On Diethylene Glycol Succinate Monoester as a Stationary Liquid Phase in Gas-chromatography of Methyl Esters of Fatty Acids and on the Chromatographic Study of Horse-mackerel Oil.

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On Diethylene Glycol Succinate Monoester as a Stationary Liquid Phase in Gas-chromatography of Methyl Esters of Fatty Acids and on the Chromatographic Study of Horse-mackerel Oil.

Shojiro MIYAHARA and Yoshiaki TABATA

The purpose of the work described below was to analyze the methyl esters of fatty acids by means of the stationary liquid phase of diethylene glycol succinate monoester in gas chromatography.

Since high vacuum grease¹ had been used as the stationary liquid phase for analysis of methyl esters of fatty acids by gas-liquid-chromatography, the various liquids have been studied by several groups of workers in recent years. Afterwards it has been found that polyesters¹²) and polyglycols⁶) give good results respectively. Above all, polyvinyl acetate⁵) and polyesters of diethylene glycol or butanediol succinate⁶⁷), glutarate⁸⁹) and adipate¹⁰¹¹) are now in use frequently. Although, in this country of past days, these compounds were difficult to obtain, and the synthetic methods were troublesome to go through. From the result of our experiments about the stationary phase which we used in place of these compounds for analysis of methyl esters of higher fatty acids, it was found in our laboratory that, when we used diethylene glycol succinate monoester, methyl esters of fatty acids containing carbon atoms from 16 to 22, could be completely separated in accordance with number of carbon atoms and unsaturated degree.

Experiments and Results

1. Preparation of diethylene glycol succinate monoester

The calculated value of well dried powder of sodium succinate was poured slowly into 2,2'-dichloro diethyl ether, and the mixture reacted to change into sticky liquid feverishly. Next, sodium sulfate anhydride was added rather excessively to the liquid. Leaving it alone overnight, its upper layer liquid was analyzed and the result obtained was as follows.

<table>
<thead>
<tr>
<th></th>
<th>calculated (as C₈H₁₂O₅)</th>
<th>found</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>51.1%</td>
<td>C</td>
</tr>
<tr>
<td>H</td>
<td>6.4%</td>
<td>H</td>
</tr>
<tr>
<td>O</td>
<td>42.5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>
molecular weight 188 190-200

In view of this result, it is evident that the above-mentioned sticky liquid is diethylene glycol succinate monoester.

2. Conditions of gas chromatography

2.1 Stationary phase

The above-mentioned diethylene glycol succinate monoester was dissolved in methylene chloride, and celite 545 (80-100 mesh) * was added and mixed up thoroughly. Finally, methylene chloride was evaporated at 70°C. In the course of this experiment, the ratio of celite and ester was kept in 4 to 1.

2.2 Methyl esters of fatty acids

Methyl esters of palmitic, stearic, oleic, linolic, linolenic and erucic acids obtained commercially were prepared by boron trifluoride method [12].

2.3 Apparatus, column temperature and flow rate, etc.

According to the apparatus and conditions listed in Table 1, the methyl esters of the six fatty acids were submitted to chromatography on the column using diethylene glycol succinate monoester as a stationary phase.

Table 1. Apparatus and Conditions.

<table>
<thead>
<tr>
<th>Apparatus</th>
<th>Yanagimoto Gas-chromatograph GCG-200</th>
<th>Hitachi Gas-chromatograph KGL-2</th>
<th>Yanagimoto Gas-chromatograph GCG-500 (High sensitivity, H2 flame type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>packing</td>
<td>20% synthetic diethylene glycol succinate monoester in celite 545</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Column length</td>
<td>2m</td>
<td>2m</td>
<td>2m</td>
</tr>
<tr>
<td>I. D.</td>
<td>5mm</td>
<td>4mm</td>
<td>5mm</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>200</td>
<td>200 (col. chamber 220)</td>
<td>200</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>H2</td>
<td>N2</td>
<td>N2</td>
</tr>
<tr>
<td>Flow rate (cc/min.)</td>
<td>54</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>(Katharometer) 72mA 8mV</td>
<td>(Katharometer) 150mA 8mV</td>
<td>(Sensitivity×Attenuator) 1/10×1/16mV</td>
</tr>
</tbody>
</table>

3. Results

Ten µl of each of the methyl esters was chromatographed and the separation curve as shown in Fig. 1 was obtained. Although Fig. 1 shows only the result of separation under the condition listed in the extremely left file of Table 1. The ratio of retention time of each of the methyl esters separated under other conditions coincided with the result of Fig. 1. Next, from the relationship between

* for gas chromatography, Yanagimoto Co., Ltd., Kyoto.
**Fig. 1.** Separation of methyl esters of fatty acids on 2m column with diethylene glycol succinate monoester. Temp. 200°C; carrier gas H₂; flow rate 54cc/min.

**Fig. 2.** Relation between retention volume and number of carbon atoms of esters.

retention volume and number of carbon atoms of fatty acids, Fig. 2 was obtained. But, for the purpose of comparison, the retention volume of methyl esters of lauric and myristic acids under the same condition is also shown in Fig. 2.
In case of temperature over 200 °C

When the separation was executed under the same conditions as listed in Table 1 except the column temperature of 230°C, ratio of retention volume was occasionally not constant, but tailing was observed rather clearly.

5. Gas chromatography of fish oil (horse-mackerel oil)

The oil of horse-mackerel (Trachurus japonicus Temminck & Schlegel, body length 18.5cm), was obtained by the extraction of its fresh flesh with ether. The oil was transesterified by the usual method and sodium methoxide method. Gas chromatography of both of these esters gave the same retention time under the same condition as stated in 2. 3.

The result is shown in Fig. 3. Identification of each peak in Fig. 3 was practised according to Fig. 2. The methyl esters of which the retention time is larger than that of erucic acid (C_{22}) are not shown in Fig. 3.

Discussion

The number of theoretical plates of this column calculated from Fig. 1 is about 1100 as far as methyl ester of linolic acid is concerned. Under other conditions in Table 1, maximum, minimum and average numbers of theoretical plates to each methyl esters are 1100, 480 and 600 respectively. Judging from these results and from the linear function between retention volume and number of carbon atoms as shown in Fig. 2, it is considered that the column using diethylene glycol succinate monoester has sufficient separability for analysis of methyl esters of fatty acids. Moreover, in case of temperature over 230°C, the separations were unsuccessful with those tailings of peaks often appearing. It is probably because the transition of stationary phase might happen occasionally, and the vapour pressure of stationary phase is comparatively higher. Therefore, the column of diethylene glycol succinate monoester is not always best for the separation of methyl esters of all fatty acids but, in general, when the separation
is practised at the temperature below 200°C, this column is sufficient to be used for analysis of methyl esters of fatty acids which contain carbon atoms up to 22. For instance, as shown in Fig. 3, in the experiment in which horse-mackerel oil is used as a sample, the separation can be practised completely.

Summary

1. After preparation of the column of diethylene glycol succinate monoester, using this substance as stationary phase, gas chromatography of methyl esters of fatty acids which contain carbon atoms from 16 to 22, was studied.

2. This column has the sufficient separability for analysis of methyl esters of fatty acids which contain carbon atoms below 22, when the separation temperature is below 200°C.

3. Using this column, horse-mackerel oils were analyzed and the separation curve as shown in Fig. 3 was obtained.

A part of this study was reported at the Meeting of the Japanese Society of Scientific Fisheries held in autumn, 1962.

Thanks are given to Mr. Takeyasu Murata and Mr. Kinsei Ishihara for their assistance in the experimental work.

References