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Note

Analysis of fatty acids in methyl ester form by using relative retention times obtained from Gas Chromatography techniques

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ABSTRACT

Fatty acid methyl esters were separated by high resolution gas chromatography on a DB－WAX fused silica capillary column, coated with polyethylene glycol and analyzed under conditions of linear temperature programming. Retention index values were calculated by using fatty acid methyl esters as standards and tabu1ated. Excellent reproducibility of the index values were obtained.

It was found that this method was very useful, convenient and not time consuming in qualitative analysis of various kinds of fatty acid of fats and oils.

Introduction

As a part of the purpose on the analysis of fats and oils, it was wanted to investigate the multivariate optimization of various types of lipid analyses. Fats and oils are tri－ester of glycerol with many kinds of fatty acids. In many cases, determination of fatty acid components are necessary to classify in the Customs Tariff Schedule.

Gas Chromatography has been an important aid in the analysis of fatty acid in fats and oils and other lipids, particularly analysis of fatty acid methyl esters. Identification using GC has been limited to those compounds for which the analyst has a reference compound to run for confirmation. Thus, this will be useful when the necessary standards are not available.

The objective of this particular study was to use this method in the analysis of fatty acid methyl esters from fats and oils by Gas Chromatography.

Gas Chromatography：

The analysis of the fatty acid methyl esters was on a Shimadzu Model GC－15A Gas Chromato－graphy, configured for capillary Chromatography with a capillary injection port using split mode and a flame ionization detector connected to a Chromatopac C－R 4 A data processor.

A DB－WAX fused silica capillalry column (3m ×0.25mmI.D.) coated with polyethylene glycol with film thickness 0.25μm. (J&W Scientific) was used. The carrier gas (Helium) flow rate is 0.5ml／min. Inject at 60℃, raise the oven temperature to 220℃at a rate of 2℃／min and keep the column at this temperature for 60min. The temperature of the detector and injection port were 250℃.

Experimentation

All materials, obtained from the Wako Pure Chemical Industries Ltd. and the Tokyo Kasei Co., were used and some of fatty acid methyl esters were prepared from fatty acids by using sulfuric―methanol method.

Conversion of fatty acids into fatty acid methyl esters：

A1g amount of fat sample is weighted into an esterification flask and 60ml of sulfuric acid―benzene―methanol solution (add 2ml of concentrated sulfuric acid to 230ml of 1:3 (v／v) benzene―methanol mixture.) are added to dissolved the sample. Heat the mixture with a reflux condenser for 2.5hr. then, cool and tansfer it to a separatory funnel and add 100ml of water. The mixture extracted twice with 50ml of petroleum ether. The combined extract is washed by extraction with water, by using 20ml of water each time, until water does not show acidity as detected by methyl orange. Dehydrate the petroleum ether solution with anhydrous sodium sulfate, filter and evaporate the solvent.

Results and discussion：

The Kovats retention index system has generally been accepted as the best way to characterize the retention behavior of chromatographically separated compounds. In order to facilitate the analytical application of GC to the investigation of samples with wide boiling point range, temperature programmed GC is one of the most important techniques and many GC analyses are carried out under this conditions.

According to definition, Kovats indices are determined at constant temperature. However, it is impossible to determine the index values of all fatty acid methyl esters in a single run at a certain constant temperature, because of the large differences in their retention times. Thus, index values were determined by using linear temperature programme.

The effect of temperature on retention was recognized in gas chromatography and increasing the temperature during the elution sequence was soon exploited in the examination of samples with wide ranges of boiling points. With linearly increasing column temperature, a nearly arithmetic rather than a logarithmic relationship.

The compounds examined in this study and their respective retention indices are presented in Table1. The chromatogram of standard fatty acid methyl esters from $C_4H_9CO_2CH_3$ and $C_{25}H_{51}CO_2CH_3$, using internal standard fatty acid methyl esters, $C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$, are showed in Fig. 1. In Table 2―Table 3, the relative retention time of fatty acid compositions of standard samples are given and Fig. 2―Fig. 15 show the actual separation of standard fat samples, run with the optimized method.

Mean retention times index values and standard deviations were calculated from five measurements. From the chromatogram, good separation of fatty acid methyl esters from methyl pentanoate to methyl hexacosanoate were obtained. Calculation of relative retention tiems from each retention time by use of this simple linear index scheme.

$$\text{Relative retention time} = \left(\frac{T_{sample} - T_{C7}}{T_{C21} - T_{C7}}\right) \times 100$$

Where T_{sample} is the retention time of the substance for which the retention index is to be determined, $T_{c7}$and $T_{c21}$are the retention times for the fatty acid methyl ester standards which bracket the substances of interest.

As the results show, the good characterization were obtained with low percentage differ from standards.

Reproducibility

Reproduced the retention indices by use of Hewlett Packard 5890 Series II and HP 3396 Series II Integrator. Column used was PEG (DB−WAX) 15 m × 0.53 mmI.D. and analyzed under the same conditions.

It was found that, retention time indices could be reproduced from hexanoic acid methyl ester to tetracosanoic acid methyl ester. As the chromatogram and results showed. in Table

4 and Fig. 16.

In Table 5, the relatuie retention time of fatty acid compositions of standard samples are given.

Conclusion

The use of Kovats retention indices has yielded a great advantage over relative retention times, that they are dependent of the film thickness, column dimensions and phase ratio. Owing to the advantages in column technology, highly reproducible column are available for some polar stationary phase.

This retention index is considered to be convenient and reliable to identify fatty acids contained in complex mixtures and this procedure also can be easily used for identification in linear temperature programme capillary gas chromatography and provide useful guides when necessary standards are not available.

Acknowledgement

The authors would like to express deeply gratitude to Mr.Uehama Takaichi and Mr.Kuwata Shin−ichiro for access to laboratory facilities and encouragement during the course of the work. Grateful acknowle−dgements also go to Central Customs Laboratory and Thai Customs Department.

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Table　1　Retention Indices of fatty acid methyl esters (Internal standards：$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

Samples	R. T	R. R. T	R. T	R. R. T	R. T	R. R. T	R. T	R. R. T	R. T	R. R. T	$\overline{R. T}$	$\overline{R. R. T}$	S. D
C_5	3.786	-6.855	3.791	-6.849	3.799	-6.853	3.803	-6.849	3.790	-6.818	3.797	-6.840	0.006
C_6	5.617	-4.183	5.624	-4.187	5.635	-4.183	5.637	-4.180	5.623	-4.171	5.627	-4.185	0.008
C_7	8.487	0.000	8.508	0.000	8.523	0.000	8.525	0.000	8.530	0.000	8.511	0.000	0.013
C_8	12.500	5.901	12.509	5.898	12.585	5.907	12.588	5.903	12.560	5.887	12.576	5.899	0.018
C_9	17.530	13.119	17.538	13.111	17.580	13.130	17.578	13.130	17.526	13.102	17.552	13.120	0.026
C_{10}	23.030	21.112	23.043	21.104	23.090	21.137	23.086	21.130	23.051	21.098	23.060	21.117	0.029
C_{11}	28.750	29.415	28.750	29.404	28.810	29.431	28.805	29.443	28.781	29.406	28.785	29.420	0.032
$C_{10(1)}$	29.154	30.002	29.158	29.998	29.218	30.023	29.210	30.035	29.187	29.998	29.192	30.011	0.030
$C_{11(10)}$	31.611	33.570	31.626	33.567	31.675	33.588	31.684	33.580	31.644	33.557	31.648	33.574	0.031
C_{12}	34.448	37.680	34.458	37.680	34.518	37.712	34.508	37.683	34.487	37.650	34.480	37.683	0.031
$C_{12(1)}$	34.830	38.276	34.836	38.272	34.896	38.304	34.975	38.284	34.808	38.303	34.852	38.288	0.026
C_{13}	39.980	45.681	39.977	45.692	40.023	45.689	40.029	45.679	39.988	45.632	39.990	45.681	0.030
$C_{13(1)}$	40.548	46.547	40.578	46.532	40.620	46.565	40.620	46.550	40.584	46.518	40.589	46.549	0.031
C_{14}	45.346	53.518	45.368	53.520	45.419	53.527	45.421	53.514	45.388	53.498	45.389	53.513	0.032
$C_{13(1)}$	45.980	54.445	46.012	54.455	46.056	54.434	46.070	54.438	46.038	54.437	46.031	54.440	0.036
$C_{14(1)}$	47.125	56.102	47.150	56.122	47.204	56.116	47.218	56.120	47.185	56.080	47.178	56.110	0.037
C_{15}	50.477	60.970	50.512	60.969	50.557	60.981	50.580	60.987	50.530	60.940	50.527	60.980	0.034
$C_{14(1)}$	51.227	62.060	51.264	62.060	51.306	62.070	51.314	62.061	51.277	62.034	51.276	62.050	0.035
C_{16}	55.426	68.138	55.452	68.181	55.509	68.165	55.522	68.178	55.503	68.151	55.484	68.163	0.044
C_{17}	60.180	75.034	60.170	75.035	60.242	75.033	60.274	75.056	60.257	75.044	60.225	75.038	0.050
$C_{16(1)}$	61.010	76.268	61.036	76.259	61.093	76.256	61.121	76.285	61.097	76.262	61.071	76.270	0.046
C_{18}	64.739	81.670	64.758	81.644	64.812	81.691	64.855	81.700	64.843	81.693	64.795	81.674	0.050
$C_{18(1)}$	65.443	82.707	65.487	82.706	65.536	82.701	65.561	82.724	65.545	82.711	65.506	82.709	0.051
$C_{18(9,12)}$	67.401	85.550	67.430	85.557	67.490	85.548	67.515	85.550	67.496	85.543	67.457	85.550	0.048
C_{19}	69.226	88.201	69.212	88.140	69.226	88.202	69.254	88.226	69.288	88.254	69.280	88.204	0.072
$C_{18(9,12,15)}$	70.194	89.553	70.192	89.563	70.258	89.559	70.276	89.563	70.281	89.540	70.229	89.559	0.048
C_{20}	73.328	94.158	73.345	94.141	73.424	94.130	73.456	94.175	73.447	94.158	73.400	94.159	0.059
$C_{20(11)}$	73.962	95.108	74.016	95.118	74.079	95.105	74.087	95.105	74.069	95.088	74.053	95.107	0.051
$C_{20(11,14)}$	75.892	97.883	75.932	97.899	75.990	97.876	76.006	97.872	75.997	97.866	75.963	97.870	0.049
C_{21}	77.350	100.000	77.380	100.000	77.450	100.000	77.472	100.000	77.469	100.000	77.425	100.000	0.056
$C_{20(11,14,17)}$	77.784	100.630	77.809	100.608	77.886	100.631	77.898	100.618	77.893	100.600	77.856	100.628	0.047
$C_{20(5,8,11,14)}$	78.482	101.644	78.520	101.674	78.585	101.640	78.580	101.626	78.578	101.606	78.554	101.636	0.048
C_{22}	80.415	104.430	80.474	104.430	80.525	104.454	80.521	104.407	80.514	104.415	80.461	104.430	0.048
$C_{20(5,8,11,14,17)}$	81.432	105.938	81.460	105.934	81.562	105.951	81.501	105.974	81.601	105.984	81.526	105.952	0.060
C_{23}	82.077	109.985	82.130	109.996	82.187	109.992	82.225	109.991	82.211	109.978	82.169	109.984	0.061
$C_{22(13)}$	85.877	112.384	85.982	112.394	86.022	112.436	86.020	112.415	86.003	112.394	85.986	112.422	0.063
C_{24}	91.742	120.962	91.858	121.032	91.943	121.021	91.873	121.031	91.847	120.982	91.892	120.984	0.094
C_{28}	108.540	145.445	108.847	145.680	108.962	145.755	108.961	145.818	108.947	145.641	108.897	145.669	0.104

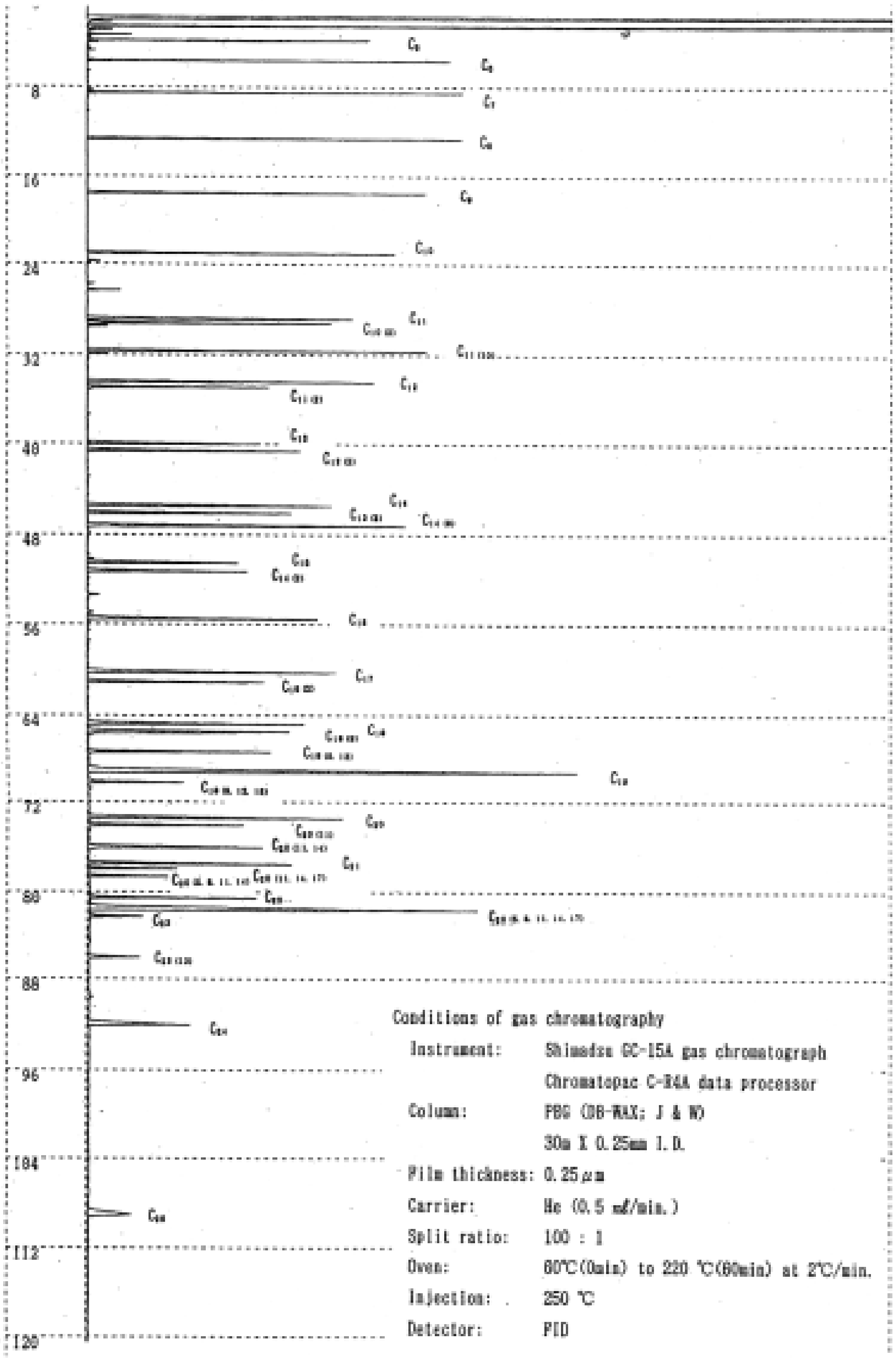

Fig. 1 : Chromatogram of standard fatty acid methyl esters from $C_4H_9CO_2CH_3$ to $C_{25}H_{5}1CO_2CH_3$
(Internal standards：$C_6H_{13}CO_2CH_3$and $C_{20}H_{41}CO_2CH_3$)

Table 2　Relative retention times of standard fat samples.(Internal standards：$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

Number of Carbon of Fatty Acids	R. R. T	Standard Samples (R. R. T)						
		COCONUT OIL	COTTON SEED OIL	PALM OIL	PEANUT OIL	LINSEED OIL	MALT EXTRACT *	RAPE SEED
C_5	-6.840							
C_6	-4.185	-4.262						
C_7	0.000							
C_8	5.899	5.876						
C_9	13.130							
C_{10}	21.117	21.122						
C_{11}	29.420							
$C_{10(1)}$	30.011							
$C_{11(1)}$	33.574							
C_{12}	37.683	38.106	37.934	37.882		38.124		
$C_{12(1)}$	38.368				38.320			
C_{13}	45.681							
$C_{13(1)}$	46.549							
C_{14}	53.513	53.856	53.370	53.346	53.560	53.520		
$C_{14(1)}$	54.446							
$C_{14(2)}$	58.110							
C_{15}	60.880							
$C_{15(1)}$	62.059							
C_{16}	68.163	68.199	68.332	68.592	68.080	68.088	68.075	68.051
C_{17}	75.038							
$C_{16(1)}$	76.270							
C_{18}	81.674	81.667	81.848	81.684	81.740	81.648	81.626	81.627
$C_{18(9)}$	82.709	82.729	82.950	83.190	82.730	82.769	82.575	82.685
$C_{18(9,12)}$	85.550	85.488	86.149	85.671	85.570	85.634	85.548	85.731
C_{19}	88.204							
$C_{18(9,12,15)}$	89.559	89.613	89.429	89.366		89.690	89.704	88.507
C_{20}	94.159			94.058	94.080			93.972
$C_{20(11)}$	95.107			95.127	95.160		95.155	95.185
$C_{20(11,14)}$	97.879							97.740
C_{21}	100.000							
$C_{20(11,14,17)}$	100.629							
$C_{20(5,8,11,14,17)}$	101.638							101.525
C_{22}	104.450							
$C_{22(4,7,10,13,16,19)}$	105.862				105.900			105.831
C_{23}	106.884							
$C_{22(13)}$	112.423							
C_{24}	120.984				121.050			
C_{26}	145.688							

Table 3　Relative retention times of standard fat samples.(Internal standards： $C_6H_{13}CO_2CH_3$ and $CO_{20}H_{41}CO_2CH_3$)

Number of Carbon of Fatty Acids	R. R. T	Standard Samples (R. R. T)						
		CAMELLIA OIL	CASTOR OIL	SESAME OIL	SOY BEAN OIL	BEEF TALLOW	LARD OIL	MILK FAT
C_4	-6.840							
C_6	-4.185			-4.131				-4.181
C_7	0.000							
C_8	5.669							5.651
C_9	13.130							
C_{10}	21.117							21.031
C_{11}	29.420							
$C_{10(9)}$	30.011							
$C_{11(10)}$	33.574				33.107			
C_{12}	37.683					37.833	37.815	37.562
$C_{12(9)}$	38.368							
C_{13}	45.681							45.387
$C_{13(9)}$	46.549							
C_{14}	53.513					53.398	53.333	53.527
$C_{13(9)}$	54.446							
$C_{14(9)}$	56.110					55.815	55.855	
C_{15}	60.690					60.739	60.653	60.698
$C_{14(9)}$	62.059			62.186				62.020
C_{16}	68.153	68.100	67.887	68.216	68.163	68.273	68.255	68.426
C_{17}	75.038					74.811	74.727	74.691
$C_{16(9)}$	76.270					78.117		
C_{18}	81.674	82.041	81.434	81.937	81.839	81.807	82.076	81.860
$C_{18(9)}$	82.709	83.356	82.513	83.150	82.939	82.908	83.097	82.788
$C_{18(9,12)}$	85.550	85.444	85.420	85.685	86.081	85.026	85.622	85.587
C_{19}	88.204							
$C_{18(9,12,15)}$	89.559		89.319		89.577		89.337	
C_{20}	94.159	94.322	93.977	93.038				
$C_{20(11)}$	95.107						94.939	
$C_{20(11,14)}$	97.879						97.695	
C_{21}	100.000							
$C_{20(11,14,17)}$	100.639							
$C_{20(8,11,14)}$	101.638							
C_{22}	104.450							
$C_{20(5,8,11,14,17)}$	105.952	106.758			105.795			
C_{23}	106.884							
$C_{22(13)}$	112.493							
C_{24}	120.994	122.479	120.539					
C_{26}	145.669		142.558					

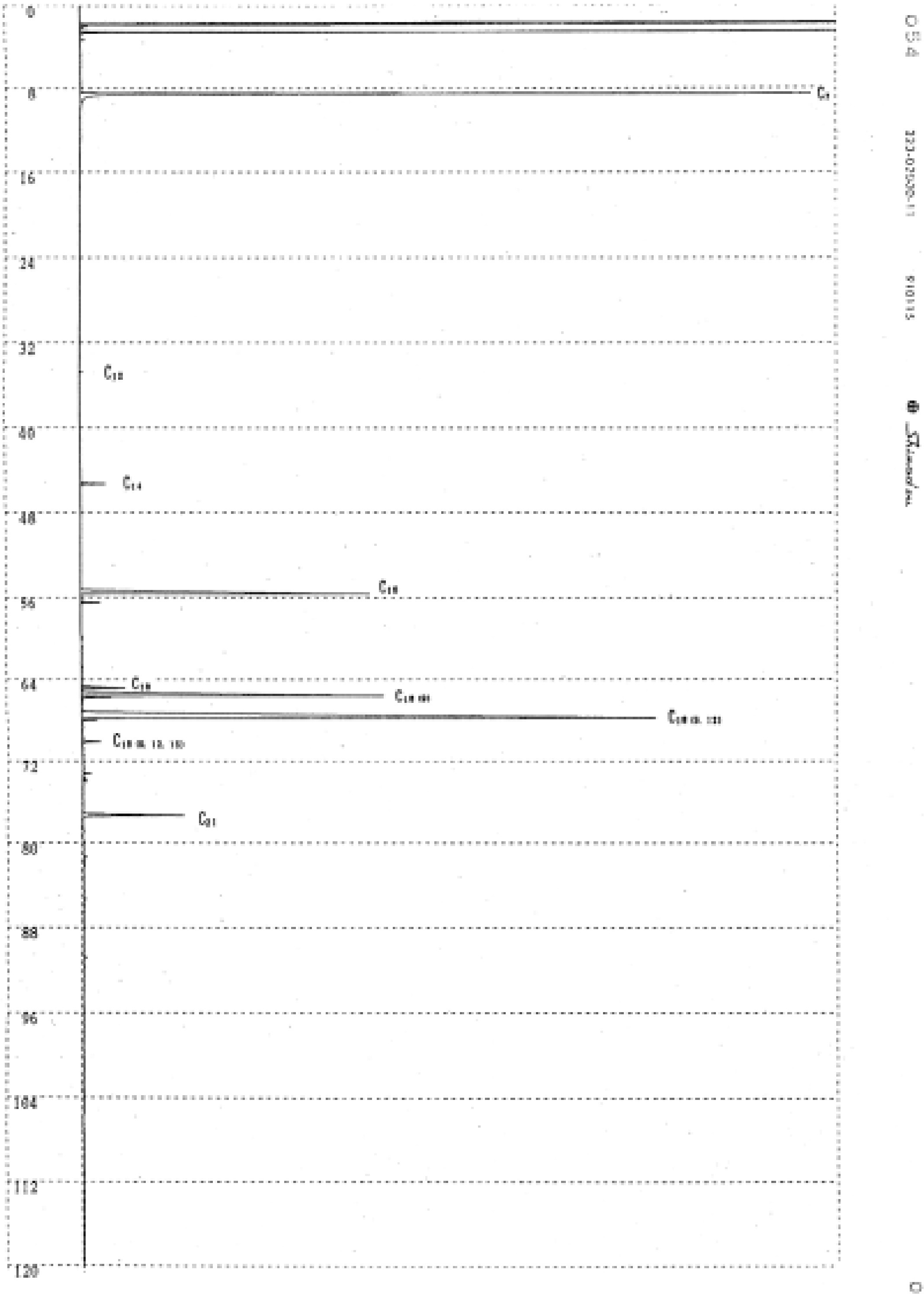

Fig. 2　Chromatogram of standard sample of coconut oil (Internal standards： $C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

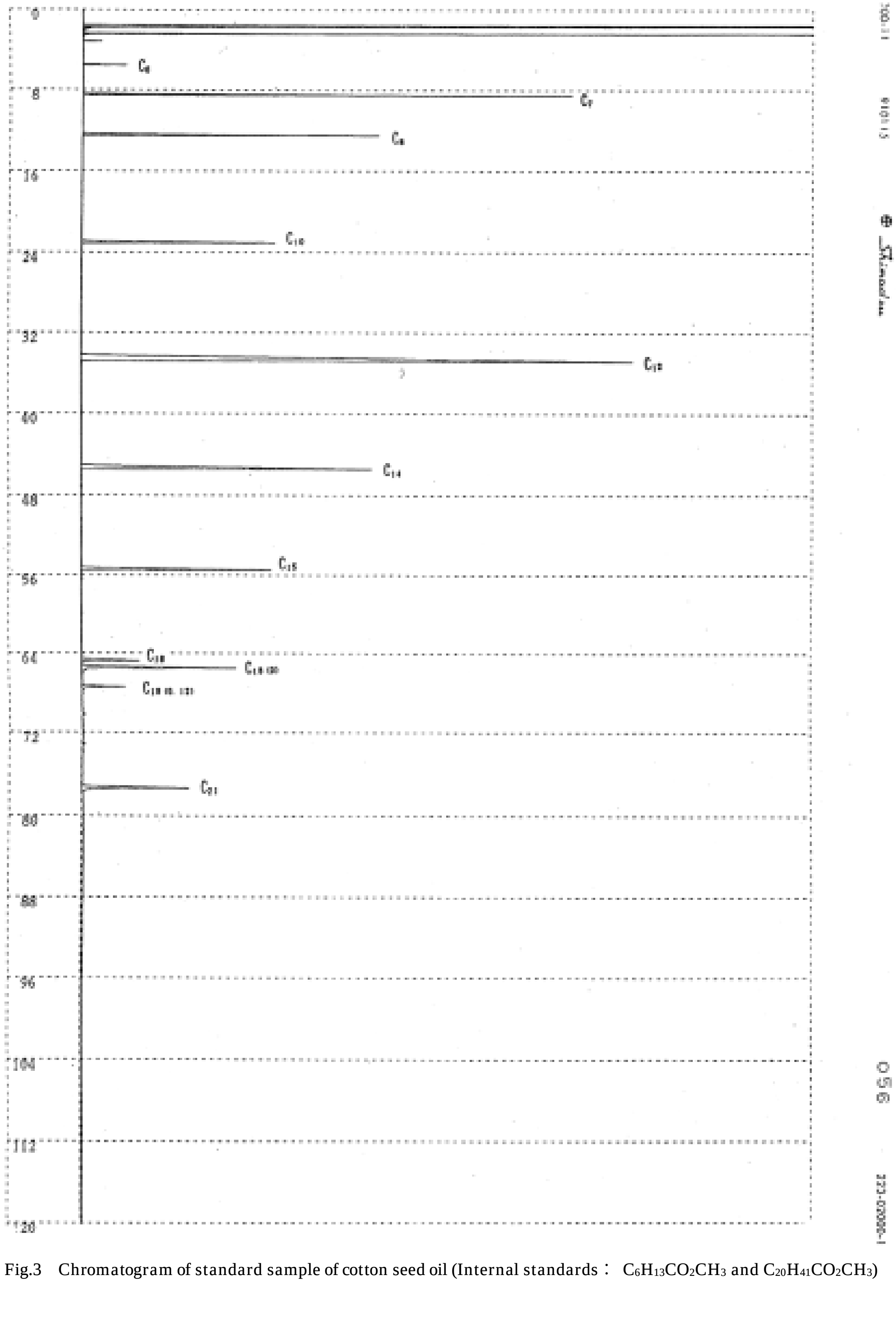

Fig.3　Chromatogram of standard sample of cotton seed oil (Internal standards：$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

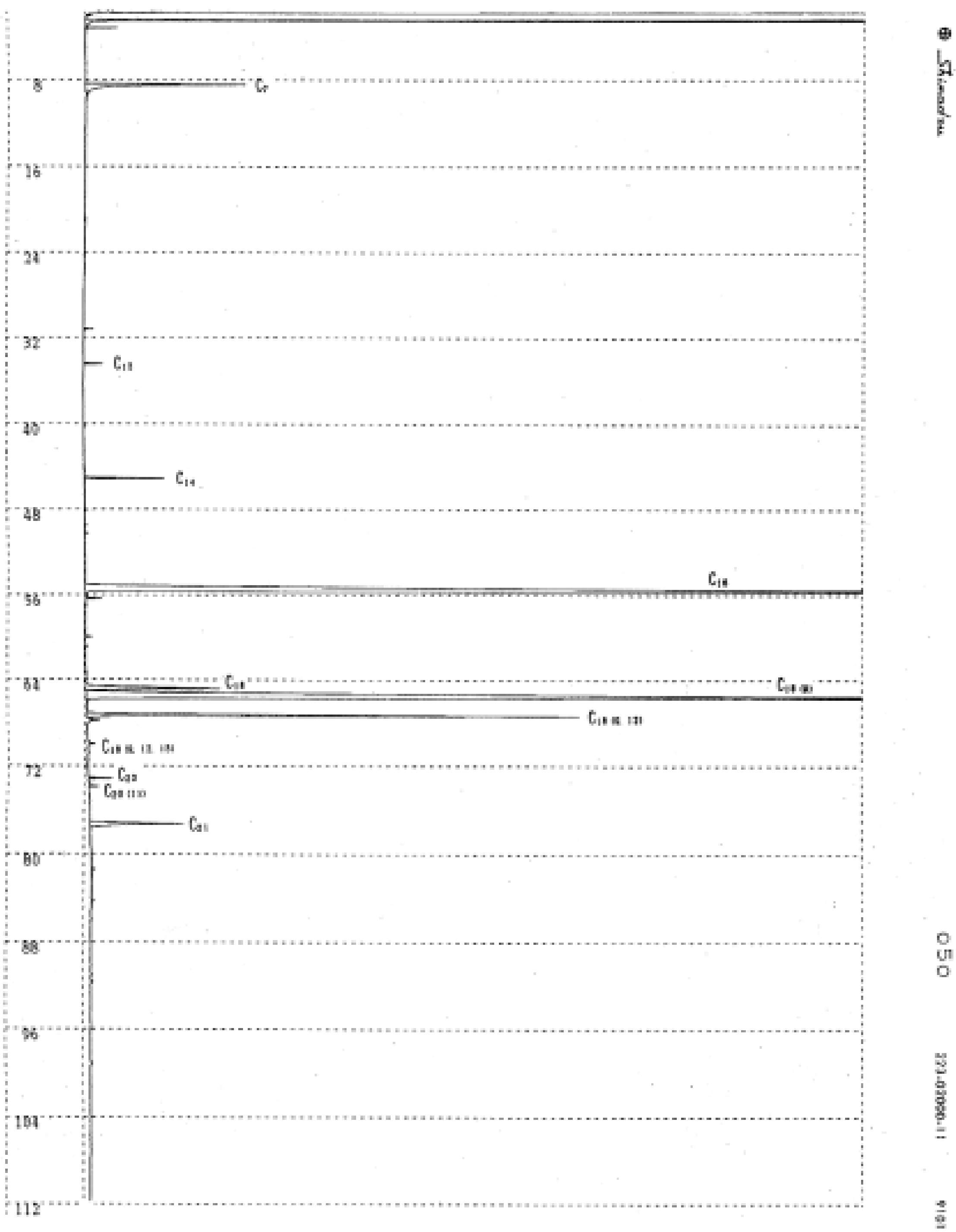

Fig. 4　Chromatogram of standard sample of palm oil (Internal standards：　$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

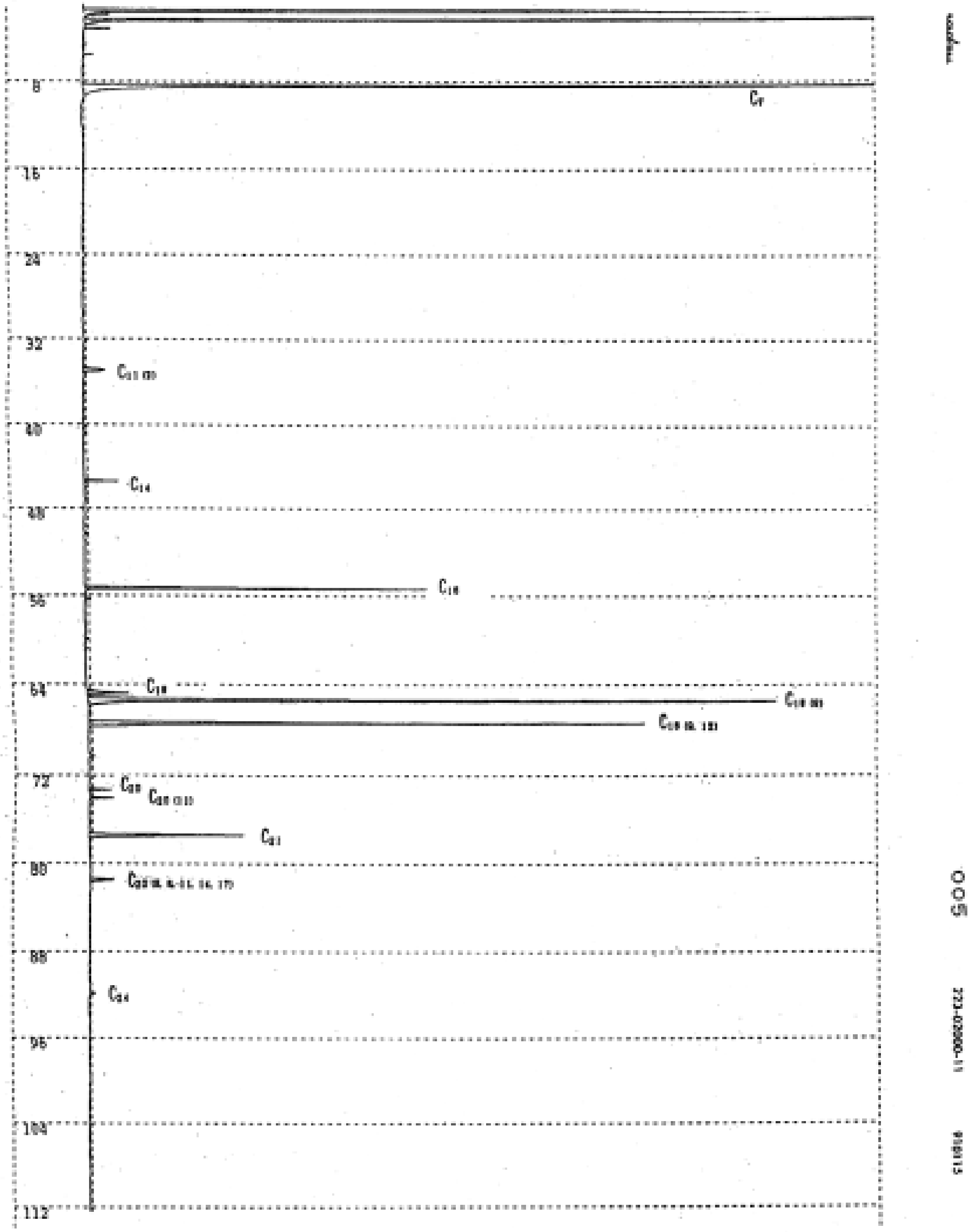

Fig. 5　Chromatogram of standard sample of peanut oil (Internal standards：$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

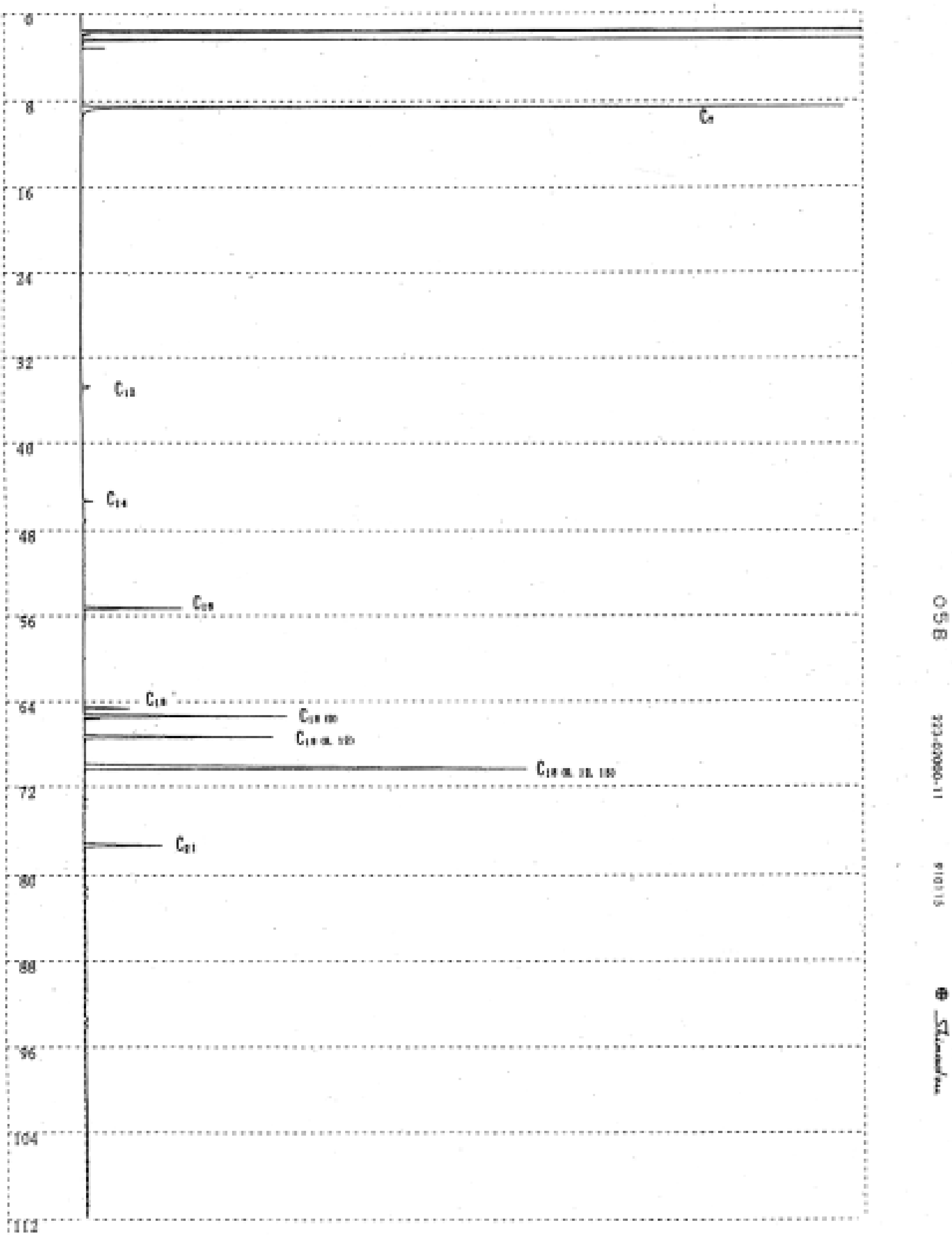

Fig. 6 Chromatogram of standard sample of linseed oil (Internal standards：$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

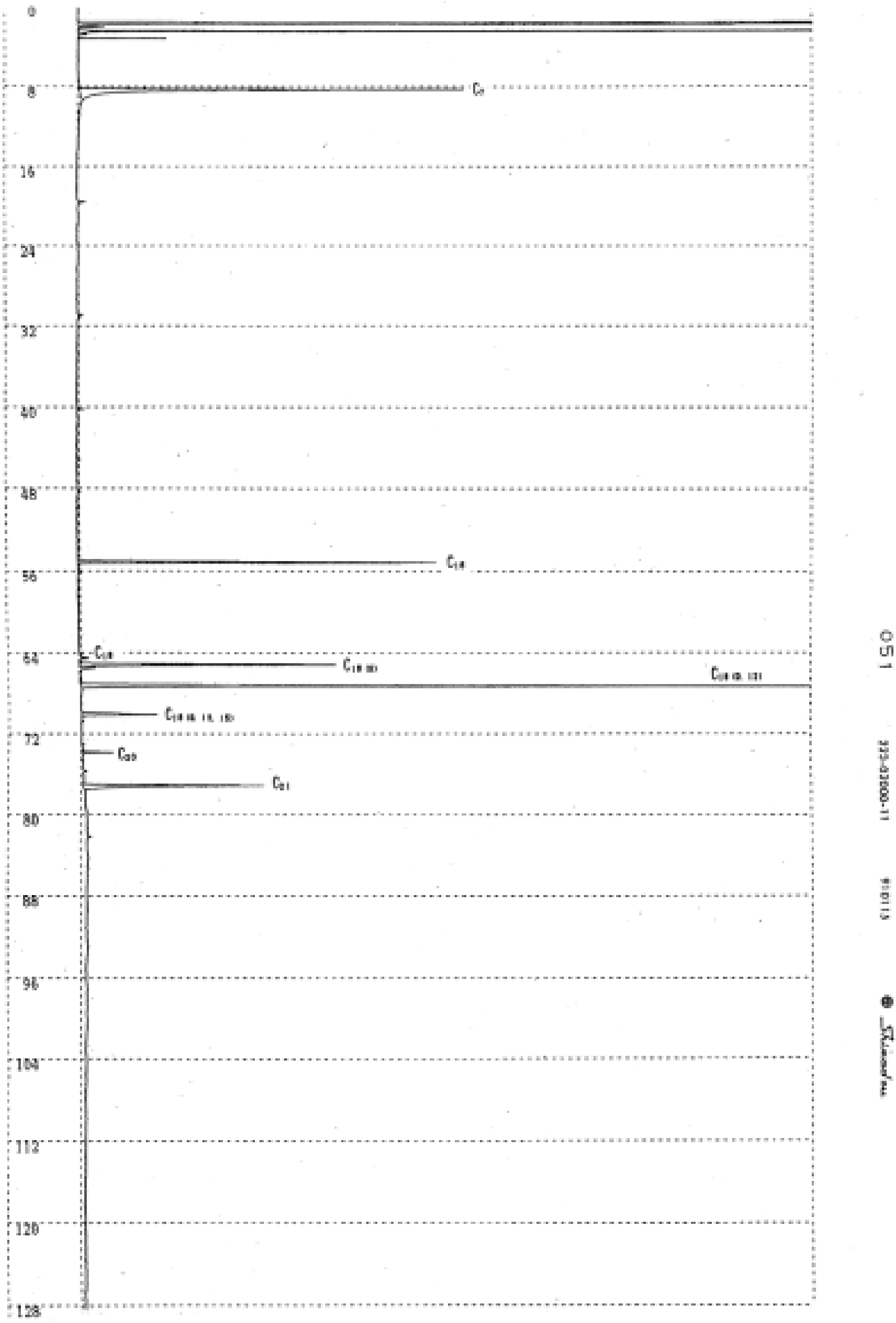

Fig. 7　Chromatogram of standard sample of malt extract oil (Internal standards:$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

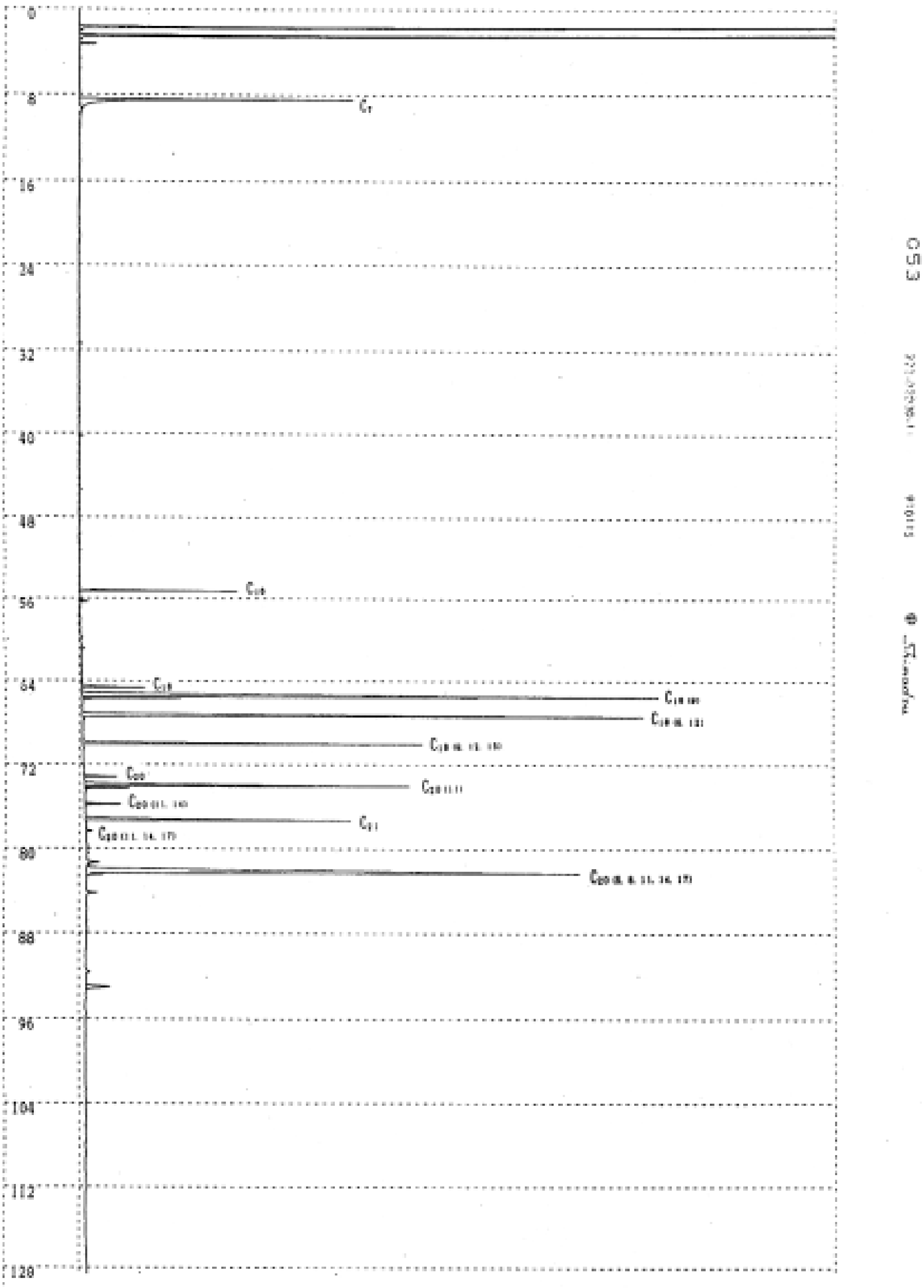

Fig. 8　Chromatogram of standard sample of rape seed oil (Internal standards:$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

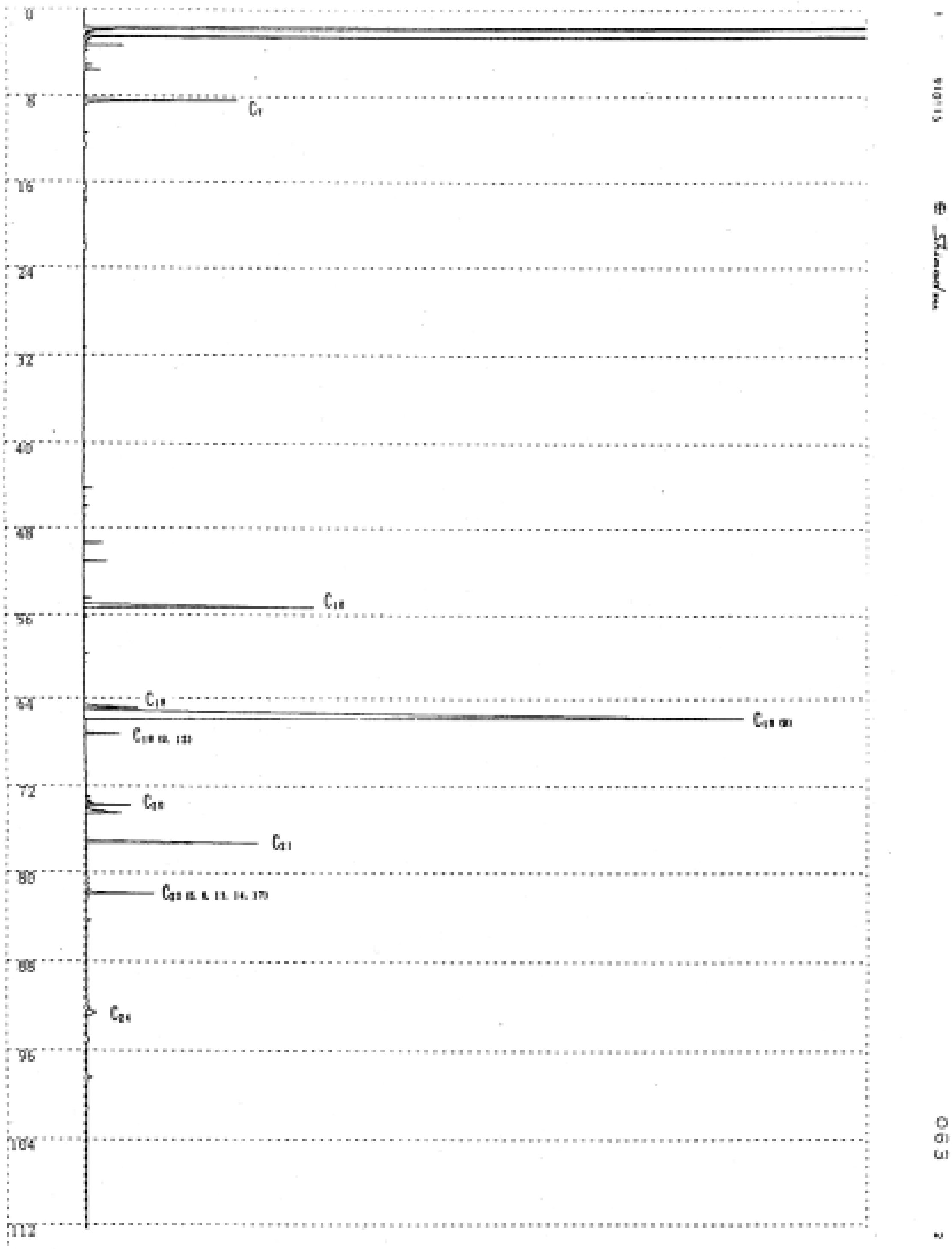

Fig. 9　Chromatogram of standard sample of camellia oil (Internal standards：$C_6H_{13}CO_2CH_3$and $C_{20}H_{41}CO_2CH_3$)

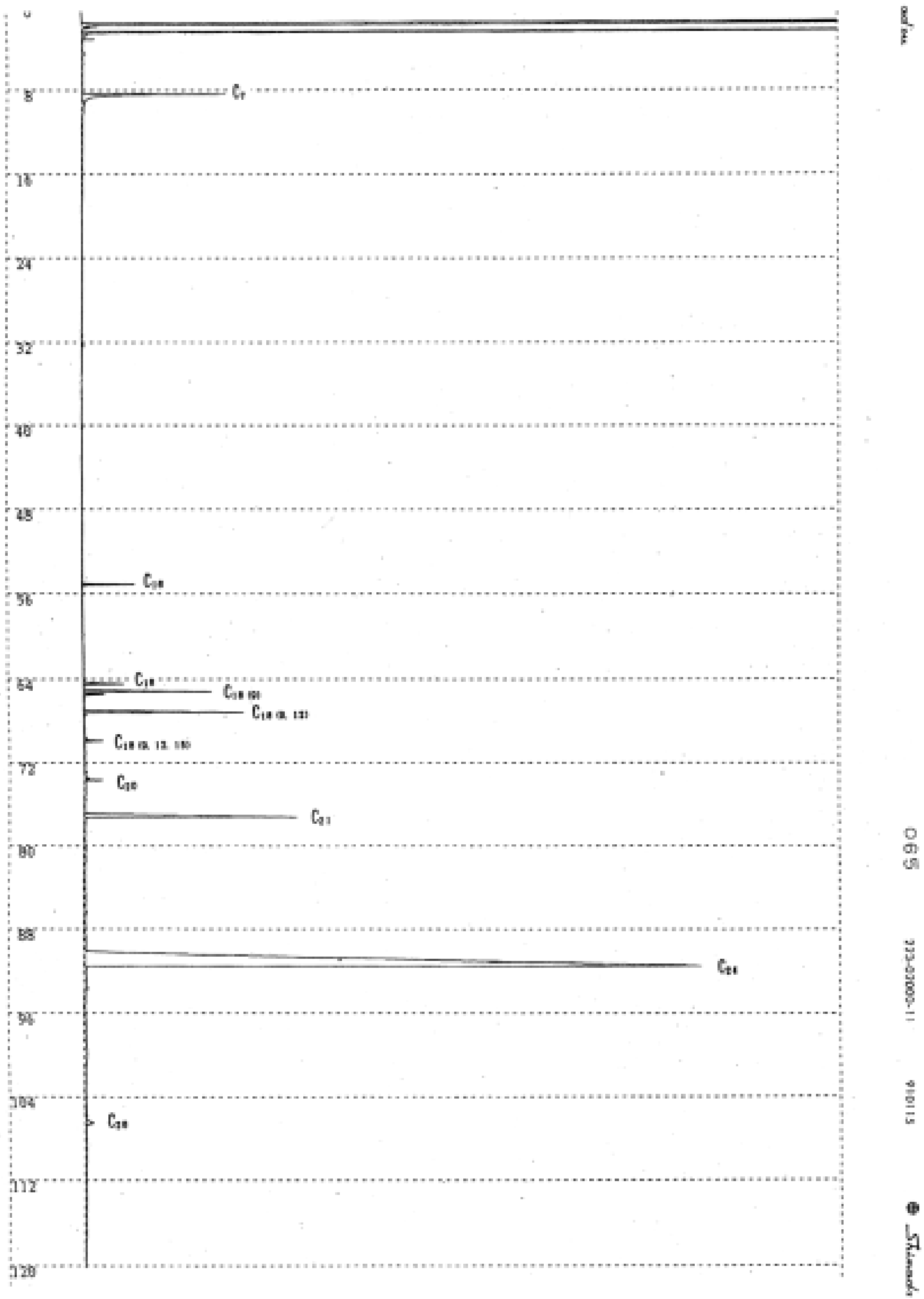

Fig. 10 Chromatogram of standard sample of castor oil (Internal standards：$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

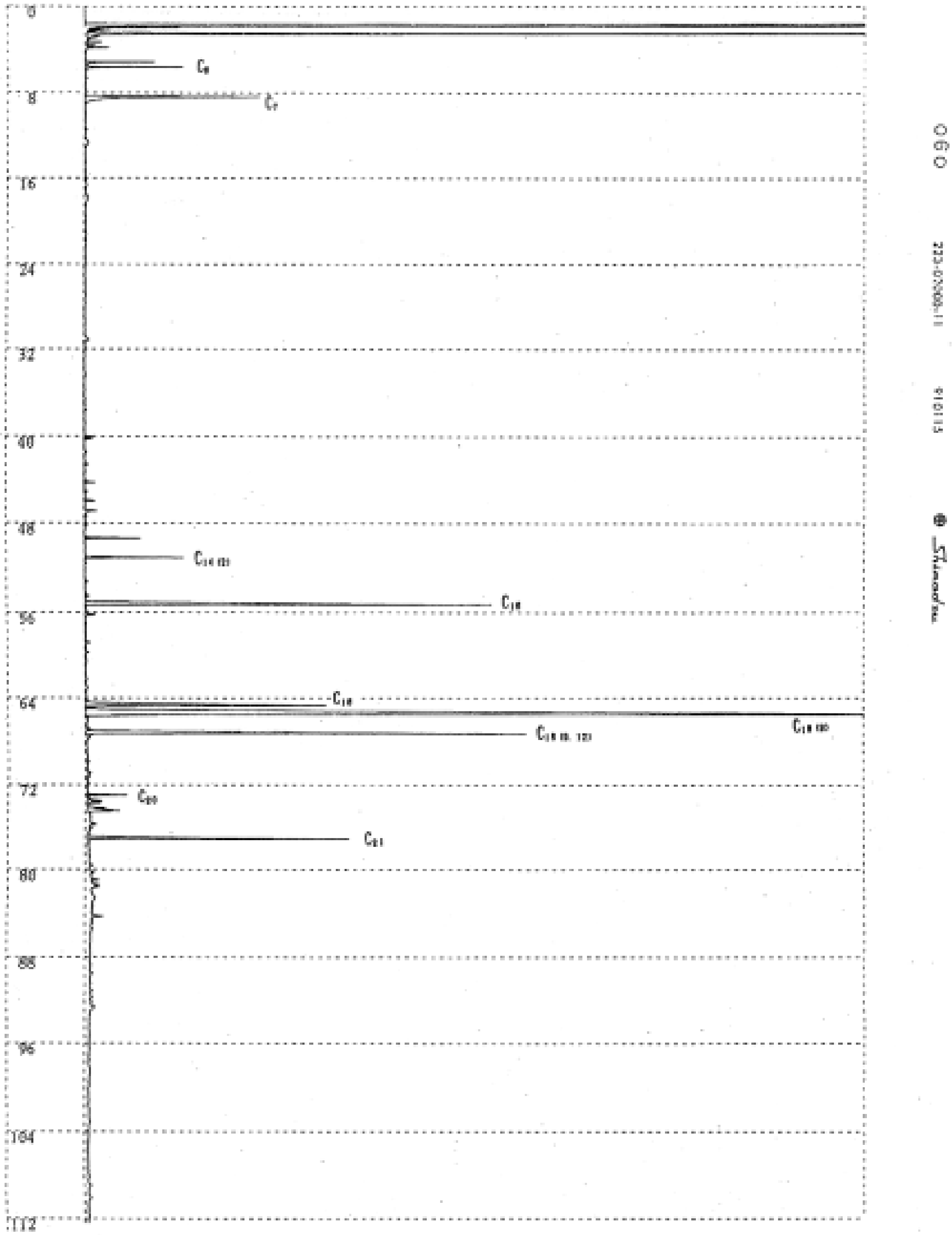

Fig. 11　Chromatogram of standard sample of sesame oil (Internal standards：$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

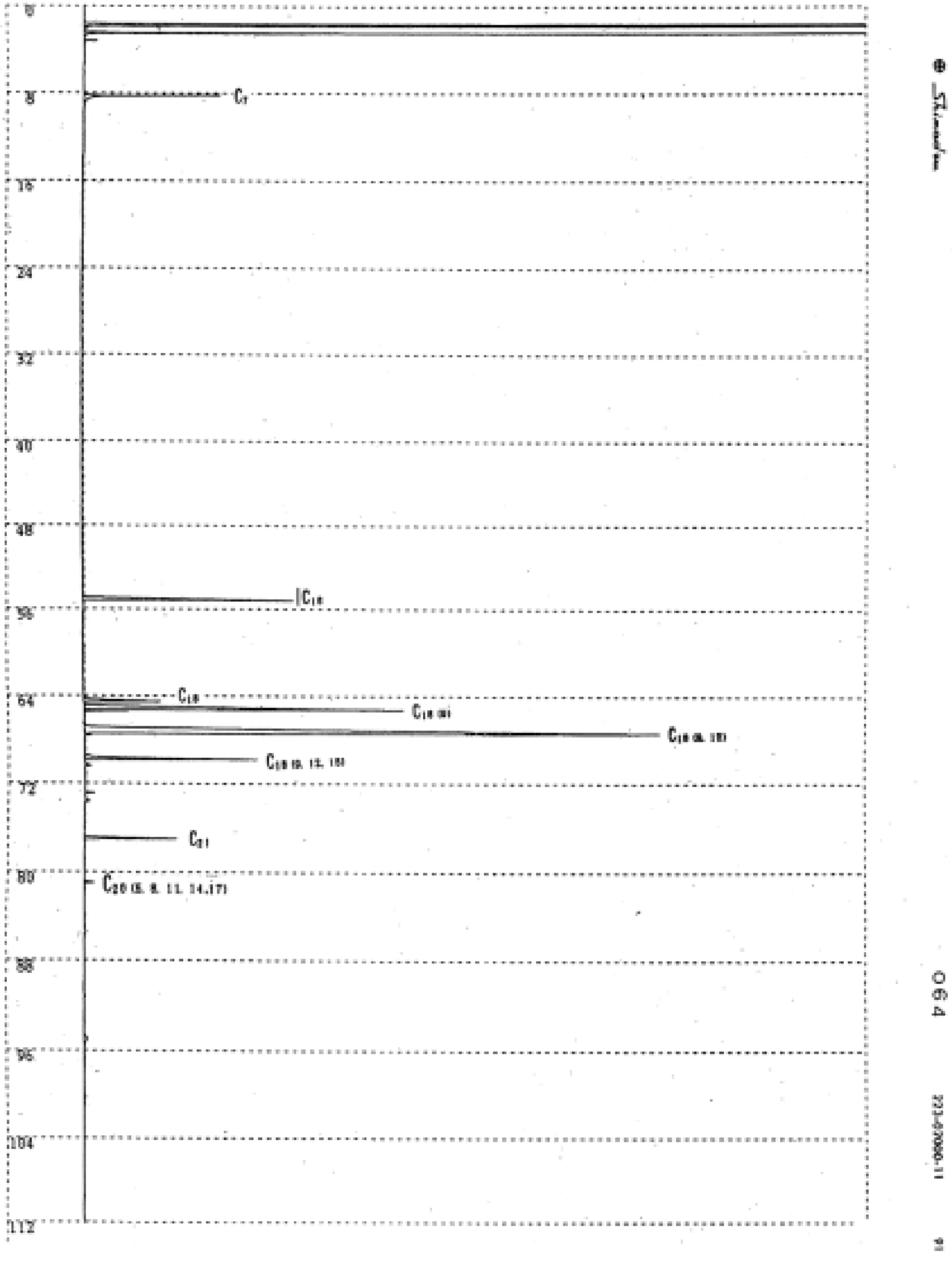

Fig. 12　Chromatogram of standard sample of soy bean oil (Internal standards：$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

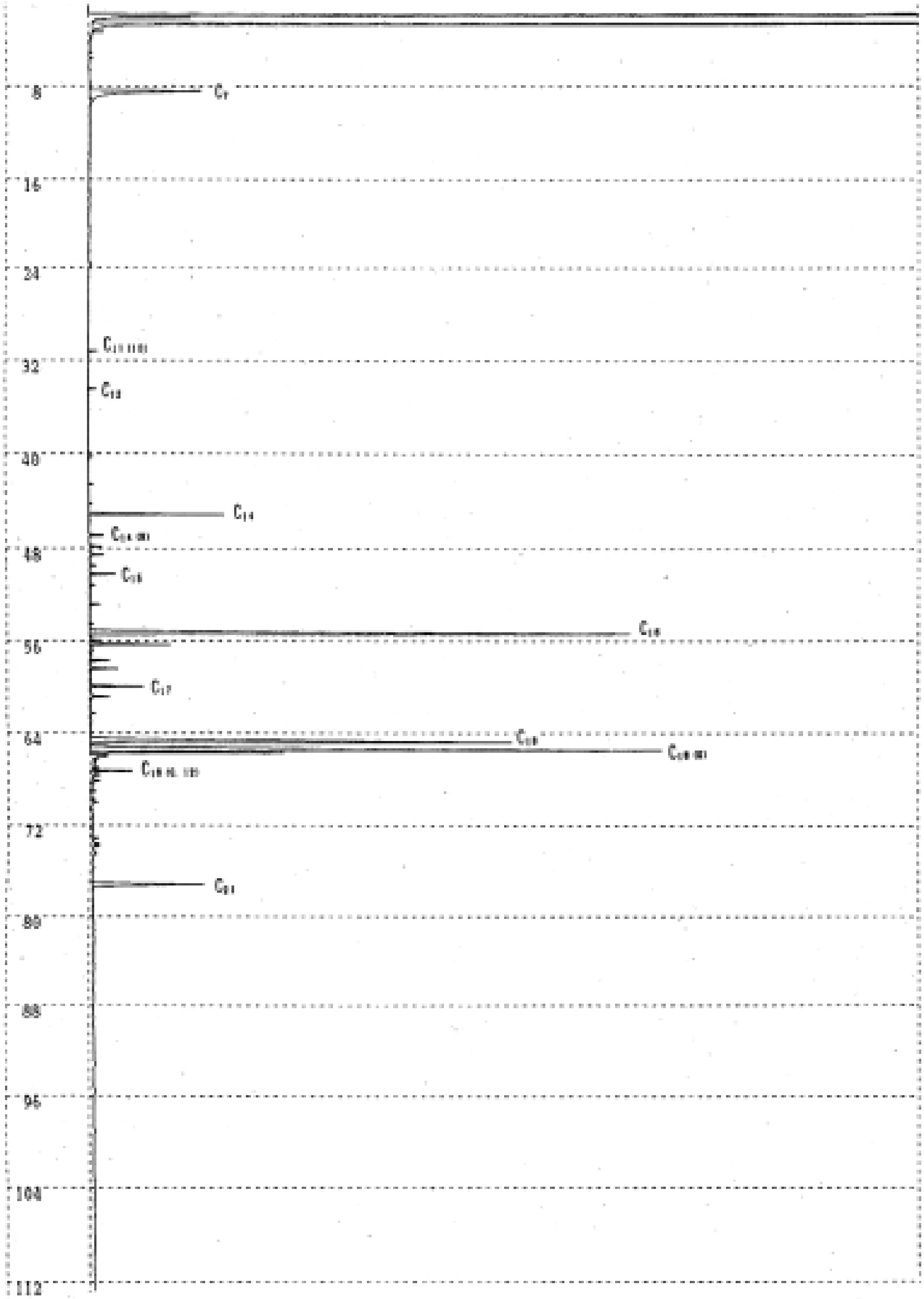

Fig. 13　Chromatogram of standard sample of beef oil (Internal standards：$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

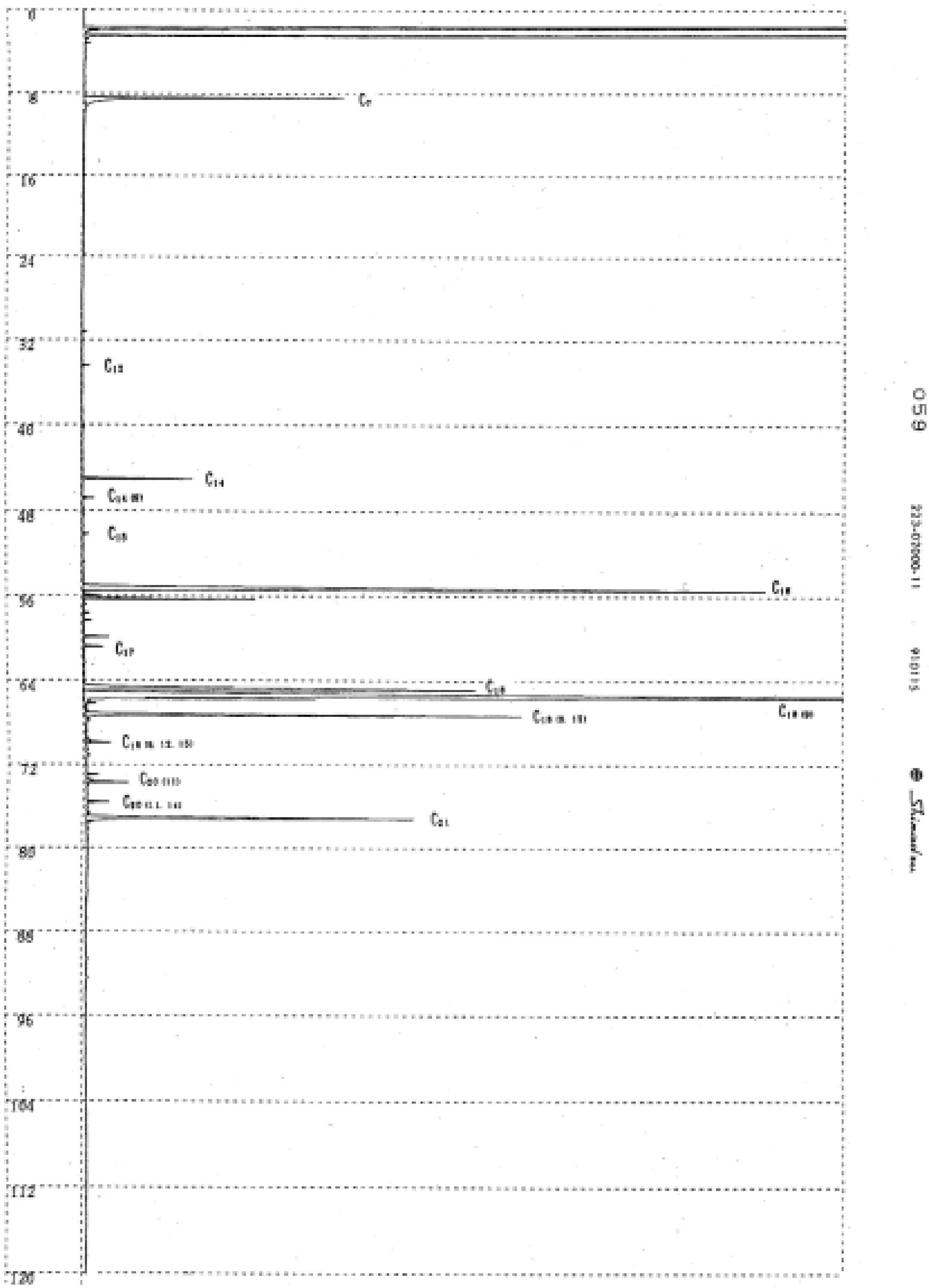

Fig. 14　Chromatogram of standard sample of lard oil (Internal standards：$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

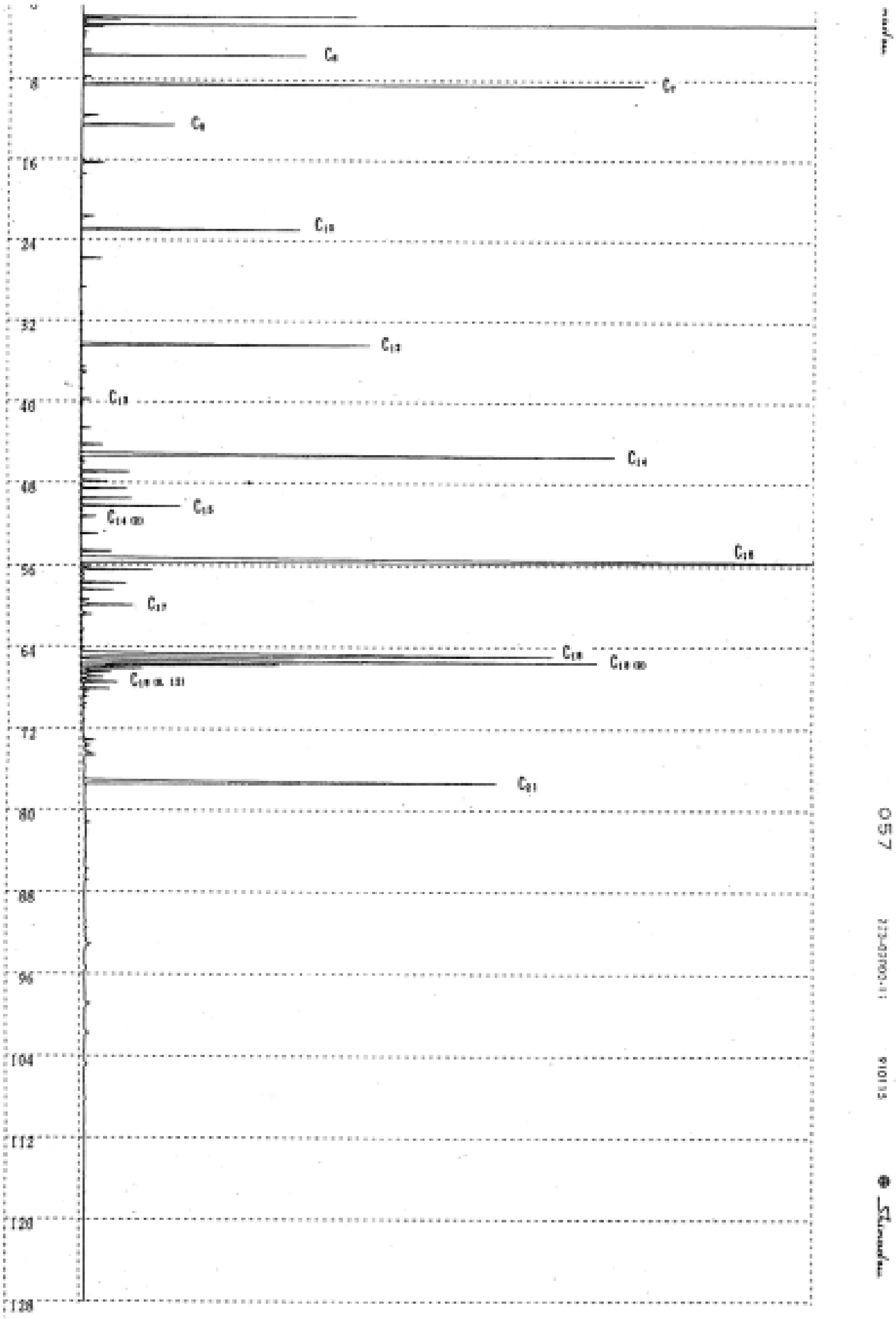

Fig. 15　Chromatogram of standard sample of milk fat (Internal standards：$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

Table 4 Retention Indices of fatty acid methyl esters. (Internal standards : $C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

Samples	R.T	R.R.T	R.T	R.R.T	R.T	R.R.T	R.T	R.R.T	R.T	R.R.T	$\overline{R.T}$	$\overline{R.R.T}$	S.D (R.T)	S.D (R.R.T)
C_6	2.000	-2.451	2.023	-2.440	2.025	-2.438	2.021	-2.437	2.036	-2.452	2.005	-2.450	0.0102	0.019
C_7	3.373	0.000	3.510	0.000	3.512	0.000	3.507	0.000	3.532	0.000	3.527	0.000	0.0287	
C_8	6.029	4.027	5.935	3.979	5.940	3.981	5.930	3.974	5.970	3.987	5.961	3.992	0.0422	0.022
C_9	9.511	9.737	9.395	9.840	9.395	9.845	9.391	9.854	9.434	9.875	9.421	9.895	0.0544	0.043
C_{10}	13.846	16.846	13.706	16.728	13.716	16.730	13.698	16.715	13.788	16.750	13.747	16.750	0.0030	0.054
C_{11}	16.736	24.848	16.572	24.714	16.580	24.714	16.572	24.709	16.644	24.778	16.620	24.752	0.0083	0.060
$C_{10 (9)}$	19.008	25.210	18.854	25.177	18.870	25.180	18.848	25.183	18.915	25.218	18.899	25.210	0.0061	0.050
$C_{11 (10)}$	21.222	29.105	21.165	28.959	21.185	28.975	21.160	28.958	21.232	29.016	21.213	29.005	0.0068	0.050
C_{12}	23.804	33.178	23.695	33.073	23.698	33.043	23.649	33.030	23.727	33.108	23.702	33.088	0.0542	0.057
$C_{12 (9)}$	24.163	33.754	24.030	33.653	24.035	33.646	24.010	33.628	24.081	33.703	24.064	33.679	0.0037	0.033
C_{14}	28.890	41.513	28.763	41.428	28.777	41.420	28.752	41.408	28.831	41.472	28.802	41.430	0.0071	0.043
$C_{14 (9)}$	29.350	42.209	29.218	42.177	29.224	42.156	29.203	42.148	29.282	42.212	29.255	42.192	0.0509	0.050
C_{15}	33.908	49.738	33.780	49.658	33.785	49.650	33.767	49.654	33.852	49.704	33.827	49.685	0.0526	0.038
$C_{16 (9)}$	34.443	50.610	34.314	50.544	34.335	50.519	34.312	50.520	34.370	50.583	34.354	50.554	0.0558	0.040
$C_{16 (11)}$	35.584	52.432	35.452	52.411	35.465	52.388	35.451	52.393	35.523	52.448	35.485	52.425	0.0078	0.043
C_{16}	38.750	57.730	38.636	57.638	38.663	57.631	38.639	57.622	38.707	57.658	38.681	57.690	0.0525	0.052
$C_{16 (?)}$	39.376	58.710	39.235	58.651	39.266	58.630	39.250	58.624	39.320	58.685	39.306	58.656	0.0552	0.030
C_{17}	43.457	65.432	43.347	65.362	43.368	65.345	43.354	65.355	43.412	65.376	43.388	65.389	0.0403	0.032
C_{17}	47.995	72.849	47.898	72.803	47.937	72.820	47.893	72.800	47.955	72.835	47.934	72.824	0.0424	0.018
$C_{18 (9)}$	48.740	74.075	48.645	74.030	48.658	74.013	48.652	74.045	48.710	74.051	48.682	74.081	0.0442	0.024
C_{18}	52.364	80.036	52.265	79.988	52.293	79.978	52.280	79.965	52.334	80.032	52.307	79.998	0.0408	0.011
$C_{18 (9)}$	53.050	81.137	52.950	81.135	52.963	81.077	52.960	81.110	53.025	81.120	52.992	81.119	0.0442	0.026
$C_{18 (9, 12)}$	54.930	84.333	54.835	84.215	54.849	84.189	54.843	84.190	54.909	84.200	54.871	84.201	0.0415	0.039
C_{20}	56.611	86.972	56.514	86.970	56.560	86.974	56.530	86.966	56.585	86.971	56.560	86.971	0.0385	0.003
$C_{18 (9, 12, 15)}$	57.635	88.651	57.535	88.646	57.558	88.605	57.738	88.610	57.584	88.635	57.571	88.629	0.0426	0.019
C_{20}	60.642	93.580	60.565	93.617	60.581	93.583	60.585	93.584	60.618	93.582	60.598	93.590	0.0287	0.015
$C_{20 (11)}$	61.252	94.582	61.174	94.610	61.197	94.578	61.165	94.589	61.221	94.573	61.202	94.582	0.0355	0.030
$C_{20 (11, 14)}$	63.139	97.680	63.048	97.691	63.059	97.659	63.048	97.656	63.098	97.644	63.078	97.656	0.0396	0.023
C_{21}	64.558	100	64.455	100	64.505	100	64.477	100	64.533	100	64.505	100.000	0.0408	0.000
$C_{20 (11, 14, 17)}$	65.671	101.829	65.590	101.852	65.609	101.810	65.598	101.819	65.645	101.823	65.620	101.828	0.0388	0.020
$C_{20 (5, 8, 11, 14)}$	67.457	104.757	67.372	104.780	67.407	104.758	67.390	104.779	67.436	104.759	67.412	104.768	0.0343	0.014
C_{22}	68.392	106.274	68.299	106.307	68.320	106.268	68.311	106.268	68.350	106.257	68.334	106.279	0.0330	0.019
$C_{20 (5, 8, 11, 14, 17)}$	68.990	107.271	68.924	107.323	68.945	107.280	68.919	107.288	68.960	107.272	68.940	107.288	0.0301	0.025
C_{23}	71.995	112.198	71.924	112.253	71.953	112.211	71.930	112.224	71.985	112.218	71.957	112.221	0.0319	0.021
$C_{22 (13)}$	73.558	118.041	73.480	118.090	73.513	118.048	73.487	118.074	73.535	118.098	73.507	118.058	0.0308	0.022
C_{24}	82.545	120.498	82.480	120.576	82.512	120.528	82.485	120.506	82.542	120.522	82.513	120.531	0.0305	0.028

Table 5　Relative retention times of standard fat samples (Internal standards：$C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

Number of Carbon of Fatty Acids	R. R. T	Standard Samples (R. R. T)				
		LARD OIL	MILK FAT	SOY BEAN OIL	PALM OIL	PEANUT OIL
C_6	-2.452					
C_7	0.000					
C_8	5.992	5.958	5.897	5.962	5.962	5.988
C_9	9.696					
C_{10}	16.700	16.602	16.680		16.690	16.757
C_{11}	24.752					
$C_{10([illegible])}$	25.210					
$C_{11([illegible])}$	29.005					
C_{12}	33.086		32.995		32.987	33.063
$C_{12([illegible])}$	33.679					
C_{13}	41.450					
$C_{13([illegible])}$	42.192					
C_{14}	49.685	49.498	49.621	49.564	49.593	49.641
$C_{14([illegible])}$	50.554					
$C_{14([illegible])}$	52.425		52.353			
C_{15}	57.650	57.496	57.609		57.601	
$C_{15([illegible])}$	58.658					
C_{16}	65.369	65.453	65.355	65.384	65.600	65.385
C_{17}	72.824	72.698	72.740	72.758	72.780	72.794
$C_{16([illegible])}$	74.051	74.011		74.088	74.107	
C_{18}	79.998	80.080	79.983	80.051	80.069	79.995
$C_{18(9)}$	81.119	81.272	81.002	81.212	81.200	81.128
$C_{18(9,12)}$	84.201	84.163	84.667		84.247	84.264
C_{19}	86.971					
$C_{18(9,12,15)}$	88.629	88.495		88.637	88.563	88.596
C_{20}	93.590	93.471		93.509	93.529	93.540
$C_{20(11)}$	94.582	94.454		94.506	94.519	94.548
$C_{20(11,14)}$	97.656	97.539		97.598		
C_{21}	100.000					
$C_{20(11,14,17)}$	101.828	101.624				
$C_{[illegible]}$	104.768					
$C_{20(5,8,11,14)}$	106.279			106.174	106.208	106.183
$C_{20(5,8,11,14,17)}$	107.288			108.036		
C_{22}	112.221			112.098		112.177
$C_{22(13)}$	118.058			117.961	118.104	118.018
C_{24}	129.531					129.477

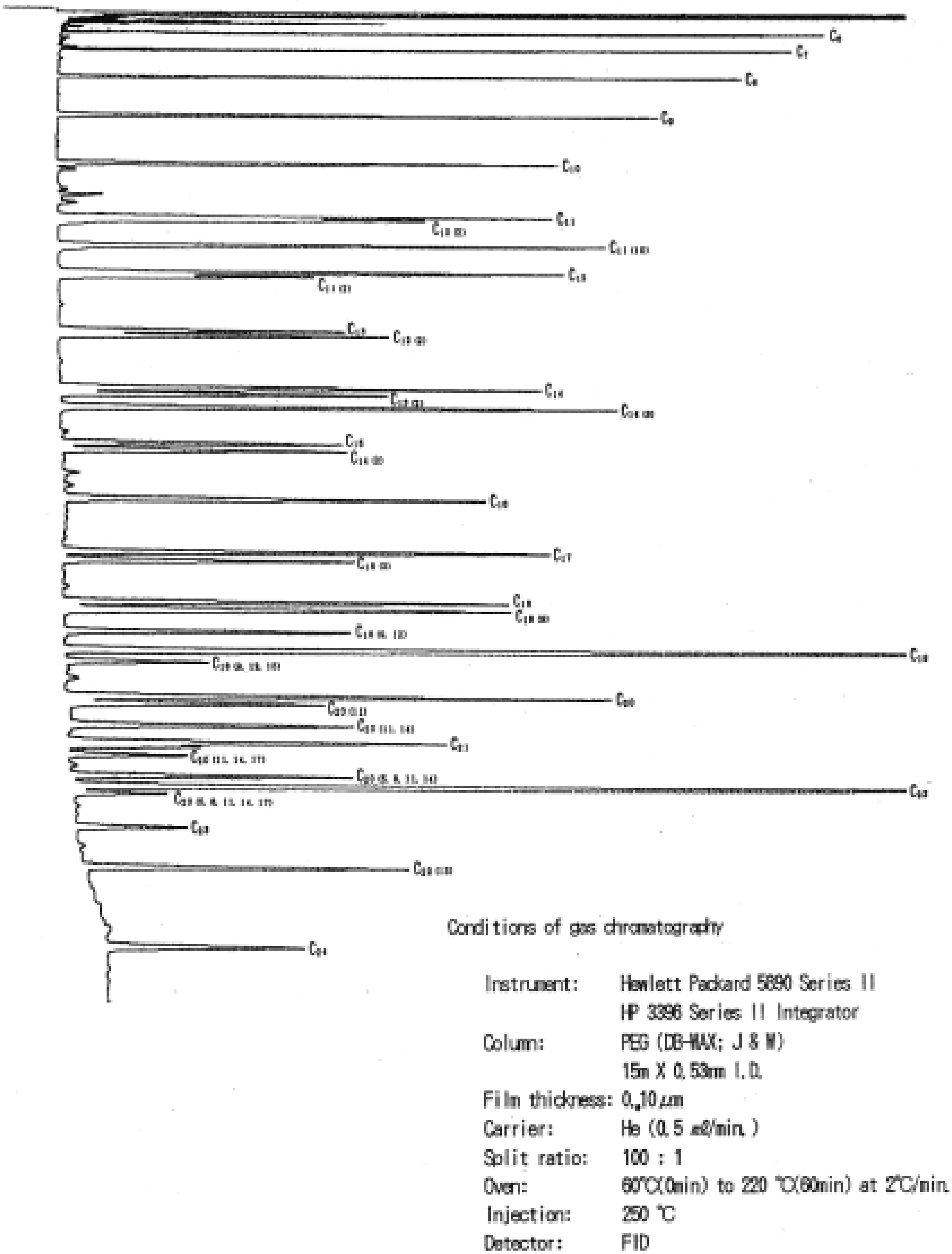

Fig. 16　The chromatogram of standard fatty acid methyl esters

(Internal standards : $C_6H_{13}CO_2CH_3$ and $C_{20}H_{41}CO_2CH_3$)

APPENDIX

C_{5}	:	Pentanoic acid methyl ester
C_{6}	:	Hexanoic acid methyl ester
C_{7}	:	Heptanoic acid methyl ester
C_{8}	:	Octanoic acid methyl ester（Methyl caprylate）
C_{9}	:	Nonanoic acid methyl ester（Methyl pelargonate）
C_{10}	:	Decanoic acid methyl ester（Methyl caprate）
C_{11}	:	Hendecanoic acid methyl ester（Methyl undecanoate）
$C_{10(2)}$	:	2－Decenoic acid methyl ester
$C_{11(10)}$	:	10－Undecenoic acid methyl ester
C_{12}	:	Dodecanoic acid methyl ester（Methyl laurate）
$C_{11(2)}$	:	2－Undecenoic acid methyl ester
C_{13}	:	Tridecanoic acid methyl ester
$C_{12(2)}$	:	2－Dodecenoic acid methyl ester
C_{14}	:	Tetradecanoic acid methyl ester（Methyl myristate）
$C_{13(2)}$	:	2－Tridecenoic acid methyl ester
$C_{14(9)}$	:	9－Tetradecenoic acid methyl ester
C_{15}	:	Pentadecanoic acid methyl ester
$C_{14(2)}$	:	2－Tetradecenoic acid methyl ester
C_{16}	:	Hexadecanoic acid methyl ester（Methyl palmitate）
C_{17}	:	Heptadecanoic acid methyl ester
$C_{16(2)}$	:	2－Hexadecenoic acid methyl ester
C_{18}	:	Octadecanoic acid methyl ester（Methyl stearate）
$C_{18(9)}$	:	9－Octadecenoic acid methyl ester（Methyl oleate）
$C_{19(9,12)}$	:	9, 12－Octadecadienoic acid methyl ester（Methyl linoleate）
C_{19}	:	n－Nonadecanoic acid methyl ester
$C_{18(9,12,15)}$	:	9, 12, 15－0ctadecatrienoic acid methyl ester（Methyl linolenate）
C_{20}	:	Eicosanoic acid methyl ester（Methyl arachidate）
$C_{20(11)}$	:	11－Eicosenoic acid methyl ester
$C_{20(11,14)}$	:	11,　14－Eicosadienoic acid methyl ester
C_{21}	:	Heneicosanoic acid methyl ester
$C_{20(11,14,17)}$	:	11,　14,　17－Eicosatrienoic acid methyl ester
$C_{20(5,8,11,14)}$	:	5,　8,　11,　14－Eicosatetraenoic acid methyl ester（Methyl arachidonate）
$C_{20(5,8,11,14,17)}$	:	5,　8,　11,　14,　17－Eicosapentaenoic acid methyl ester
C_{22}	:	Docosanoic acid methyl ester（Methyl behenate）
C_{23}	:	Tricosanoic acid methyl ester
$C_{22(13)}$	:	13－Docosenoic acid methyl ester（Methyl erucidate）
C_{24}	:	Tetracosanoic acid methyl ester
C_{26}	:	Hexacosanoic acid methyl ester