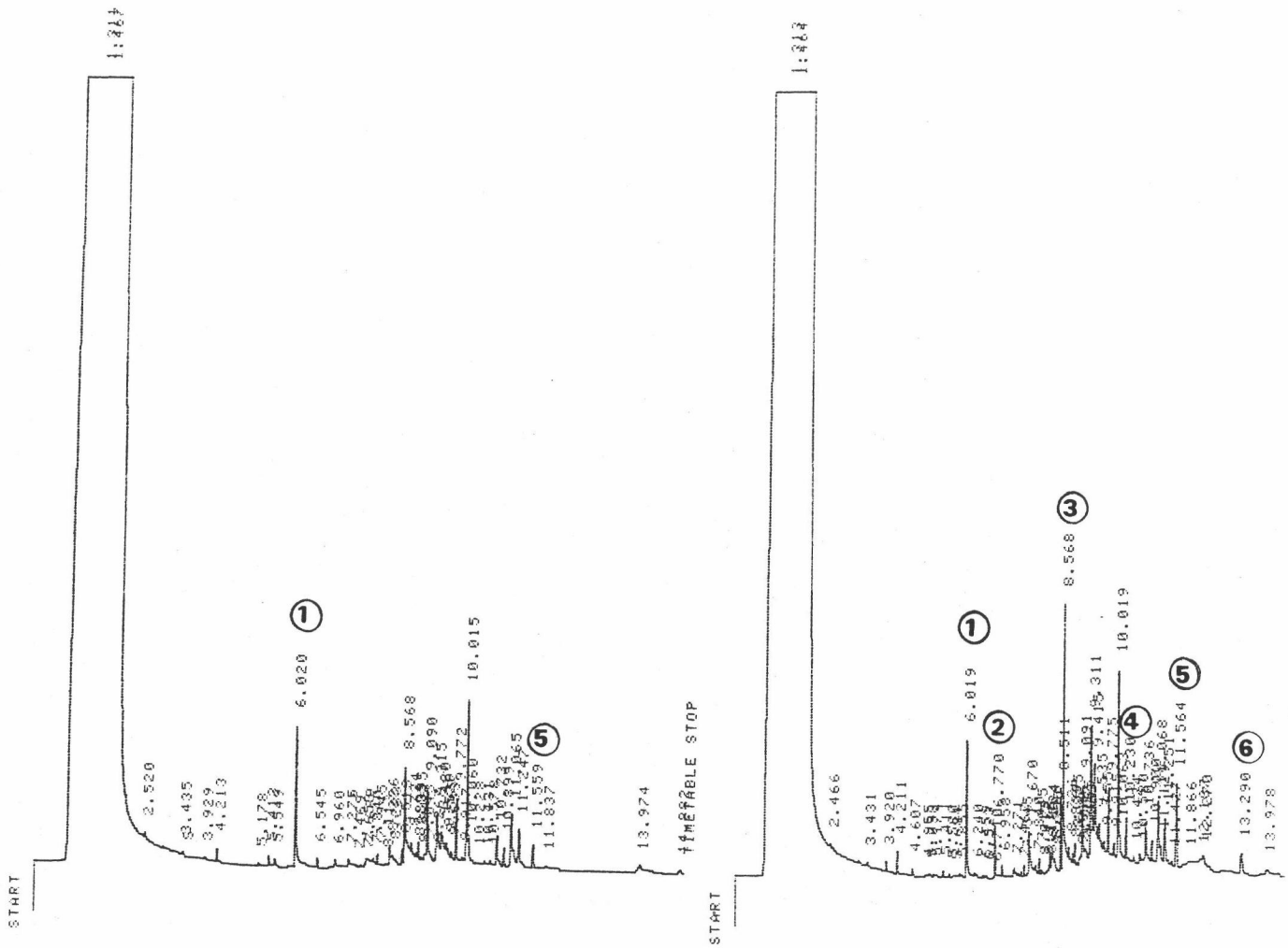
Figure 4.39 The gas chromatogram of standard mixture solution in milk by method 1

GC-condition : described in Table 3.1. Integrator Att = 5

concentration of DMP: 78.21 mg/kg DEP: 74.51 mg/kg

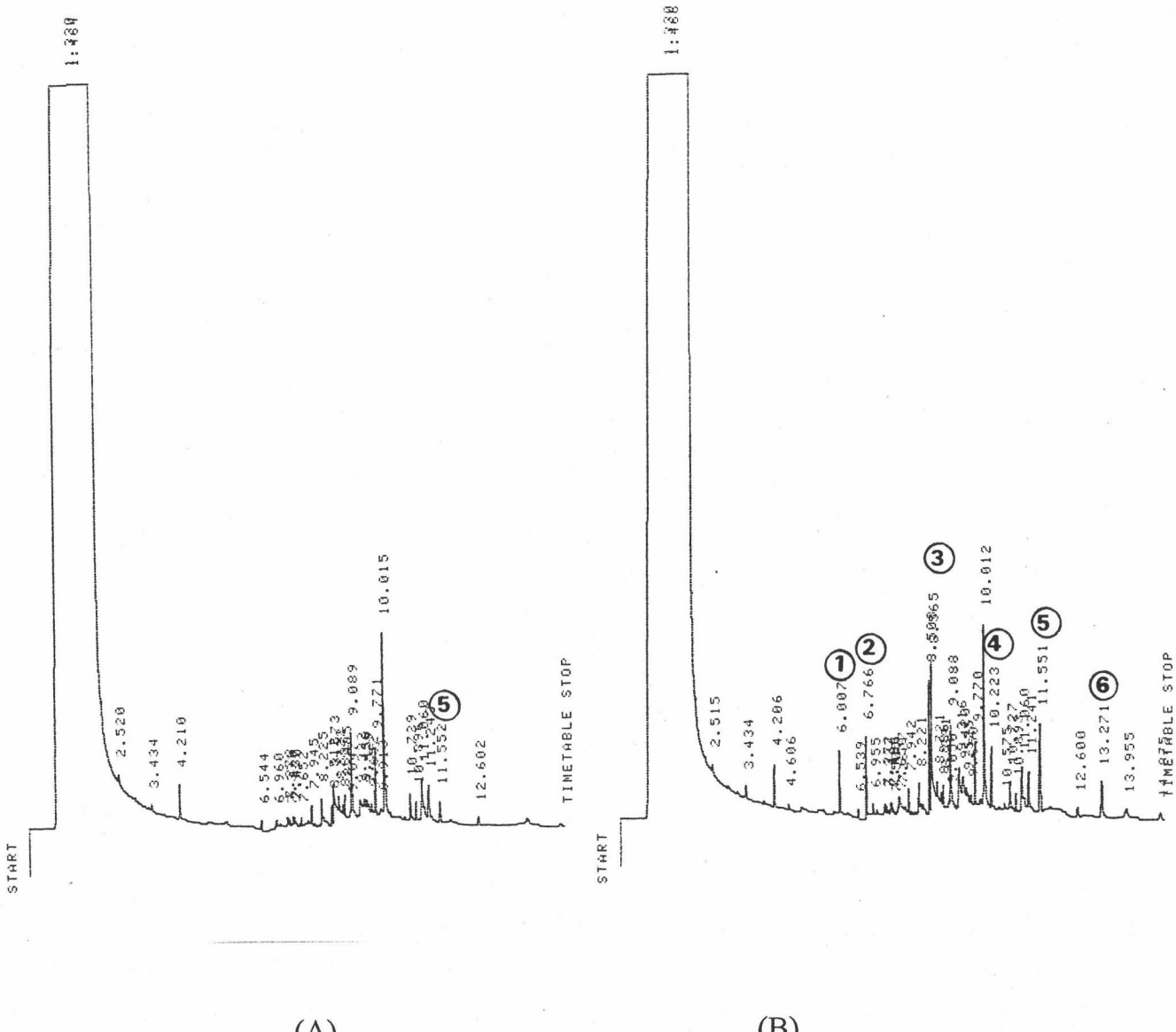
DBP : 20.72 mg/kg BBP: 21.77 mg/kg

DEHP: 10.86 mg/kg DOP: 12.94 mg/kg



(A) (B)

Figure 4.40 The gas chromatogram of
(A) Yakult (milk)
(B) Yakult (milk) + standard mixture solution
condition
GC/FID : described in Table 3.1
Integrator : att 2



(A) (B)

Figure 4.41 The gas chromatogram of
(A) Thai-Dennish (milk)
(B) Thai-Dennish (milk) + standard mixture solution
condition
GC/FID : described in Table 3.1
Integrator : att 1

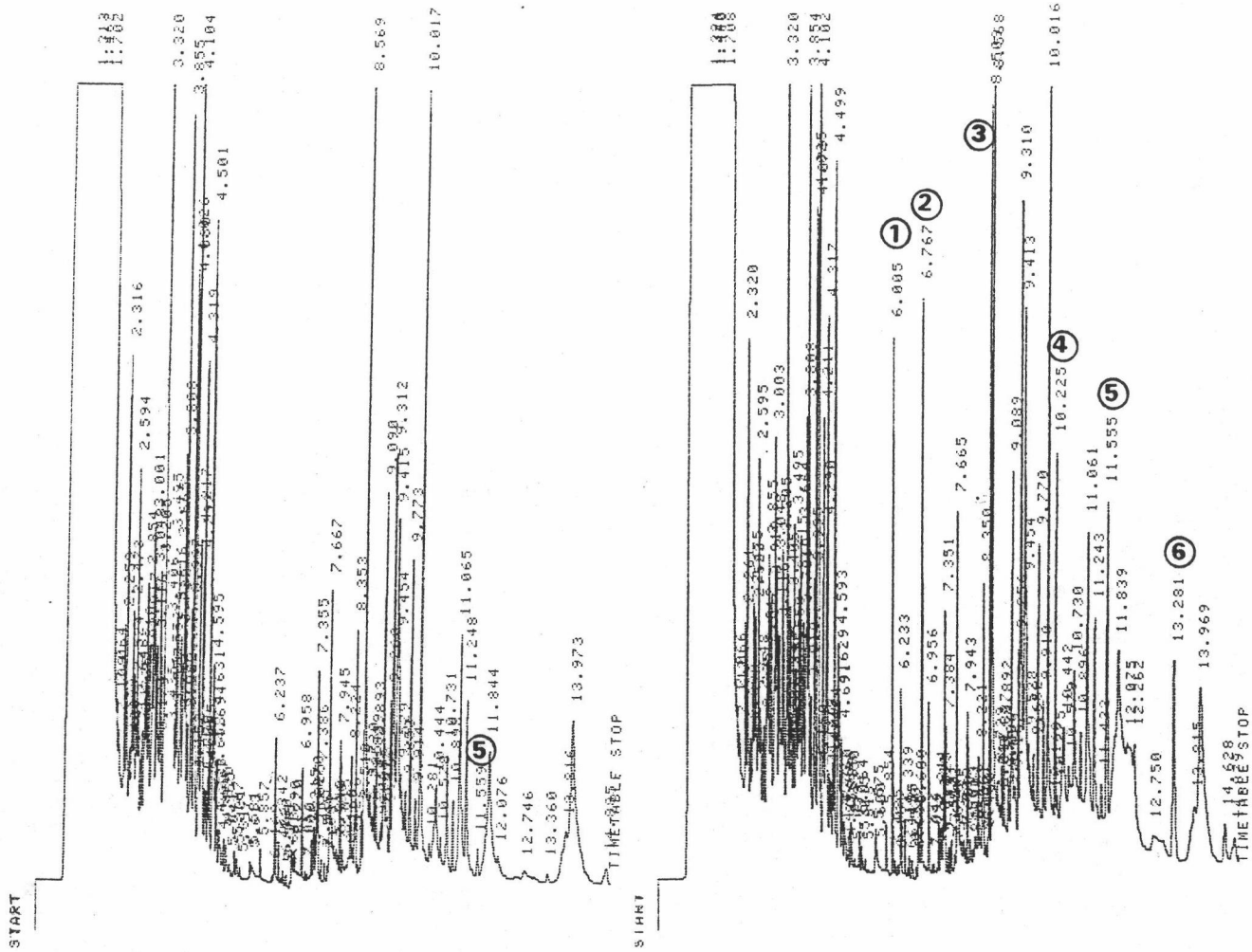
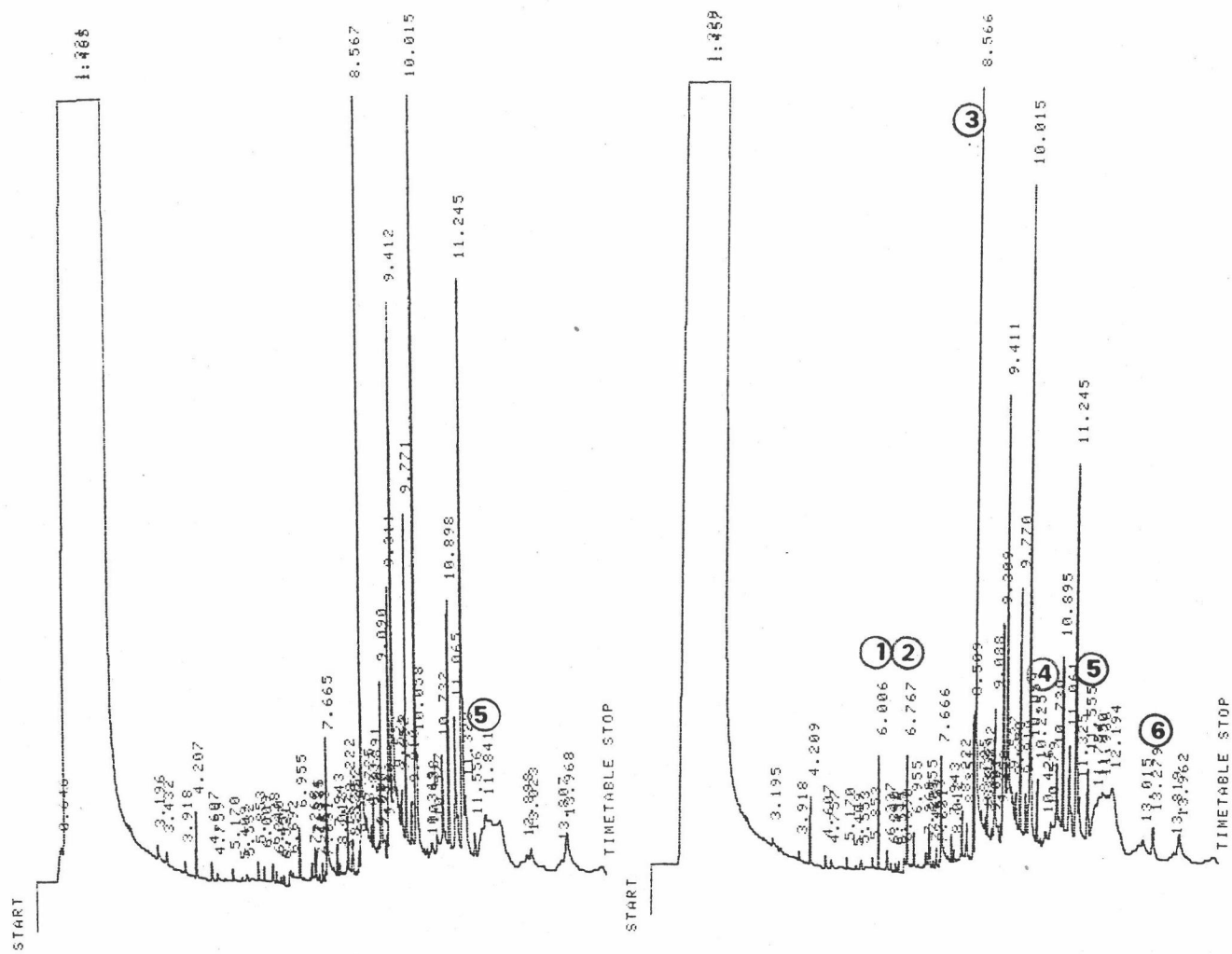
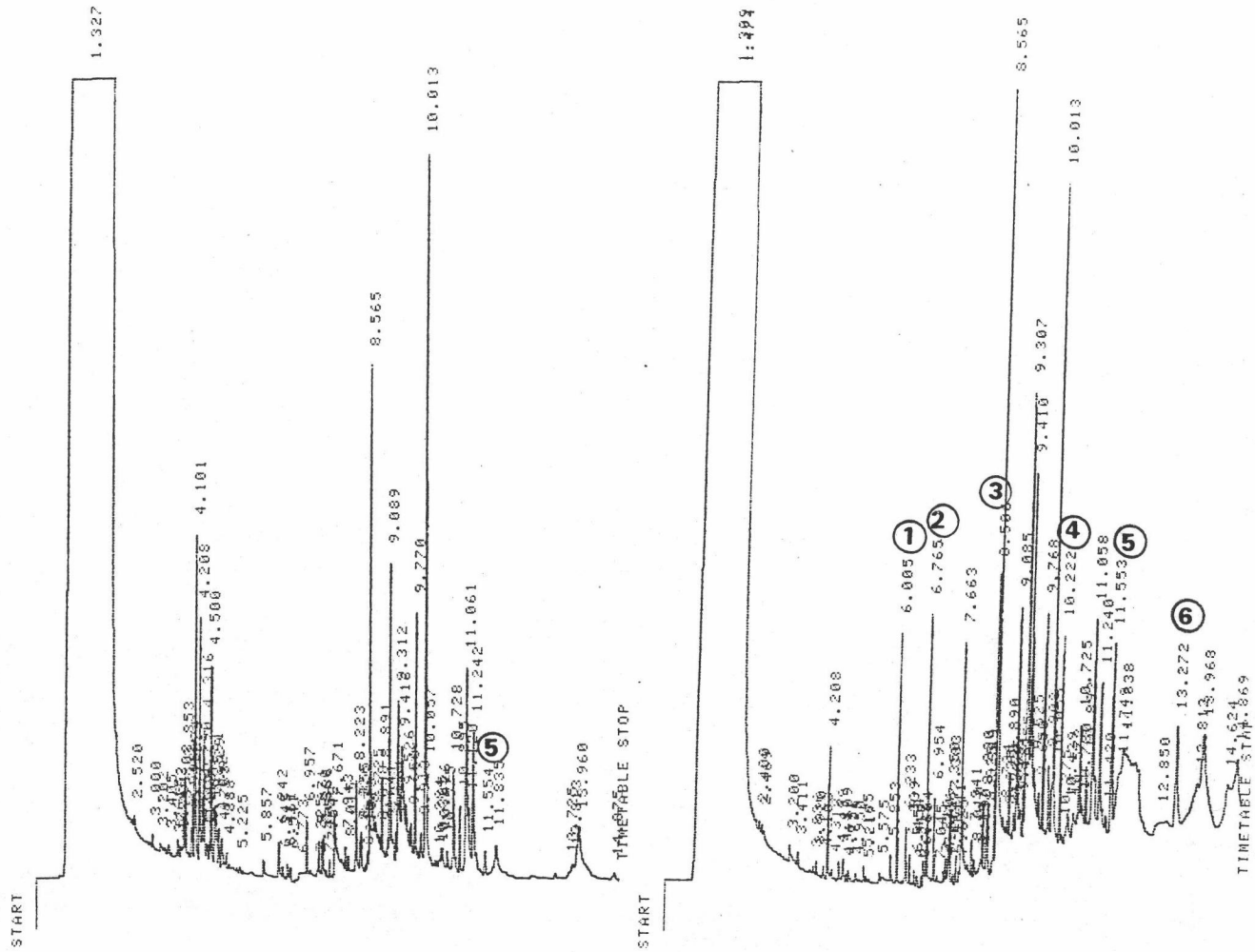


Figure 4.42 The gas chromatogram of
(A) Yomost (yogurt)
(B) Yomost (yogurt) + standard mixture solution
condition
GC/FID : described in Table 3.1
Integrator : att 1



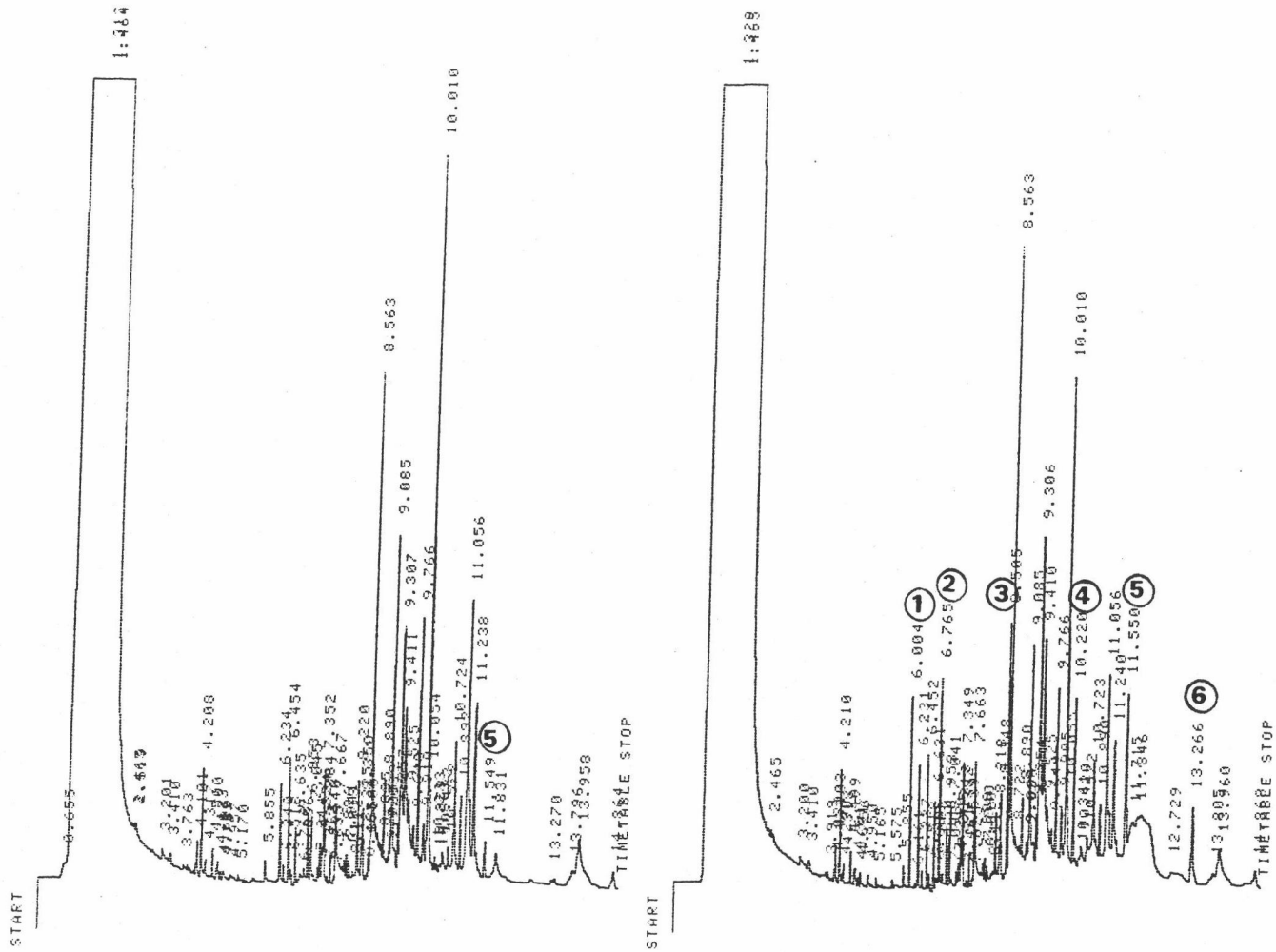
(A) (B)

Figure 4.43 The gas chromatogram of
(A) Meiji (yogurt)
(B) Meiji (yogurt) + standard mixture solution
condition
GC/FID : described in Table 3.1
Integrator : att 1



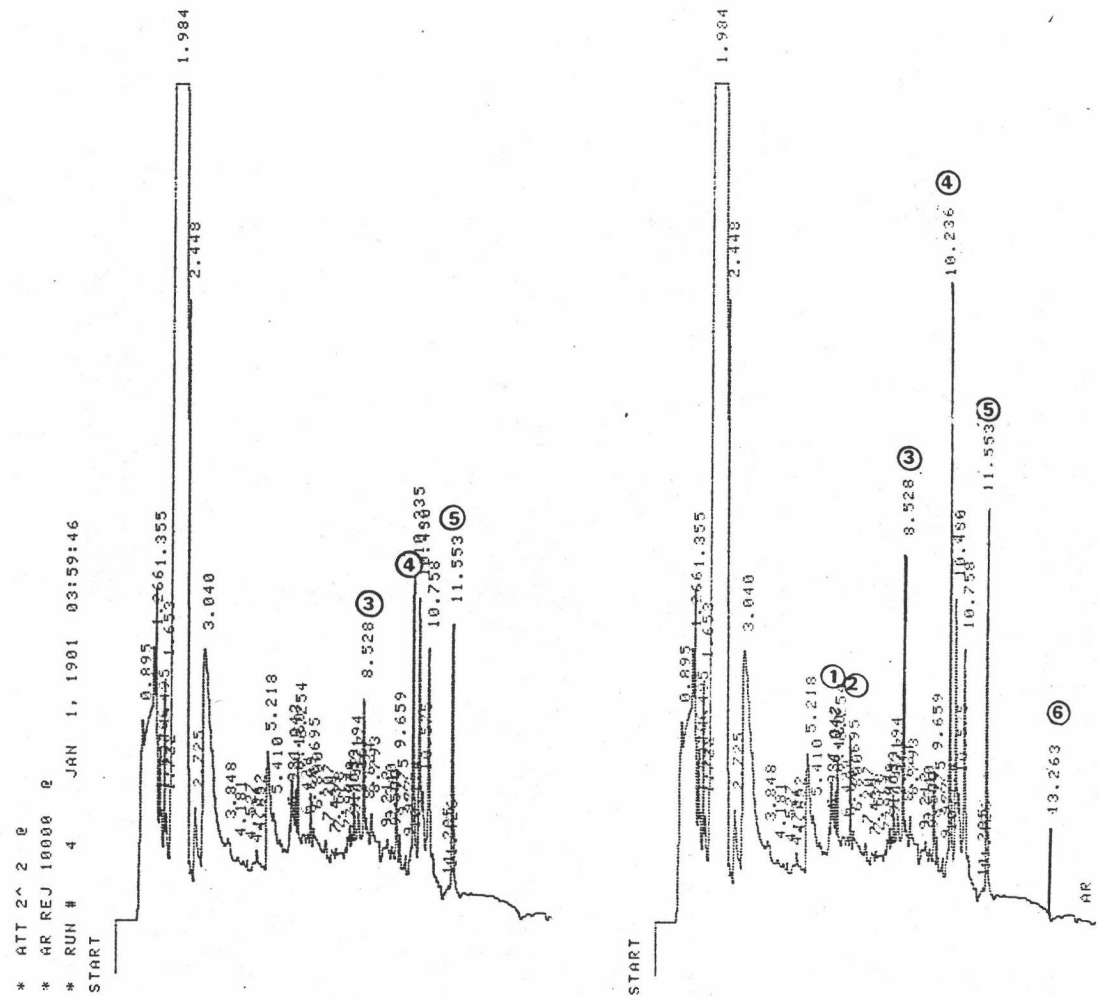
(A) (B)

Figure 4.44 The gas chromatogram of
(A) Yophalt (yogurt)
(B) Yophalt (yogurt) + standard mixture solution
condition
GC/FID : described in Table 3.1
Integrator : att 1



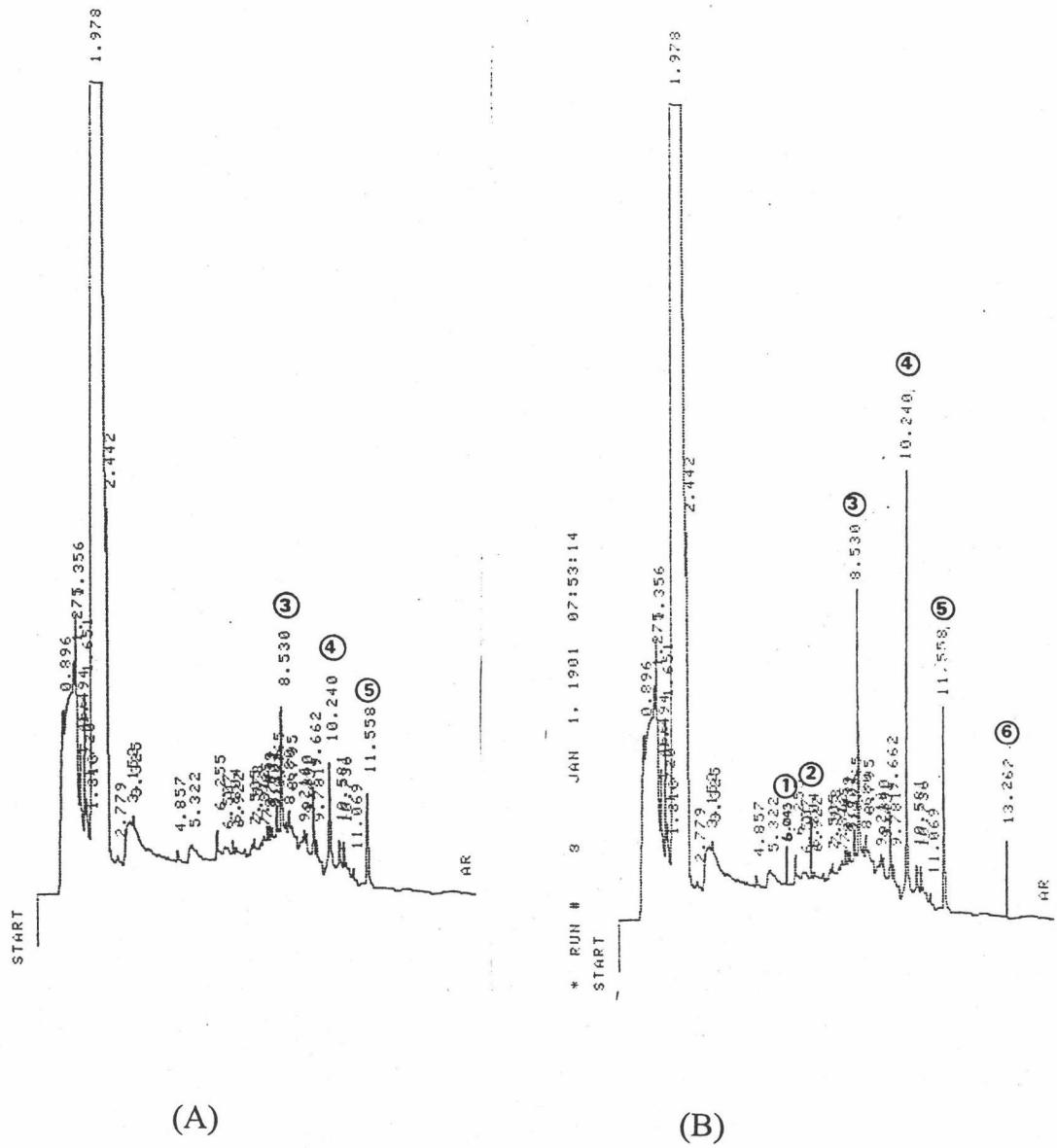
(A) (B)

Figure 4.45 The gas chromatogram of
(A) Duchmill (yogurt)
(B) Dutchmill (yogurt) + standard mixture solution
condition
GC/FID : described in Table 3.1
Integrator : att 1



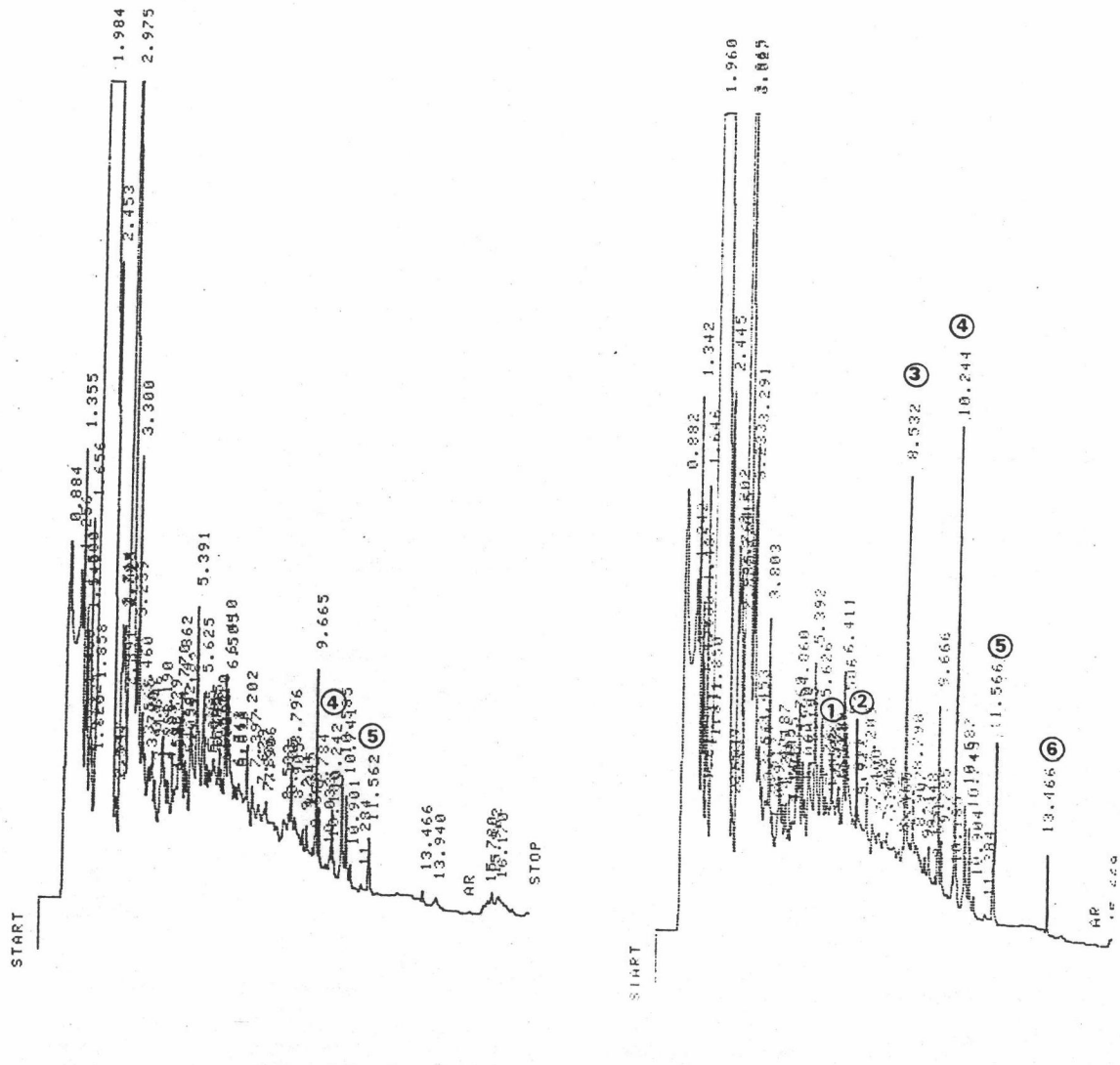
(A) (B)

Figure 4.46 The gas chromatogram of
(A) Yakult (milk)
(B) Yakult (milk) + standard mixture solution
condition
GC/ECD : described in Table 3.2
Integrator : att 2



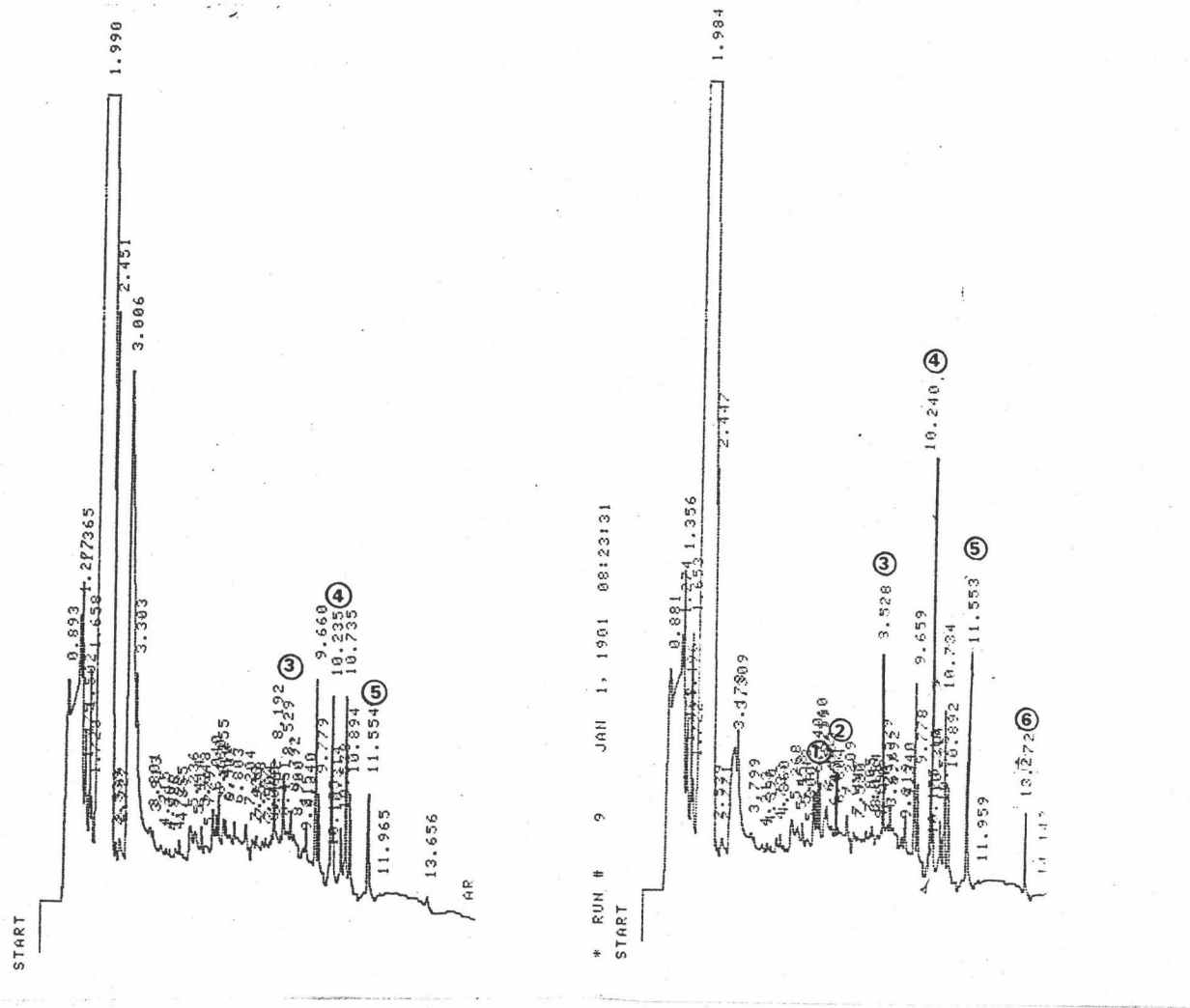
(A) (B)

Figure 4.47 The gas chromatogram of
(A) Thai-Dennisht (milk)
(B) Thai-Dennish + standard mixture solution
condition
GC/ECD : described in Table 3.2
Integrator : att 2



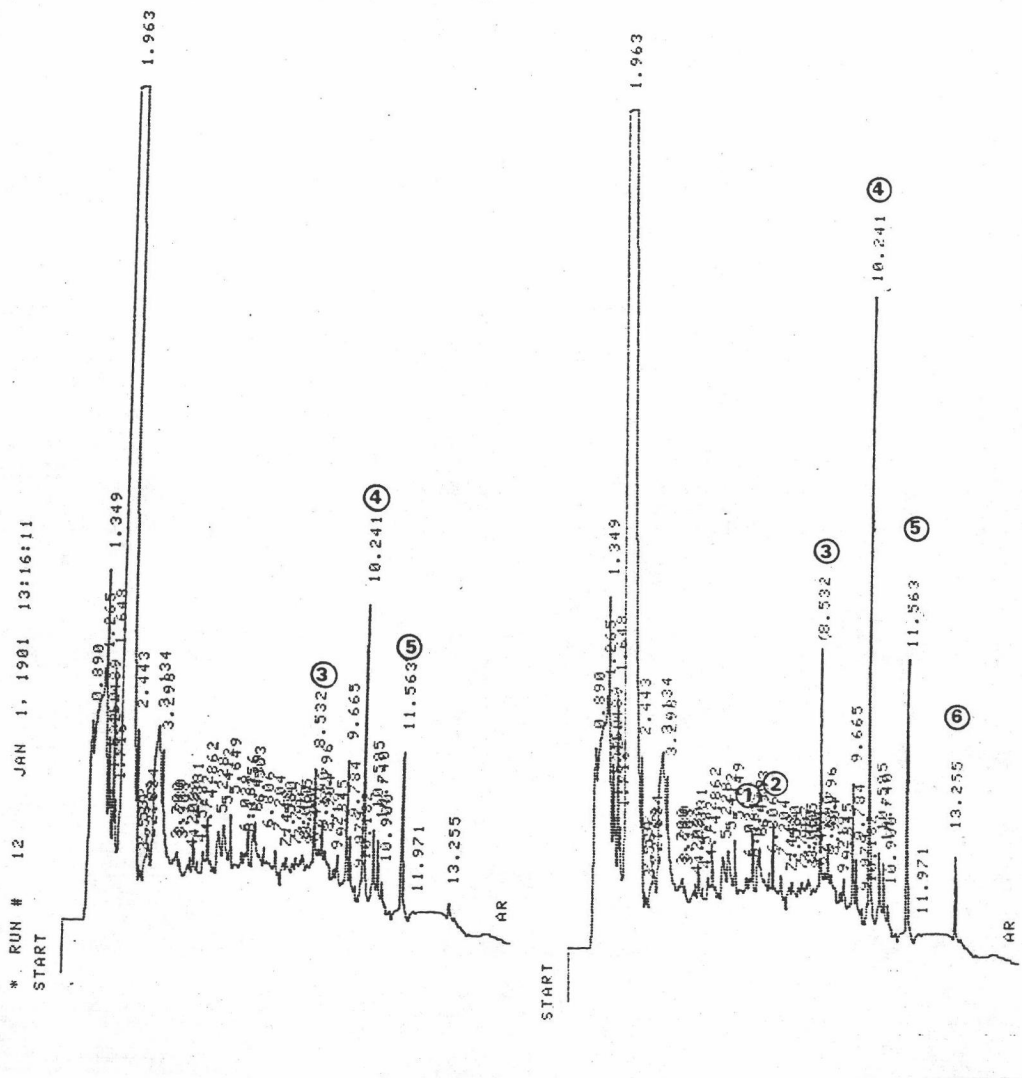
(A) (B)

Figure 4.48 The gas chromatogram of
(A) Yomost (yogurt)
(B) Yomost (yogurt) + standard mixture solution
condition
GC/ECD : described in Table 3.2
Integrator : att 2



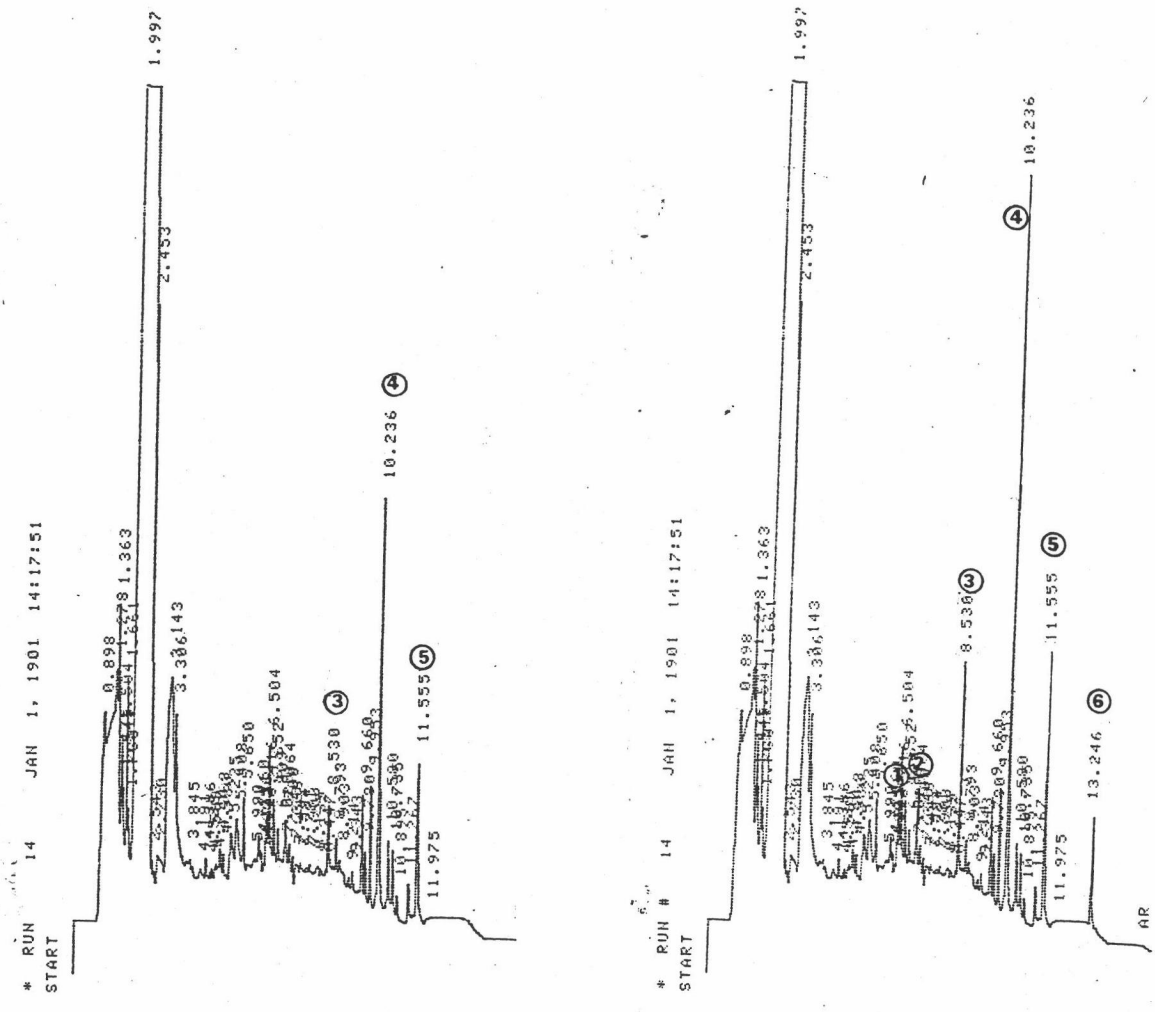
(A) (B)

Figure 4.49 The gas chromatogram of
(A) Meiji (yogurt)
(B) Meiji (yogurt) + standard mixture solution
condition
GC/ECD : described in Table 3.2
Integrator : att 2



(A) (B)

Figure 4.50 The gas chromatogram of
(A) Yaphalt (yogurt)
(B) Yophalt (yogurt) + standard mixture solution
condition
GC/ECD : described in Table 3.2
Integrator : att 2



(A) (B)

Figure 4.51 The gas chromatogram of
(A) Duchmill (yogurt)
(B) Duchmill (yogurt) + standard mixture solution
condition
GC/ECD : described in Table 3.2
Integrator : att 2

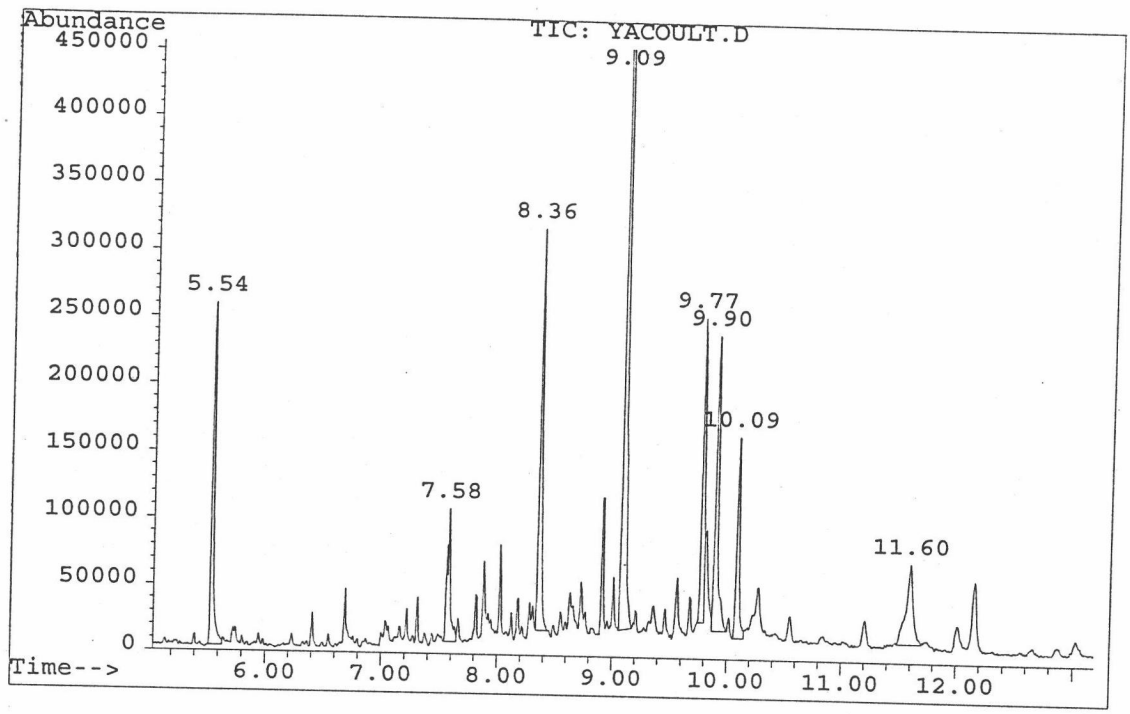


Figure 4.52 The gas chromatogram with MSD detection for an extract of a sample 1(Yakult)
GC/MSD condition : described in Table 3.3

Library Searched : C:\DATABASE\JO.L
Quality : 91
ID : 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) est
er

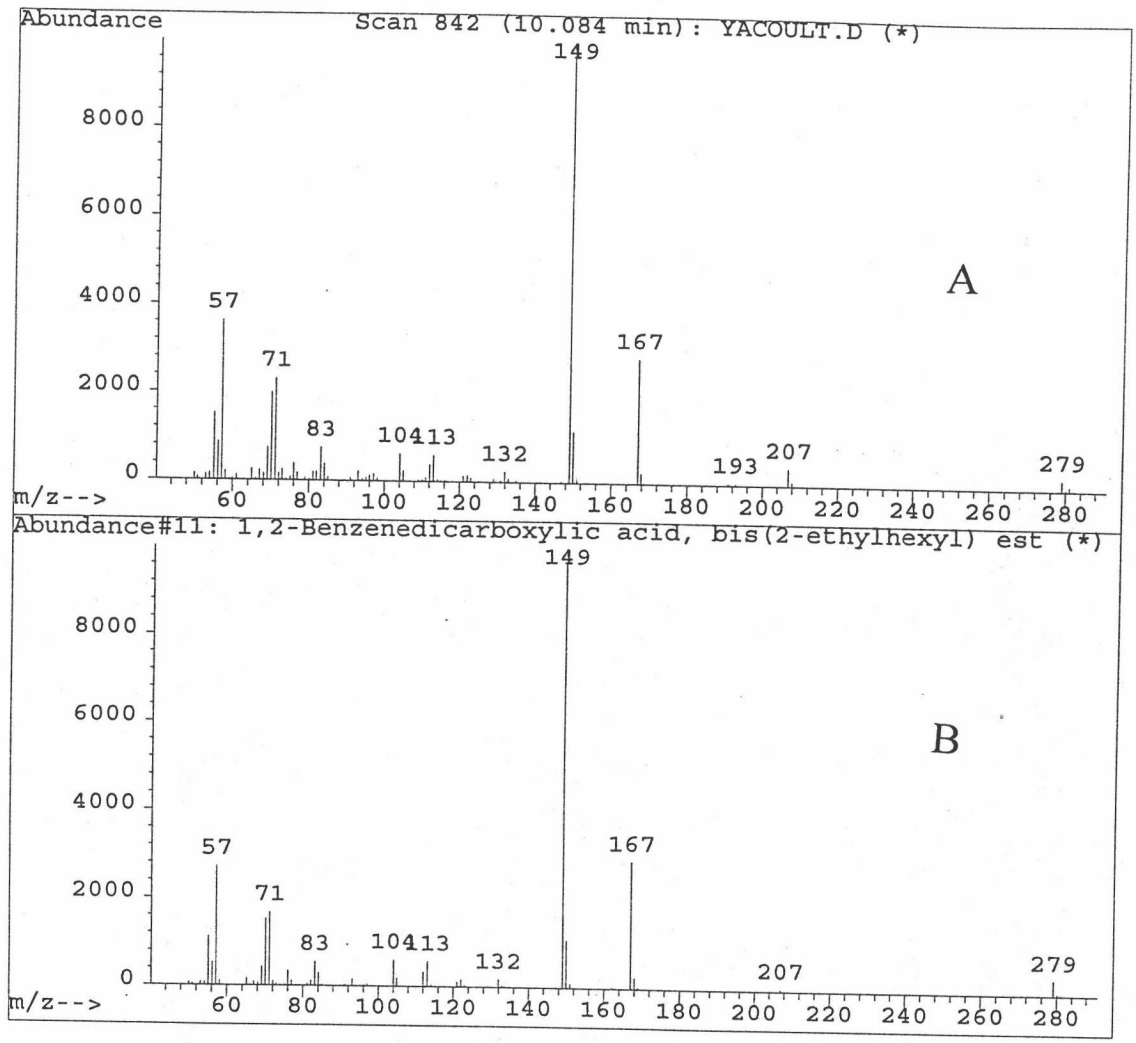


Figure 4.53 The mass spectrum for an extract of sample 1 with the retention time of 10.084 min from the gas chromatogram in Figure 4.52
(A) scan
(B) Library searched

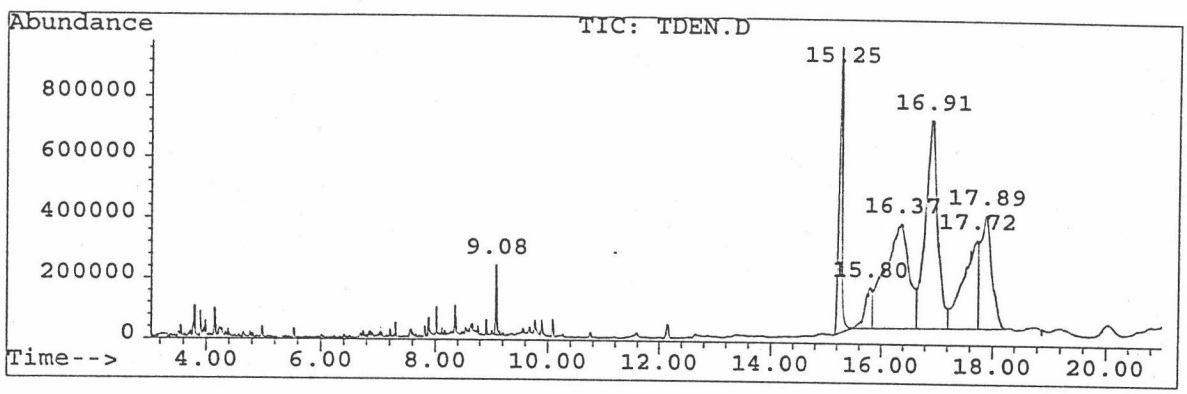


Figure 4.54 The gas chromatogram with MSD detection for an extract of a sample 2 (Thai-Dennish)
GC/MSD condition : described in Table 3.3

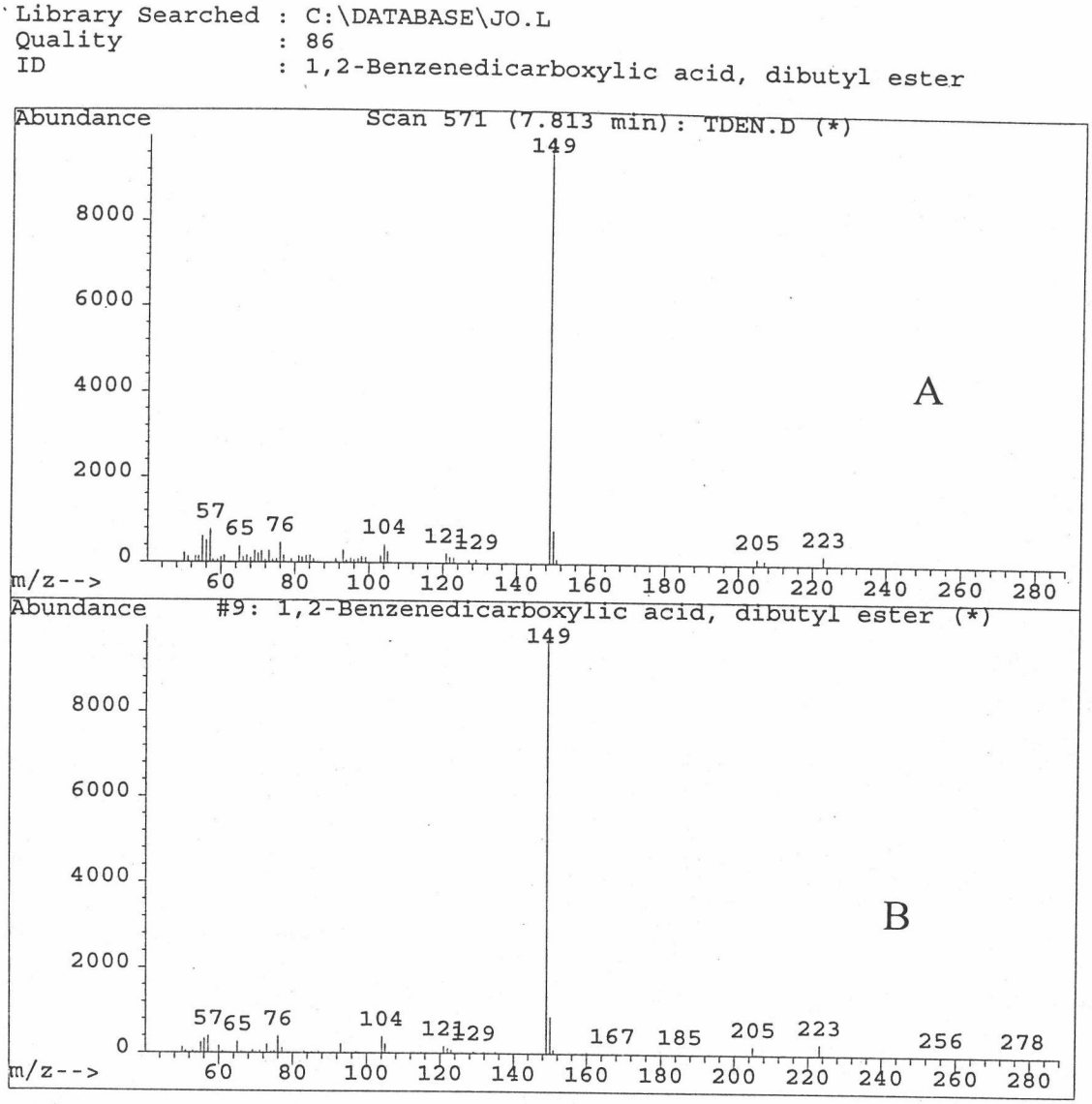


Figure 4.55 The mass spectrum for an extract of sample 2 with the retention time of 7.813 min from the gas chromatogram in Figure 4.54
(A) scan
(B) Library searched

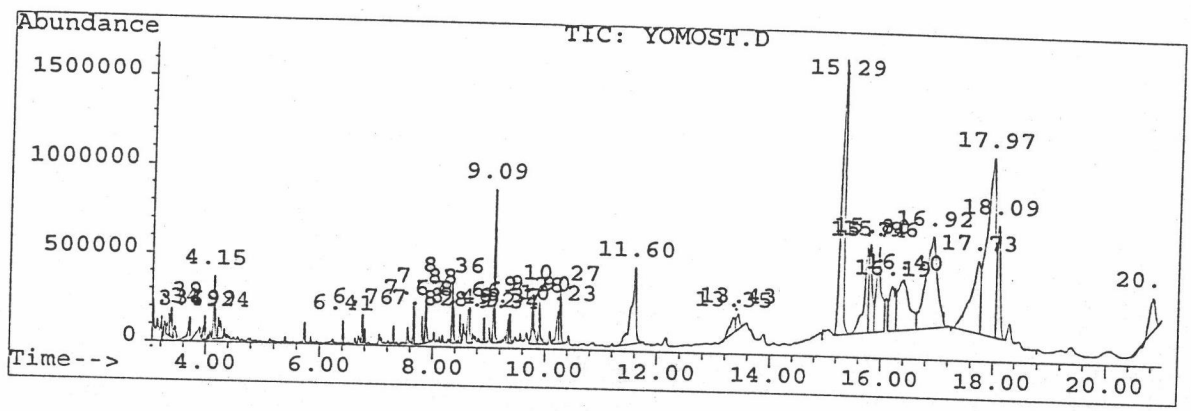


Figure 4.56 The mass spectrum for an extract of sample 2 with the retention time of 10.090 min from the gas chromatogram in Figure 4.54
(A) scan
(B) Library searched

Library Searched : C:\DATABASE\JO.L
Quality : 90
ID : 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) est
er

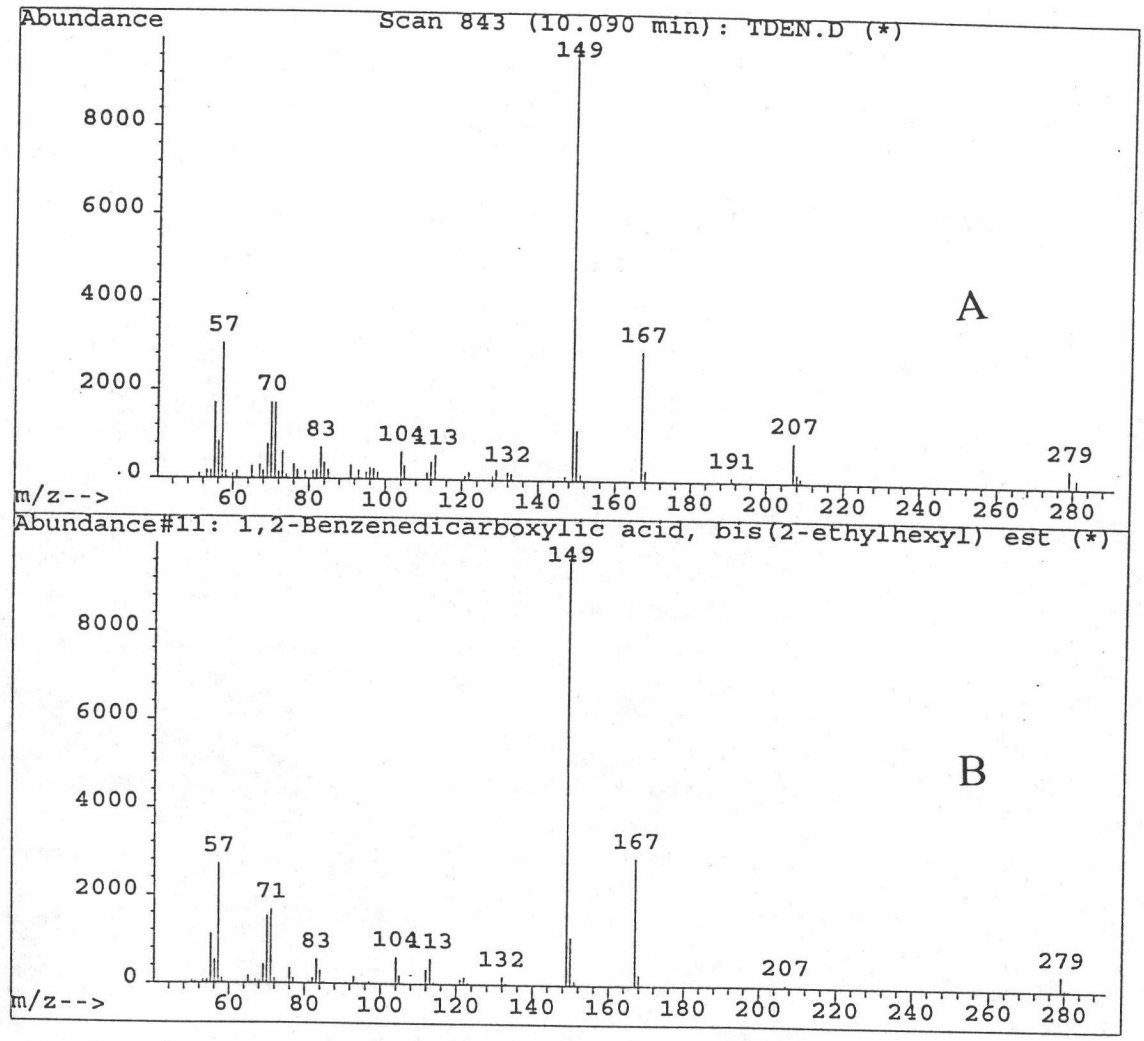


Figure 4.57 The gas chromatogram with MSD detection for an extract of a sample 3 (Yomost)
GC/MSD condition : described in Table 3.3

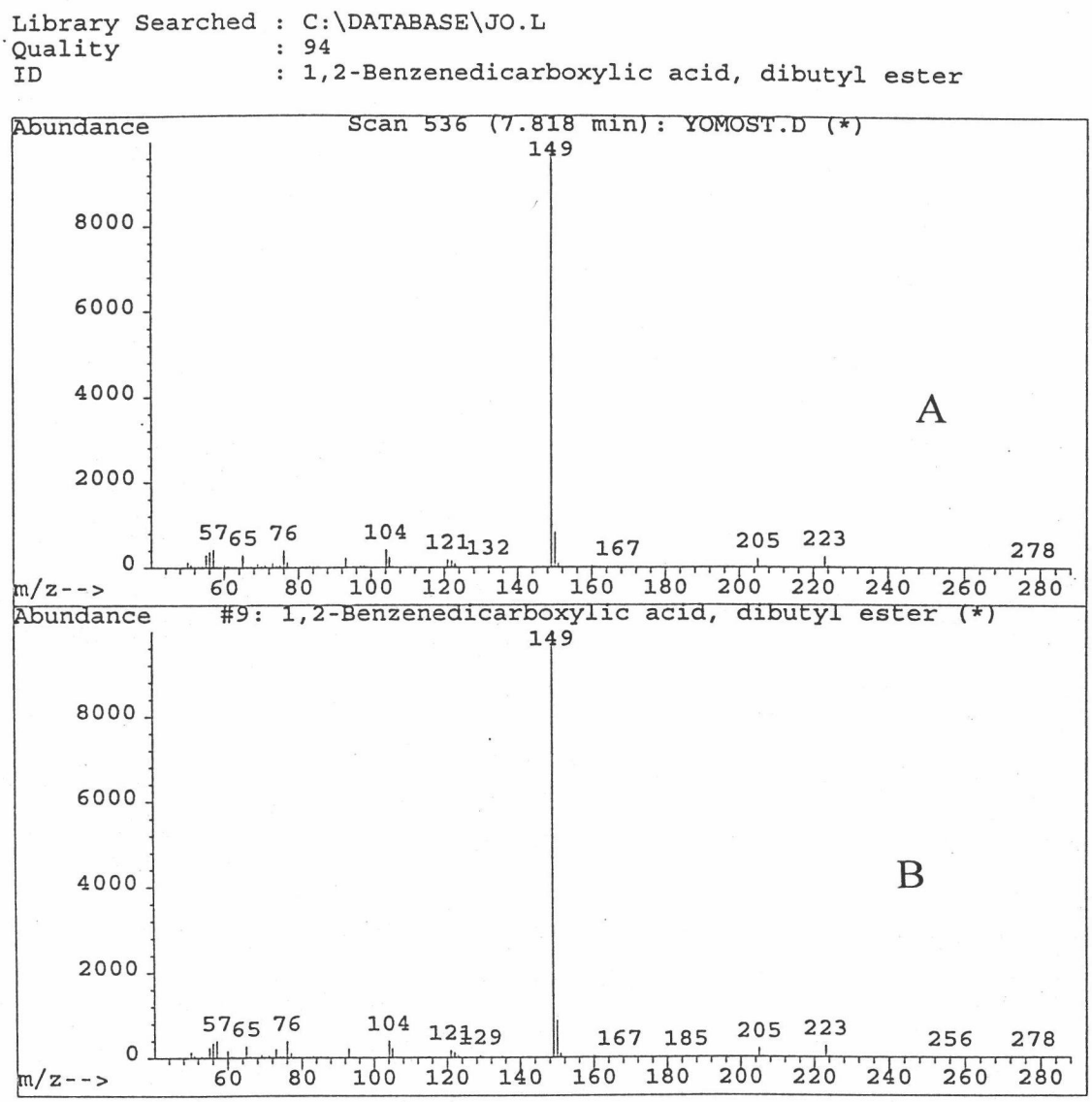


Figure 4.58 The mass spectrum for an extract of sample 3 with the retention time of 7.818 min from the gas chromatogram in Figure 4.57
(A) scan
(B) Library searched

Library Searched : C:\DATABASE\JO.L
Quality : 76
ID : 1,2-Benzenedicarboxylic acid, butyl phenylmethyl es ter

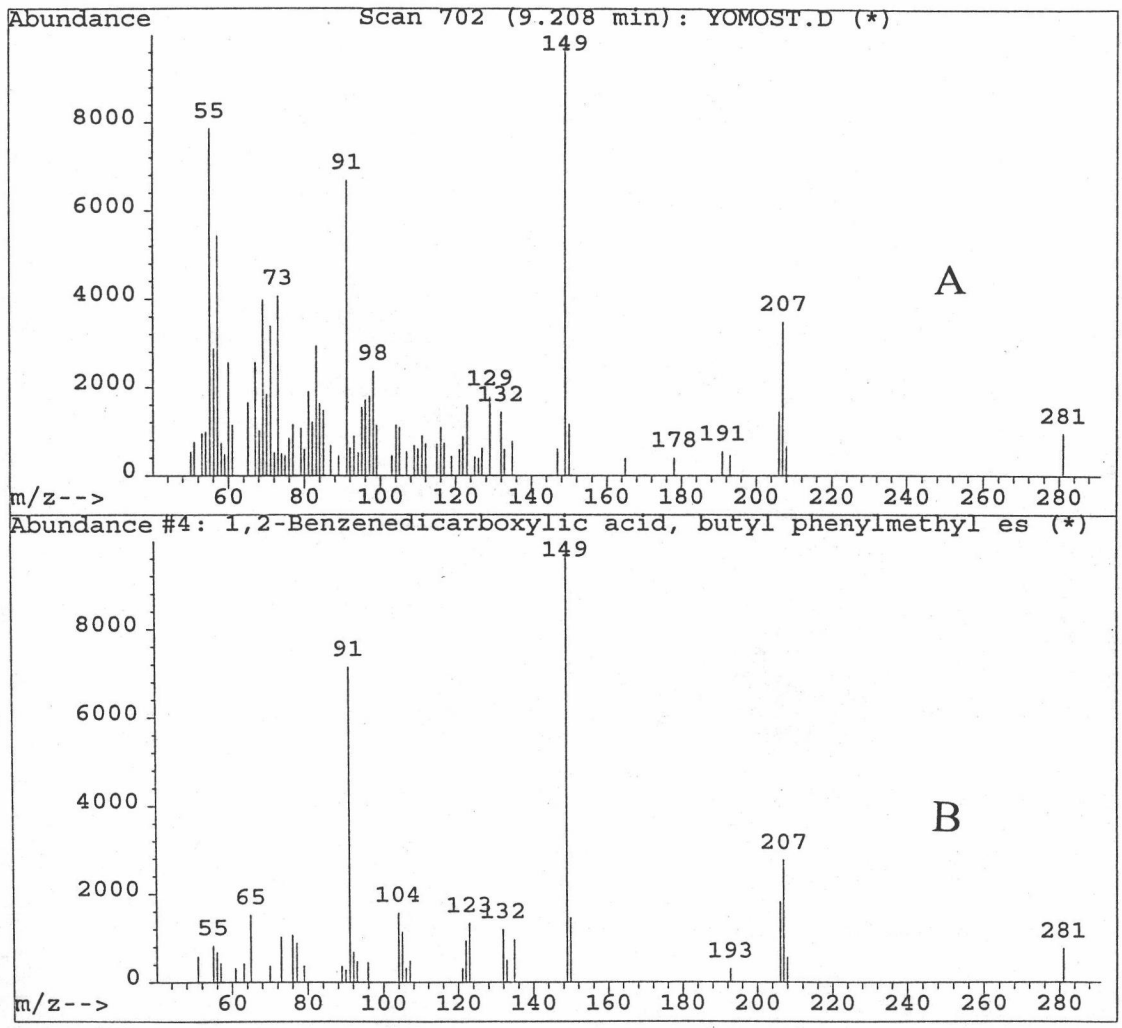


Figure 4.59 The mass spectrum for an extract of sample 3 with the retention time of 9.208 min from the gas chromatogram in Figure 4.57
(A) scan
(B) Library searched

Library Searched : C:\DATABASE\JO.L
Quality : 90
ID : 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) est
er

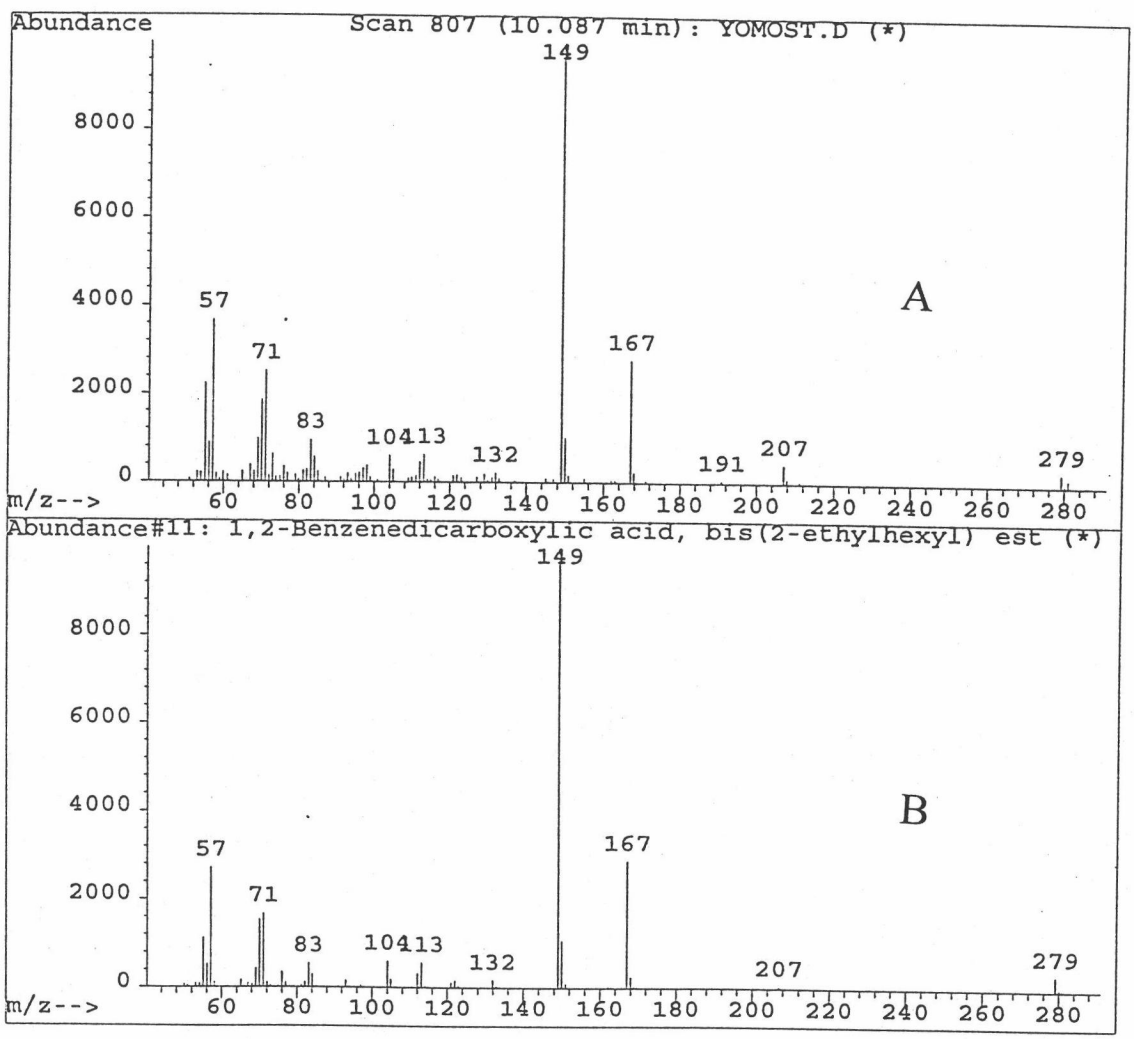


Figure 4.60 The mass spectrum for an extract of sample 3 with the retention time of 10.087 min from the gas chromatogram in Figure 4.57
(A) scan
(B) Library searched

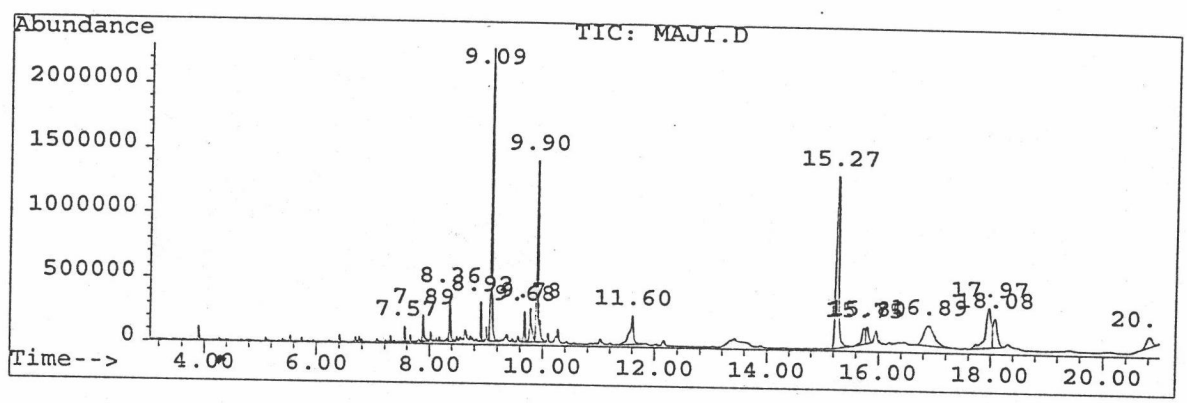


Figure 4.61 The gas chromatogram with MSD detection for an extract of a sample 4 (Meiji)
GC/MSD condition : described in Table 3.3

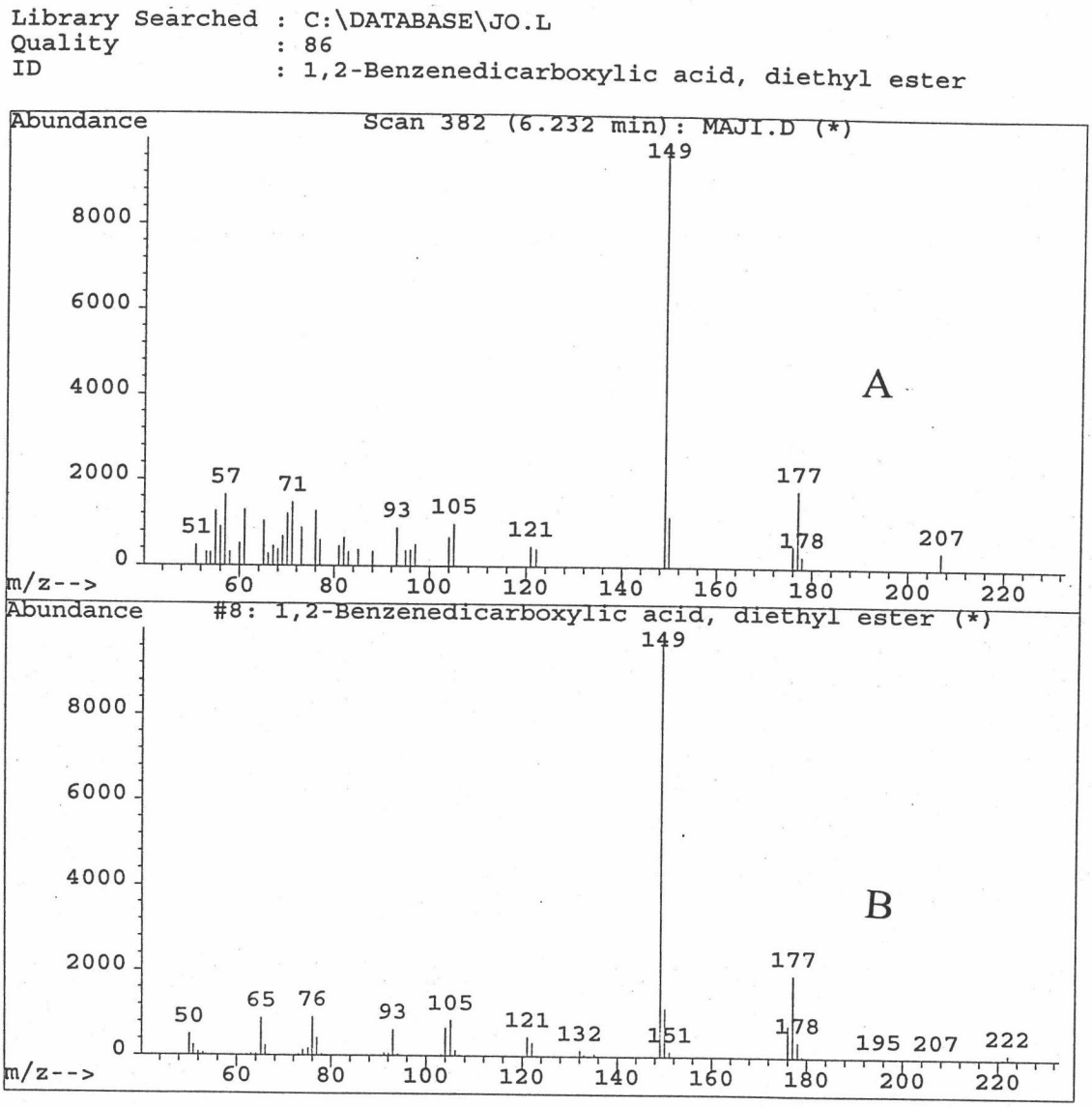


Figure 4.62 The mass spectrum for an extract of sample 4 with the retention time of 6.232 min from the gas chromatogram in Figure 4.61
(A) scan
(B) Library searched

```
Library Searched : C:\DATABASE\JO.L
Quality          : 90
ID               : 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) est
                   er
```

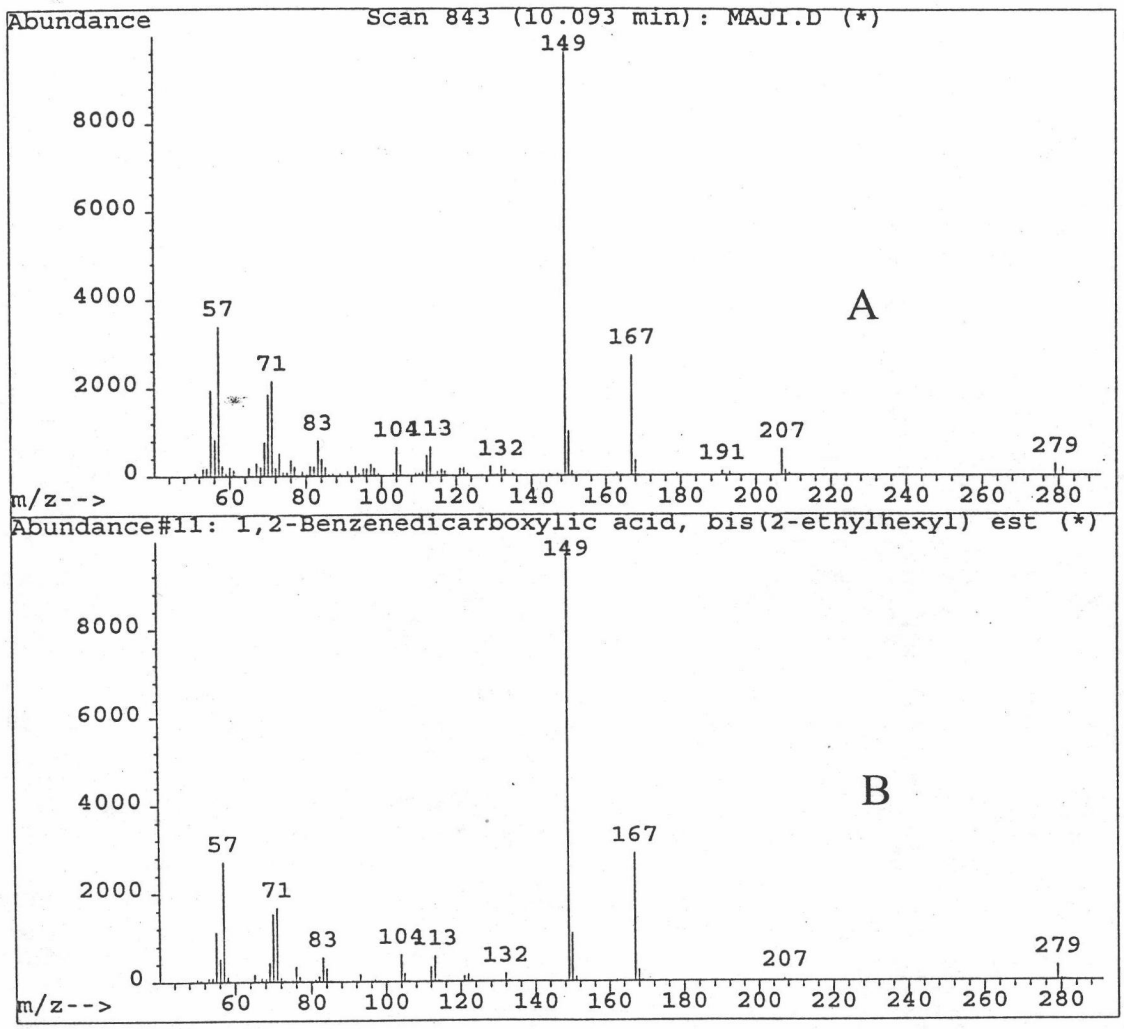


Figure 4.63 The mass spectrum for an extract of sample 4 with the retention time of 10.093 min from the gas chromatogram in Figure 4.61
(A) scan
(B) Library searched

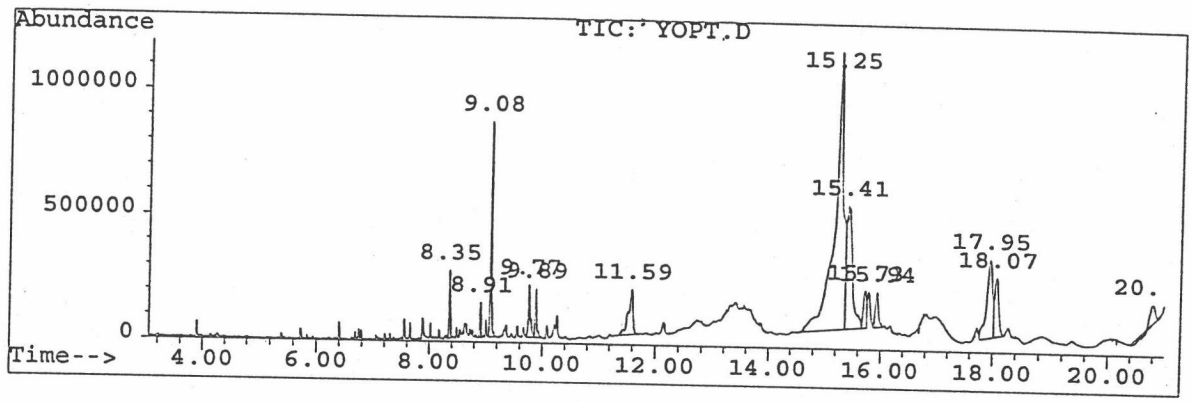


Figure 4.64 The gas chromatogram with MSD detection for an extract of a sample 5 (Yopalt)
GC/MSD condition : described in Table 3.3

Library Searched : C:\DATABASE\JO.L
Quality : 90
ID : 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) est
er

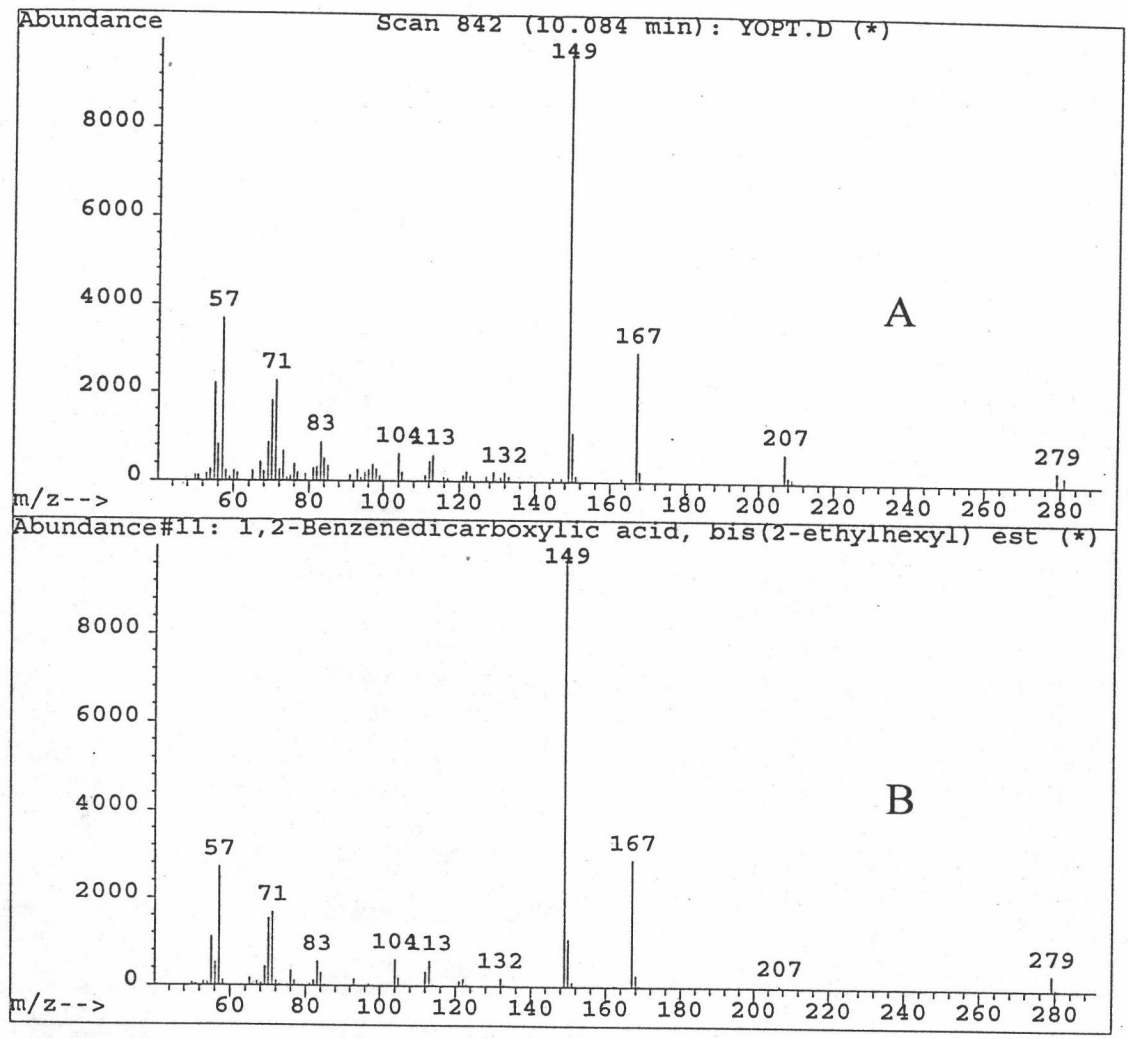


Figure 4.65 The mass spectrum for an extract of sample 5 with the retention time of 10.084 min from the gas chromatogram in Figure 4.64
(A) scan
(B) Library searched

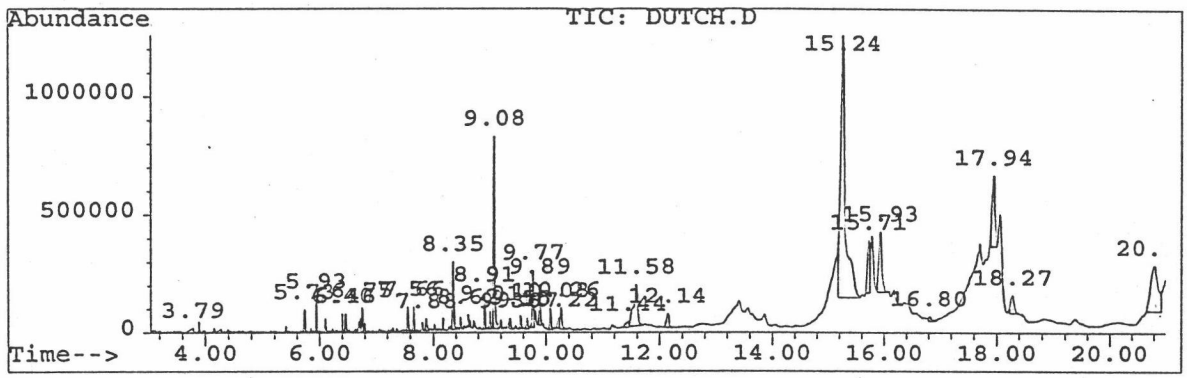


Figure 4.66 The gas chromatogram with MSD detection for an extract of a sample 6 (Dutchmill)
GC/MSD condition : described in Table 3.3

Library Searched : C:\DATABASE\JO.L
Quality : 86
ID : 1,2-Benzenedicarboxylic acid, dibutyl ester

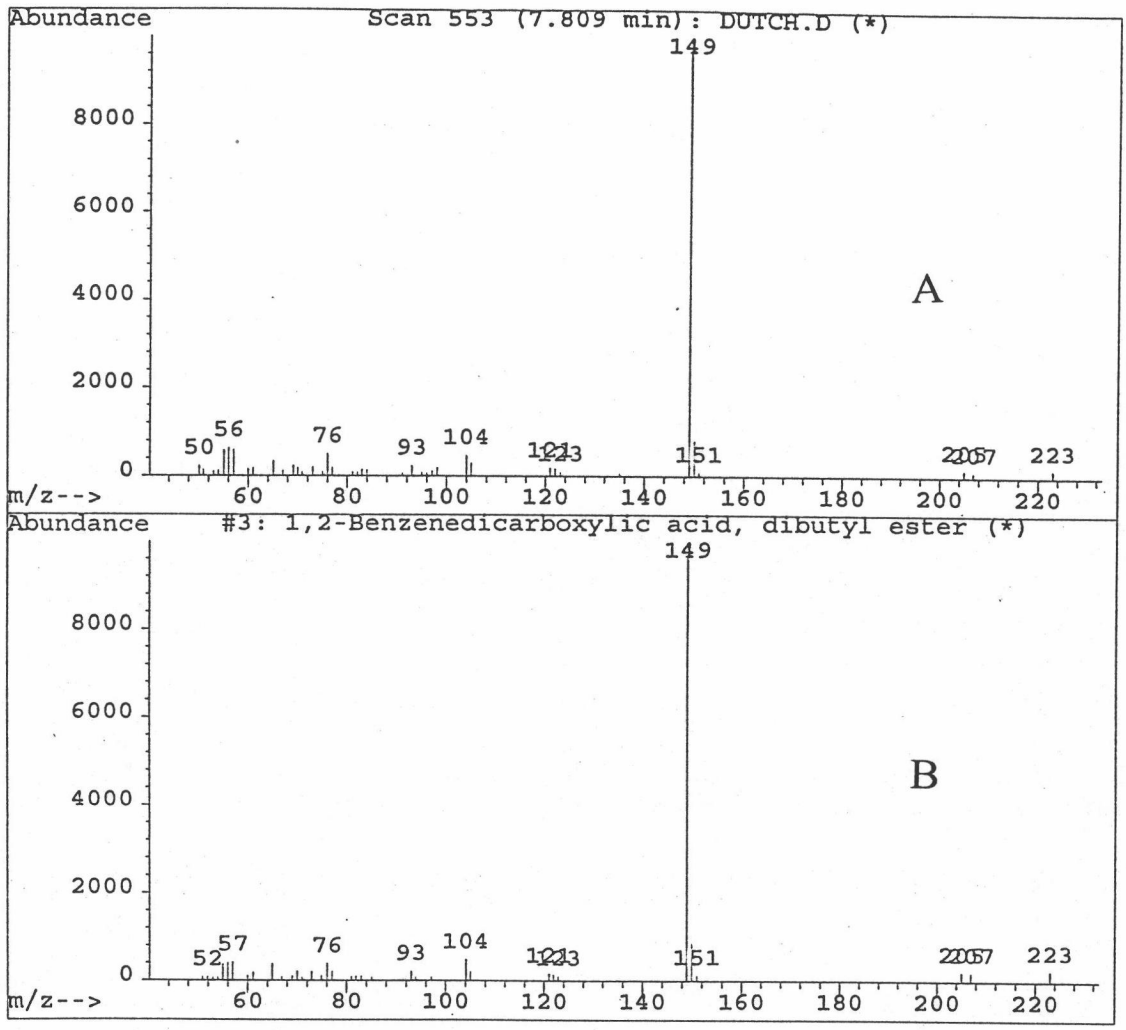


Figure 4.67 The mass spectrum for an extract of sample 6 with the retention time of 7.809 min from the gas chromatogram in Figure 4.66
(A) scan
(B) Library searched

Library Searched : C:\DATABASE\JO.L
Quality : 87
ID : 1,2-Benzenedicarboxylic acid, butyl phenylmethyl es ter

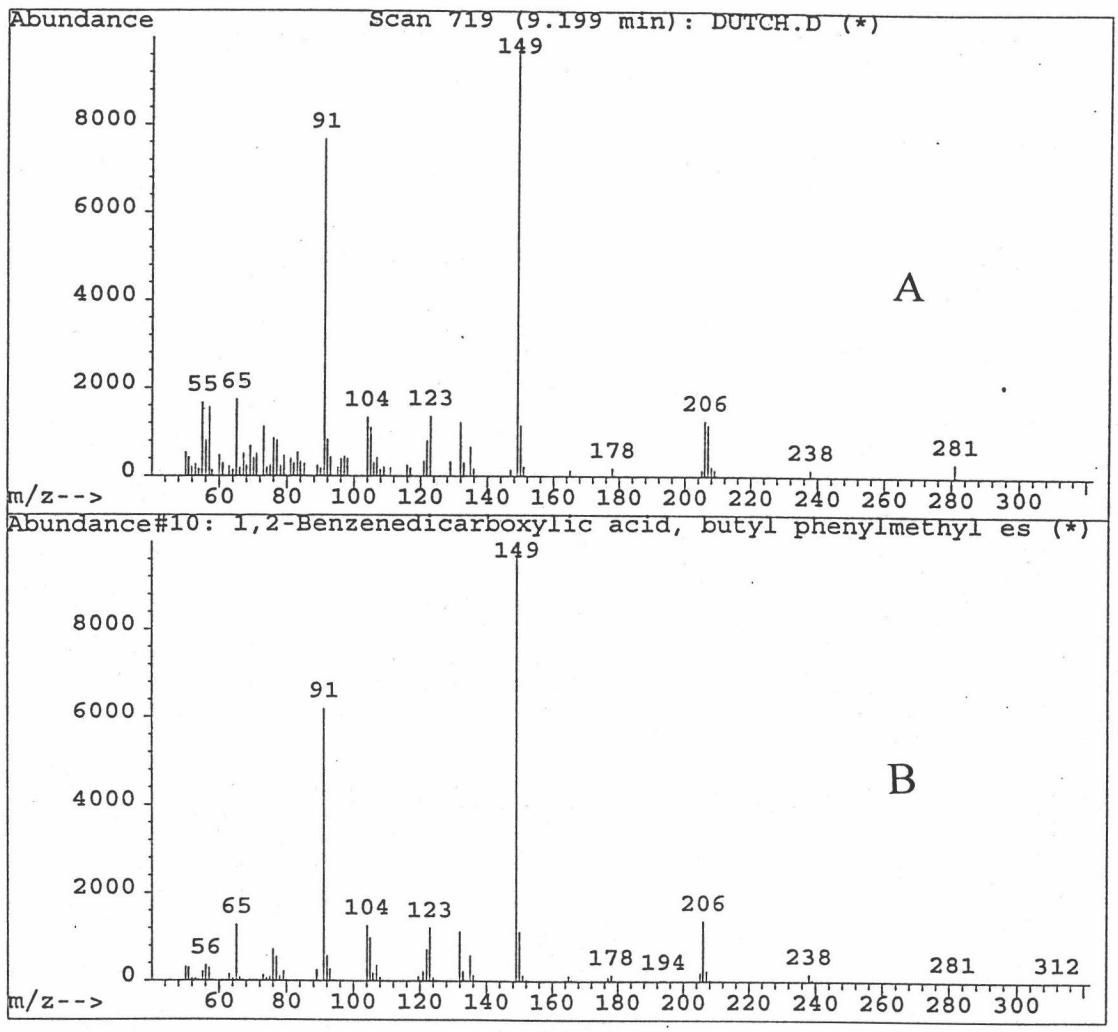


Figure 4.68 The mass spectrum for an extract of sample 6 with the retention time of 9.199 min from the gas chromatogram in Figure 4.66
(A) scan
(B) Library searched

Library Searched : C:\DATABASE\JO.L
Quality : 90
ID : 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) est
er

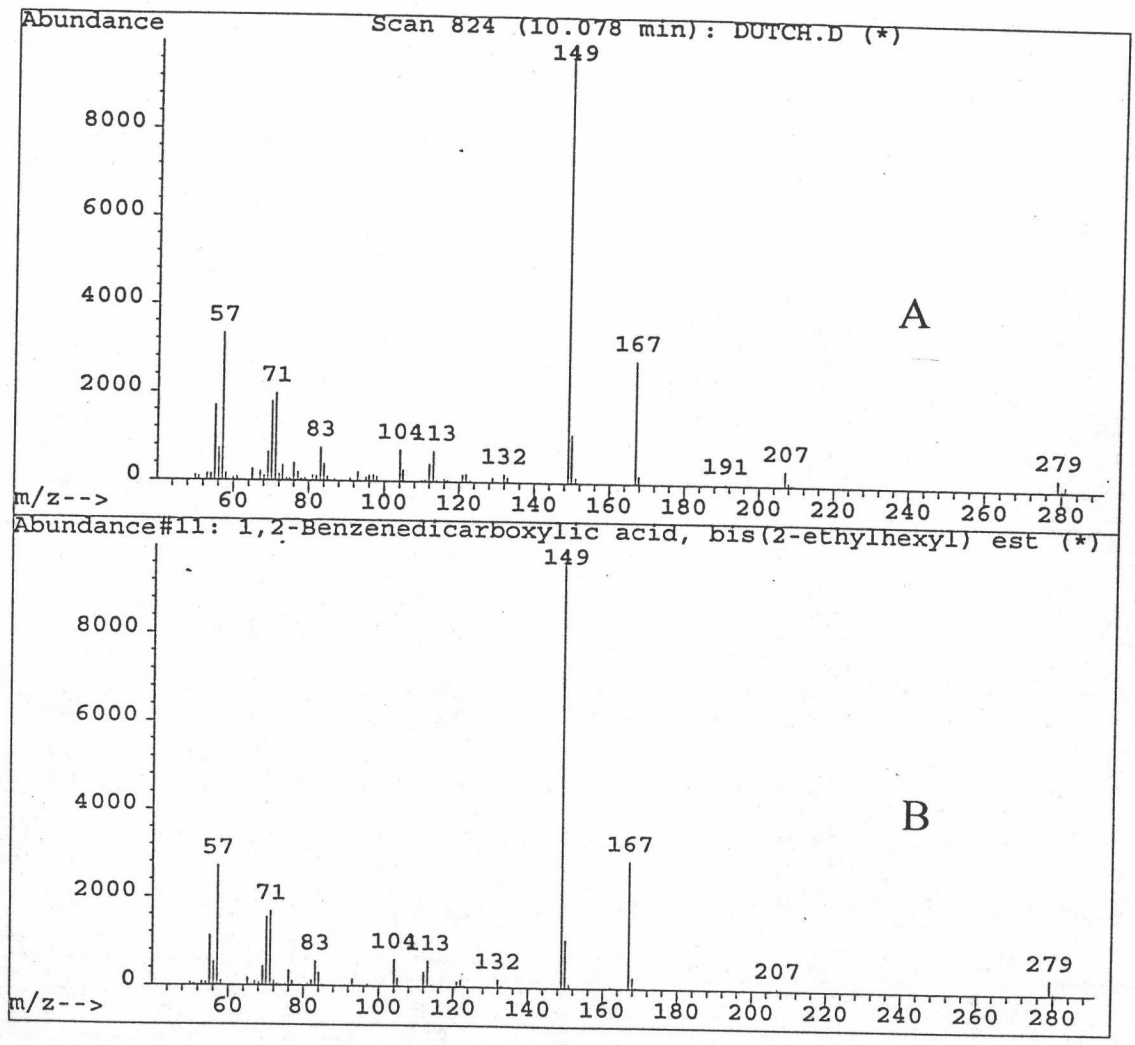


Figure 4.69 The mass spectrum for an extract of sample 6 with the retention time of 10.078 min from the gas chromatogram in Figure 4.66
(A) scan
(B) Library searched