IATROSCAN APPLICATION

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Dye (Food dye, Napthol quinone, Azo dye), Hormons (Pregandiol), Ginseng Saponin, Liquid Crystal, Capsaicine, Cosmetic Cream, Rubber Antioxidant, Polymer
APPENDIX  I

Published Scientific References
**General Information**

The IATROSCAN is an automatic detector that performs quantitative analysis on organic mixtures separated on thin layer chromatography (TLC) and detected by Hydrogen Frame Ionization System (FID). The separation of components is performed on an exclusive thin layer chromatography media (CHROMAROD) in the same manner of normal phase TLC. Its FID system has high sensitivity for almost all organic components. The detection selectivity applies to a wide variety of samples. Particularly, lipid analysis, monitoring reaction rates in organic synthesis and fractionation of residues of crude oil and asphalt are easily analyzed with the IATROSCAN. Ten CHROMARODs are held in a single rack allowing up to 10 samples to be applied at a time. All 10 are processed simultaneously and detected in series to obtain the final results quickly.

**Principle of Operation**

When a sample(s) is developed and separated on the CHROMAROD (thin layer quartz rod) and scanned directly into the Hydrogen Flame at the rated speed, organic components separated on this thin layer surface are ionized by the energy of Hydrogen Flame. The ions generated are charged both negative and positive. The negative ions (-) flow to the Burner and the positive ions (+) flow to the Collector Electrode due to the electric field loaded between the FID electric poles {Burner positive (+) and Collector negative (-)}. These ion currents flow between the Burner and the Collector proportionally to the mass of components being ionized in the Hydrogen Flame. The ion current is amplified by the FID circuit, and the components are quantitatively measured and recorded by the data processing unit.
Reference
CLASSIFICATION

1. Overview

2. Biochemical and Natural Organic Chemical
   2.1. Lipids
   2.2. Fats and Oils
   2.3. Carbohydrates

3. Industrial Chemical
   3.1. Heavy Oils and Coal Liquefaction Products
   3.2. Polymers
   3.3. Surfactants

4. Food Chemical

5. Pharmaceuticals and Medicine

6. Agriculture

7. Others
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3.2 Polymers


3.3 Surfactants


4. Food Chemistry


5. Pharmaceutics and Medicines


6. Agriculture


7. Others


APPENDIX Ⅱ

IATROSCAN Application Data
IATROSCAN ANALYSIS

1A

Keys to Analytical Procedure
IATROSCAN Analysis (1A)  Keys to Analytical Procedure

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   B) Storage of CHROMAROD
These “Keys to Analytical Procedure” are for your better IATROSCAN® analysis.

1. Development Solvent

A) Preparation of development solvent
Even a slight difference in composition of development solvent has a great influence on chromatogram pattern. Therefore, preparation of development solvent shall be precisely made with clean measuring cylinder and/or measuring pipette.

In addition, the development solvent shall be newly prepared everyday since the composition of prepared development solvent will be gradually varied by its evaporation as time goes on.

B) Volume of development solvent
To always keep constant the distance from the level of development solvent to the sample spotting point, the volumes of development solvent and tank shall always be constant. Needless to say, a clean development tank shall always be used. As for the volume of development solvent, please refer the table below.

<table>
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<th>Development Tank</th>
<th>Recommended Volume of Development Solvent</th>
</tr>
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<tbody>
<tr>
<td>DT−150</td>
<td>70mL</td>
</tr>
</tbody>
</table>

C) Setting of development tank
Set the development tank on the level table at almost constant temperature, which shall be kept from direct sunlight and air turbulence. If the temperature of whole tank is not constant, stable separation of components in the sample can not be obtained because the development tank can not be filled up by solvent vapor homogeneously.
D) Temperature
Development work shall be executed always under constant temperature. Should there be some difference of temperature in the course of development, separation pattern and development time will be varied (see Analytical Example-1). Analyst should always confirm if there is no difference between the temperature of development solvent and that of circumstance where the tank is set. Because, sometimes heat generation will be caused during preparation of mixed development solvent.

a) Development at 20°C  
b) Development at 30°C

Analytical Example-1: Separation pattern affected by development temperature

The above chromatograms are the examples of analyzing lipids at different temperature (a) 20°C, (b) 30°C, where phosphatidyl choline and phosphatidyl inositol are separated at 20°C, but overlapped each other at 30°C.

**Sample**: Standard Mixture

**Stationary Phase**: Chromarod-SIII

**Mobile Phase**: Chloroform:Methanol:Water:25%Ammonia 47:20:2.5:0.28

**Measurement conditions**: Hydrogen 160mL/min, Air 1.5L/min, Scan Speed 40sec/scan

E) Saturating inside of development tank with solvent vapor
It is very important to always establish constant atmosphere inside the tank saturated with solvent vapor in order to acquire good analytical result and reproducibility. To accomplish this, therefore, perform the following procedures.

① Erect a new filter paper inside development tank and wet it with solvent.
   • DT－150: After erecting a L-shaped filter paper along one side of the tank, wet it with solvent and then cover the tank with its lid
2. Leave the tank with filter paper and solvent as it is until it become saturated with solvent vapor.

3. Wet again the filter paper with solvent
   As time passes, the solvent vapor filled inside the tank tends to move downward, resulting in poor saturation in its upper portion even if the filter paper wetted with solvent is in it. If the development were executed under above mentioned state, the development time is possibly prolonged and the separation pattern vary. Accordingly, make sure to wet again the filter paper with solvent in advance to development.

2. Chromarod
   Chromarod, developed exclusively for IATROSCAN, is a thin layer in the form of thin quartz rod evenly applied and sintered inorganic binder and adsorbent (silica gel or alumina) on it. Usually, the cleaning and activation of Chromarods can be achieved by Blank scan on IATROSCAN, which enables Chromarods to be used repeatedly. However, their repeated use of Chromarod is possible in the limited case where the development solvent with same composition is used, because Chromarod has a memory effect to fix a bit acidic and/or alkaline substances on it. Therefore, the composition of development solvent should be always same for repeated use of Chromarod. Especially, in case of analyzing a sample having radical(s) dissociation constant affected by pH, good reproducibility will not be achieved as the case may be. For instance, the separation pattern of the sample developed by neutral solvent on a new Chromarod is different from that of the Chromarod treated with acidic development solvent. The expected number of repeat use of Chromarod is approx. 20, which depends on how the rod is used.

Chromarod is made of quartz glass. Handle it with thorough care.
A) Activation of Chromarod

Activation of Chromarod can be easily conducted only by Blank scan once to twice on IATROSCAN, the conditions of which shall be same as those of actual analysis (ex. hydrogen 160mL/min, air 2.0L/min, scan speed 30sec/scan). Activation and cleaning of Chromarod can be performed at the same time.

The activation of Chromarod shall be performed immediately before sample spotting. And, start development without delay right after spotting.

Organic compounds originated from the shipping package (cushion material and box) are being adsorbed on the Chromarods. These organic compounds are on the parts of both ends of Chromarod as well, which the hydrogen flame can not reach. Therefore, if the Chromarods are used as they are, these organic compounds will cause the ghost peak in the course of measurement. In consideration of the above, remove the adsorbed matters on the Chromarods in the shipping package in a following manner.

① Place the Chromarod(s) in position on the rod holder.

② Put the rod holder bearing Chromarod(s) in a development tank containing the same development solvent as that to be used for actual analysis, and execute development to the extent that the solvent front crosses the sample spotting point.
③ Take out the set of rod holder and Chromatod(s), and vaporize the development solvent on them [see 6. Removal of Development Solvent (page 10)].

④ Conduct Blank Scan twice in order to remove the organic compounds on the Chromarod(s), and clean and activate the latter.

B) Treatment of Chromarod for analyzing phosphorous compound
Phosphorous component sometime remains on the rod even after IATROSCAN analysis due to its interaction with Chromarod. Accordingly, the pretreatment and washing of Chromarod(s) is required for selective measurement of phosphorous by FPD of IATROSCAN MK-6. For the details, refer the following.

・ IATROSCAN Analysis（4A）[Chromarod-S III Treatment Procedure for FPD Analyzing Phosphorous Compound]

C) Preparation of Chromarod specially treated
Even special types of thin-layer treated with silver or cupper nitrate or boric acid are required, they are easily obtained by easy pretreatment as shown in the following references for further details.

・ IATROSCAN Analysis（12）[Analysis of glyceride isomers by Chromarod treated with boric acid]
・ IATROSCAN Analysis（16）[Analysis of molecular type of glycerides by Chromarod treated with silver nitrate]
・ IATROSCAN Analysis（22）[Analysis of lipids by Chromarod-SIII treated with cupper nitrate]
3. Sample spotting

Minimizing the size of sample spot is a must for successful separation. The following points should be kept in mind in the spotting procedure.

A) Sample preparation
① Solvent for dissolving sample
Select a solvent of the lowest possible boiling point and polarity among those which can sufficiently dissolve the sample. The spot tends to spread more when a solvent of higher boiling point or polarity is used. Generally, hexane, benzene and chloroform are suitable solvents.

② Concentration of sample solution
Prepare a sample solution with the concentration of approx. 2~5 μg/peak. In case the concentration of the sample concerned is unknown, adjust the concentration of sample to 1~2% (10~20mg/mL). It is recommended to keep the sample solution prepared in a vial with a screw-type of organic solvent-proof lid.

B) Sample spotting
Spot the sample solution on the rod(s) by means of micro-dispenser attached. The appropriate spotting size for better quantification is 1 μl. Firstly, remove the set of Rod Holder and activated Chromarods from the scanning stage and place it onto the attached Spotting Guide. Then, lift up a bit the upper part of Rod Holder so as to have the lower ends of Chromarods touch the bottom of Rod Holder.

Spot samples solution on the origin points of Chromarods (i.e. Zero point graduation of Rod Holder) with the guide of white spotting line of Spotting Guide.
Spotting shall be carefully conducted since analytical results will remarkably be affected by spotting reproducibility. To make the spotted width as narrow as possible, eject the sample bit by bit in several installments. Broad spotting width will result in worse separation.

During spotting, if the tip of micro-dispenser does not contact the Chromarod, some part of sample solution will climb up the outside of needle, which, as a result, makes it impossible to spot whole sample solution sucked in on the Chromarod. To minimize spotting error, therefore, eject the sample solution under the state of micro-dispenser tip contacting Chromarod, which enables to transfer the weighed sample solution onto the rods. And, in this case, **do not pressurize the Chromarod** since it is fragile and dangerous in case of breakage.

In case that the spotting width are broaden due to the nonvolatile solvent of sample solution, blow air (cool) upon the spotted area to accelerate its vaporization by hand dryer in each interval of a bit spotting.

C) Removal of sample dissolving solvent

Upon spotting, remove the sample dissolving solvent on the rod for certain. Blowing the rod by means of hand-dryer is recommended to remove the dissolving solvent with high boiling point.

4. **Relation between Chromarod performance and humidity**

In case of using development solvent with relatively high polarity, there can be observed little affection of humidity on the development pattern. But, the development is affected in a large way by the solvent with low polarity. Therefore, the development work shall be executed as quickly as possible upon completing sample spotting.
5. Development

Put the Chromarods spotted the sample on them in the development tank saturated enough with the vapor of development solvent (see Item 1., “Development Solvent” in the page 3), and continue the development until the solvent front reaches the targeted development distance.

In case of using the development tank DT-150, the adsorption of solvent to the Chromarods is improved by leaning the rod holder against the filter paper in.

The development distance shall always kept constant. Should the development distance be varied, the counts of peak area measured will be changed due to the transformation of the peak shape (see the AnalyticalExample-2, below).

When a multi-development is executed, proceed with the next development upon removing the solvent by letting the Chromarods alone for 2 minutes at room temperature or by blowing them by means of had-dryer (cool wind).

<table>
<thead>
<tr>
<th>Development Distance (cm)</th>
<th>Count (Area)</th>
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<tbody>
<tr>
<td>a 2.5cm</td>
<td>9428</td>
</tr>
<tr>
<td>b 5.0cm</td>
<td>7735</td>
</tr>
<tr>
<td>c 7.5cm</td>
<td>7597</td>
</tr>
<tr>
<td>d 10.0cm</td>
<td>6744</td>
</tr>
</tbody>
</table>

Analytical example-2: Relation of Peak Area with its Development Distance

The above data are the example of analyzing Cholesterol ester (2 μg) corresponding to the different development distance, where the peak width becomes broader and its height becomes lower in proportion to the development distance. The longer development distance is, the smaller the peak area counts are.

Stationary Phase: Chromarod-SIII
Mobile Phase: Hexane:Diethyl ether 63:7 (20°C)
Measuring Conditions: Hydrogen 160mL/min, Air 2.0L/min, Scan Speed 30sec/scan
6. **Removal of Development Solvent from Chromarod**

Remove the development solvent from the Chromarods after completion of development. If the measurement is started with the Chromarods still adsorbing the solvent on them, it will cause the fluctuation of baseline and show the smaller peak area counts. The remained solvent shall be entirely remove by keeping the Chromarods in the Rod Dryer TK-8 exclusively used for Chromarods or in the appropriate drying oven, or by blowing cool air against the Chromarods. However, it is enough to remove the solvent by air-dry (for 2 minutes) at room temperature in case of volatile solvents like hexane and ether.

![Rod Dryer TK-8](image)

7. **FID / FPD Measurement**

The detection sensitivity of FID or FPD is varied by the flow rate of air and/or hydrogen and scanning speed. Therefore, **set the hydrogen flow rate to 160mL/min**. There is a possibility that some parts of separated components will remain unburned on the rods under the low hydrogen flow rate even after scanning. On the other hand, under the high hydrogen flow rate, the rods will unnecessarily be overheated and the correlation between the peak area counts and concentration of separated component will be damaged.

A) **FID (Flame Ionization Detector) Measurement [Single detection]**

Following measurement conditions shall be applied for FID single measurement.

- Hydrogen 160mL/min, Air 2.0L/min, Scan Speed 30sec/scan

The peak shape and intensity of each component will be varied by the boiling point of component, its interaction with the rod, scanning conditions and so on. Therefore, the comparison of analytical data shall be made among those obtained under the same scanning conditions.
B) FID/FPD (Flame Photometric Detector) Measurement [Dual detection]

When analyzing sulfur (S) or phosphorous (P) by means of FPD, the FPD selectivity for detecting S or P depends on the flow rates of hydrogen and air and scanning speed. Therefore, the recommended conditions to selectively detect S or P are as follows:

- **Selective detection of S by FPD**
  - Interference filter: 394nm
  - Hydrogen 160mL/min, Air 0.5L/min, Scan Speed 30sec/scan

- **Selective detection of P by FPD**
  - Interference filter: 526nm
  - Hydrogen 160mL/min, Air 1.5L/min, Scan Speed 40sec/scan

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**8. Washing and storage of Chromarod(s)**

A) Washing of Chromarods

Usually, there is no need to wash Chromarods. The Chromarods can be used again for a next sample measurement in succession to the previous measurement as the Chromarods are cleaned and activated at the same time of measurement on the Iatroscan. The Blank Scan on the Iatroscan is enough to clean the Chromarods.

The expected number of repeated use of Chromarod is approx. 20 times. However, in case of selective measurement of P by FPD of Iatroscan MK-6, the pretreatment and washing of Chromarods are required.

For further information, refer the Iatroscan Analytical Method titled 「4A: Chromarod-S III Treatment Procedure for FPD Analyzing Phosphorous Compound」.

- **IATROSCAN Analysis (4A) 「Chromarod-S III Treatment Procedure for FPD Analyzing Phosphorous Compound」**

The phosphorous or metallic component(s) remained on the Chromarod(s) can not be removed by the scanning only. Therefore, the cleaning procedure below should be followed to eliminate those residues. Should there still be some noise on the chromatograph even after such cleaning procedure, replace the Chromarod(s) with new one(s).
① Removal of P compound(s)
Following procedure is for removing P compound(s) by using ammonia water. However, note that the separation pattern to be obtained after the cleaning will sometimes be different from that of previous measurement as the development conditions may be, because the ammonia used will possibly remain on the rod(s) even after finishing this cleaning.

In addition, during this cleaning, the silica gel as adsorbent of Chromarod·SIII will be gradually dissolved due to its poor alkali resistance. Accordingly, the Chromarods shall not be soaked in ammonia solution for long time, which will cause inferior separation due to silica gel degradation.

1) Immerse the Chromarods in the 2.5% ammonia solution for 10 minutes.
2) After taking out the Chromarods from the 2.5% ammonia solution, thoroughly rinse them with deionized water.
3) Remove the water by drying the Chromarods for one (1) hour at 120°C, or by conducting Blank scan twice after drying them for three (3) minutes at 120°C.

② Removal of metallic salt(s)
As for the metallic salts such as NaCl and CaCl₂ which can not be removed by scanning, remove eliminate them in accordance with the following procedure. Nevertheless, note that the separation pattern to be obtained after the cleaning will sometimes be different from that of previous measurement as the development conditions may be

1) Wash the Chromarods lightly with deionized water.
2) Soak them in the concentrated nitric acid all the night through.
3) Upon taking out the Chromarods from the concentrated nitric acid, thoroughly rinse them with deionized water.
4) Remove the water by drying the Chromarods for one (1) hour at 120°C, or by conducting Blank scan twice after drying them for three (3) minutes at 120°C.

③ Removal of organic compound(s)
Most organic compounds can be eliminated by the Blank Scan. Execute the Origin Scan several times to remove the organic compound(s) remained on the origin point. In case that the organic compounds are remaining on the whole of Chromarod, conduct the Blank Scan twice at the scanning speed of 60sec/scan.

If the organic residuals can not be removed by the scanning, wash the Chromarods with concentrated sulfuric acid in the manner described below. However, note that the separation pattern to be obtained after the cleaning will sometimes be different from that of previous measurement as the development conditions may be, because the sulfuric acid used will possibly remain on the rod(s) even after finishing this cleaning.
1) Wash the Chromarods lightly with deionized water.
2) Soak them in the concentrated sulfuric acid all the night through.
3) Upon taking out the Chromarods from the concentrated sulfuric acid, thoroughly rinse them with deionized water.
4) Remove the water by drying the Chromarods for one (1) hour at 120°C, or by conducting Blank scan twice after drying them for three (3) minutes at 120°C.

B) Storage of Chromarods
Keep the Chromarods in a clean glass vessel with a lid to avoid their contamination caused by adsorption of organic/inorganic matter coming from the dust in the air.
Never storage them in the vessel containing drying agent. MITSUBISHI CHEMICAL MEDIENCE CORPORATION is ready to supply the Rod Storage Chamber DE-3 as an optional item.

![Rod Storage Chamber DE-3](image)

Do not expose Chromarods to high humidity and/or water, which damages their thin layers.

Products Guide:

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Product Name</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3248</td>
<td>Chromarod-SIII (silica type)</td>
<td>10pcs/box</td>
</tr>
<tr>
<td>3201</td>
<td>Development Tank DT-150</td>
<td></td>
</tr>
<tr>
<td>3215</td>
<td>Pre-cut Filter Papers for DT-150 Development Tank</td>
<td>100pcs</td>
</tr>
<tr>
<td>3221</td>
<td>Rod Storage Chamber DE-3</td>
<td></td>
</tr>
<tr>
<td>5101</td>
<td>Rod Dryer TK-8</td>
<td></td>
</tr>
<tr>
<td>6321</td>
<td>Rod Holder SD-6</td>
<td></td>
</tr>
<tr>
<td>6100</td>
<td>Microdispenser</td>
<td></td>
</tr>
</tbody>
</table>
IATROSCAN ANALYSIS

2A

Type Analysis for Heavy Oil
IATROSCAN Analysis (2A)  Type analysis for Heavy oil

1. Heavy oil type analysis by Iatroscan®

Heavy oil is composed of a various complex mixture. The heavy oil analysis is done by the whole characterization (such as elementary analysis or NMR), the type analysis and so forth. The type analysis by Iatroscan is one of composite analysis. The heavy oil is separated into 4 groups (saturated hydrocarbons, aromatic hydrocarbons, resins, asphaltenes) by multiple development using 3 kind of development solutions different polarity. Compare with the type analysis by liquid chromatography, this analysis is short time/easy to handle.

2. An example of the heavy oil analysis by Iatroscan

A) The method of the heavy oil analysis

Sample                      : Heavy oil\( ^1 \) 10mg/mL (dissolved by dichloromethane) and 5, 10, 20, 30mg/mL (for response curve)
Spotting Volume             : 1 \( \mu \) L (using the Micro-dispenser : Code No.6100)
Stationary phase            : Chromarod®-SIII (Code No.3248)
Development tank            : DT-150 (Code No.3201)
Mobile phase\( ^2 \)          : 1st. Hexane 100% (60mL) 10 cm (20°C)
                               2nd. Toluene 100% (60mL) 6 cm (20°C)
                               3rd. Dichloromethane: Methanol 57:3 2.5cm (20°C)
                               (After each development, proceed with the next development after removing the development solvent from the Chromarods by air-dry for 2 minutes at room temperature.)
Measurement conditions      : Hydrogen 160mL/min, Air 2.0L/min, Scan Speed 30sec/scan
Detector                    : FID

First, conduct Blank Scan   (Hydrogen 160mL/min, Air 2.0L/min, Scan Speed 30sec/scan) in order to clean and activate the Chromarods. 1 \( \mu \) L of the sample solution 10mg/mL was spotted\( ^3 \) on the Chromarod by means of the micro-dispenser. Upon spotting, remove the sample dissolving solvent on the Chromarods by air-dry for 2 minutes at room temperature.

1) Before sampling a heavy oil, be sure to mix it very well because these components of the heavy oil are broken up equally. It is recommended to keep the sample solution prepared in a vial with a screw-type of organic solvent-proof lid.

2) The grade of organic solvents should be liquid chromatography or more pure than it.

3) To make the spotted width as narrow as possible, eject the sample bit by bit in several installments(8-10times/1 \( \mu \) L). To minimize spotting error, eject the sample solution under the state of micro-dispenser tip contacting Chromarod, which enables to transfer the weighed sample solution onto the rods. And, in this case, do not pressurize the Chromarod since it is fragile and dangerous in case of breakage.
Put the Chromarods spotted the sample on them in the first development tank (Hexane) saturated enough with the vapor of the development solvent, and continue the development until the solvent front reaches 100-point graduation of the Rod Holder. After the development, remove the development solvent on the Chromarods by air-dry for 2 minutes at room temperature. Next, the Chromarods were developed in Toluene until the solvent front reaches 60-point graduation of the Rod Holder, and dried at room temperature for 2 minutes. In the third stage of development, the Chromarods were developed in Dichloromethane:Methanol(57:3) until the solvent front reaches 25-point graduation of the Rod Holder.

The Chromarods were then dried at room temperature for 2 minutes and measured\(^4\) by FID (Hydrogen 160mL/min, Air 2.0L/min, Scan Speed 30sec/scan).

The heavy oil sample was measured using ten (10) Chromarods and calculated each percentage of 4 groups (saturated hydrocarbons, aromatic hydrocarbons, resins, asphaltenes) of the heavy oil. The chromatogram is shown in Figure-1. The data and the reproducibility of daily error are shown in Table-1 and Figure-2 respectively. Also the response curves for the heavy oil (5~30μg) are shown in Figure-3.

![Chromatogram of heavy oil](image)

**Figure-1** Chromatogram of heavy oil

Sample: Heavy oil

Stationary Phase: Chromarod-SIII
Mobile Phase:
1st, Hexane 100% 10cm
2nd, Toluene 100% 6cm
3rd, Dichloromethane:Methanol 57:3 2.5cm

Measurement conditions: Hydrogen 160mL/min, Air 2.0L/min, Scan Speed 30sec/scan

\(^4\) Asphaltenes are not eluted and are hence susceptible to the problem of rod rotation which influences results. Rotate the rod and the asphaltenes that remain at the point of application are oriented toward the burner with respect to the detector for valid quantification.
Table 1) Measured values of 10 Chromarods

<table>
<thead>
<tr>
<th>Chromarod No.</th>
<th>Peak 1: Saturated HC (%)</th>
<th>Peak 2: Aromatic HC (%)</th>
<th>Peak 3: Resins (%)</th>
<th>Peak 4: Asphaltenes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.4</td>
<td>43.7</td>
<td>12.1</td>
<td>5.7</td>
</tr>
<tr>
<td>2</td>
<td>35.0</td>
<td>45.3</td>
<td>13.3</td>
<td>6.4</td>
</tr>
<tr>
<td>3</td>
<td>37.6</td>
<td>43.8</td>
<td>12.0</td>
<td>6.6</td>
</tr>
<tr>
<td>4</td>
<td>36.7</td>
<td>43.6</td>
<td>13.6</td>
<td>6.0</td>
</tr>
<tr>
<td>5</td>
<td>35.1</td>
<td>45.0</td>
<td>13.3</td>
<td>6.6</td>
</tr>
<tr>
<td>6</td>
<td>36.0</td>
<td>44.2</td>
<td>13.6</td>
<td>6.2</td>
</tr>
<tr>
<td>7</td>
<td>36.5</td>
<td>44.5</td>
<td>13.3</td>
<td>5.8</td>
</tr>
<tr>
<td>8</td>
<td>34.9</td>
<td>44.5</td>
<td>14.3</td>
<td>6.4</td>
</tr>
<tr>
<td>9</td>
<td>37.0</td>
<td>43.3</td>
<td>12.8</td>
<td>6.9</td>
</tr>
<tr>
<td>10</td>
<td>38.2</td>
<td>42.7</td>
<td>12.8</td>
<td>6.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>(\bar{X})</th>
<th>SD</th>
<th>CV (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>36.5</td>
<td>1.3</td>
<td>3.5</td>
<td>44.1</td>
<td>0.8</td>
<td>1.8</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.3</td>
</tr>
</tbody>
</table>

Figure 2) Reproducibility of daily error
Prepared heavy oil sample solutions (5, 10, 20, 30 mg/mL) and measured with Iatroscan by the above conditions. The response curves of each group were found to be linearly proportional to the amount of heavy oil.

Figure 3) Response curves for heavy oil
Analysis of Biodegraded Crude Oil by IATROSCAN
The tracing biodegradation process of crude oil has been carried out mainly by GC/FID and HPLC/UV and so forth. However, such conventional analyzers are unsuitable for such reaction tracing in case of samples with high boiling points, which requires quick response and high throughput analysis. Because, GC is problematic to analyze sample with high boiling point and HPLC has some problem with degradation of stationary phase and rather long analyzing time.

In case of IATROSCAN, it is comparatively easy to trace such biodegradation process in a short time without such aforementioned problems with GC and HPLC.

1. Experimental Procedure

1.1 Preparation of sample solution [10 ~ 20 mg/ml, hexane/toluene (6 : 4) soln.]

(1) For biodegradation of crude oil:

① Pour 0.05g of crude oil and 100ml of NSW medium (see [Note]) into a 500ml Erlenmeyer flask.
② Add 10ml of sea water to the Erlenmeyer flask.
③ Shake the Erlenmeyer flask by a reciprocal shaker at 20°C for 2 weeks to incubate the prepared sample solution.
④ Extract the sample solution with 200ml of the solvent (hexane : toluene = 6 : 4) containing stearyl alcohol (25ppm) as an internal standard.
⑤ Concentrate the extracted solution by means of a rotary evaporator.
⑥ Dilute the concentrated solution up to 2 ~ 5ml with hexane/toluene (6 : 4) solution.


<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄NO₃</td>
<td>1.0g</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>0.02g</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.02g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.5g</td>
</tr>
<tr>
<td>Aged sea water</td>
<td>800ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>200ml</td>
</tr>
<tr>
<td>pH 7.8</td>
<td></td>
</tr>
<tr>
<td>crude oil, kerosene, light gas oil, A-heavy oil or n-tetradecane</td>
<td>50μl/10ml</td>
</tr>
</tbody>
</table>
(2) For biodegradation of spilt crude oil drifted ashore

1. Crude oil in the sample shall be extracted for analysis since the spilt oil drifted ashore contains water and sand. Generally, the extraction is conducted by Soxhlet apparatus using chloroform (and/or CS2) and/or reciprocal shaker using hexane/toluene (6:4) solution.
2. After extraction, remove water with sodium sulfate anhydride and solvent by rotary evaporator.
3. Weigh out 10 ~ 20mg of the extract.
4. Add to the weighed extract 1ml of hexane/toluene (6:4) solution containing 2,500 ppm of stearyl alcohol as internal standard.

1.2 IATROSCAN Analysis

(1) Execute blank scan for cleaning Chromarod-SIII.
(2) Spot 1 μl of sample on Chromarod-SIII
(3) First development : development distance ; 10cm
   development solvent ; hexane
(4) Second development : development distance ; 5.5cm
   development solvent ; hexane/toluene (2:8) soln.
(5) Measurement under PPS mode (partial pyrolysis scan mode)
   → continue measuring until stearyl alcohol (internal standard) is detected,
      where the saturates and aromatics can be detected as well.
(6) Third development : development distance ; 10cm
    development solvent ; dichloromethane/methanol
    (95:5) soln.
    → develop the remaining components not separated well under the above conditions.
(7) Measurement under normal scan mode :
    → for detecting the resins and asphaltenes.
2. Results

The example of tracing biodegradation process of crude oil is shown below.

First Chromatogram

saturates

aromatics

IR

origin

manual termination of scanning

Second Chromatogram

resin asphaltene and unknown

scanning direction


In conclusion, we wish to express our sincere appreciation to Dr. Takao Matumoto of SHOWA SHELL SEKIYU KABUSIKIKAISHA (Central Laboratory) for their having provided us with useful and helpful suggestions.

[ Reference ]


IATROSCAN ANALYSIS

5

Analysis of Lipid by Iatroscan
1. Serum lipid  
2. Red blood cell membrane lipid  
3. Lipid standard mixture  
4. Lipid in amniotic fluid  
5. Lecithin  
6. Egg yolk lecithin  
7. Glycolipid (1)(2)  
8. Skin surface lipid  
9. Lipid of marine product  
10. Lipid of rat liver  
11. Lipid of rat sciatic nerve  
12. Hepatic microsomal phospholipid of guinea pig  
13. Hepatic microsomal neutral lipid of guinea pig  
14. Lipid in brain tissue  
15. Lipid of fungi (1)(2)(3)  

Lipid components are expressed in the following abbreviation.  

<table>
<thead>
<tr>
<th>Component</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceramidedihexoside</td>
<td>CDH</td>
</tr>
<tr>
<td>Ceramidemonohexoside</td>
<td>CMH</td>
</tr>
<tr>
<td>Ceramidetrihexoside</td>
<td>CTH</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Cho</td>
</tr>
<tr>
<td>Cholesterol acetate</td>
<td>Cho.A</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>Cho.E</td>
</tr>
<tr>
<td>Diglyceride</td>
<td>DG</td>
</tr>
<tr>
<td>Fatty acid</td>
<td>FA</td>
</tr>
<tr>
<td>Monoglyceride</td>
<td>MG</td>
</tr>
<tr>
<td>Phosphatidyl choline</td>
<td>PC</td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine</td>
<td>PE</td>
</tr>
<tr>
<td>Phosphatidyl serine</td>
<td>PS</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>SM</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>TG</td>
</tr>
</tbody>
</table>
1. Serum lipid

Component
Stationary phase: CHROMAROD-SⅢ
Mobile phase
1st Chloroform : Methanol : Water
50 : 20 : 2.5 5 cm
2nd n-Hexane : Diethyl ether : Formic acid
65 : 5 : 0.15 10cm

2. Red blood cell membrane lipid

Component
Stationary phase: CHROMAROD-SⅢ
Mobile phase
1st Chloroform : Methanol : Water
50 : 20 : 2.5 5 cm
2nd n-Hexane : Diethyl ether : Formic acid
65 : 5 : 0.15 10cm

3. Lipid standard mixture

Component
Stationary phase: CHROMAROD-SⅢ
Mobile phase
1st Chloroform : Methanol : Water
50 : 20 : 2.5 5 cm
2nd n-Hexane : Diethyl ether : Formic acid
65 : 5 : 0.15 10cm

4. Lipid in amniotic fluid

Component
3. PC 9. SM
Stationary phase: CHROMAROD-SⅢ
Mobile phase
1st Chloroform : Methanol : Water
50 : 20 : 2.5 5 cm
2nd n-Hexane : Diethyl ether : Formic acid
65 : 5 : 0.15 10cm
5. Lecithin

Component
5. PC
Stationary phase: CHROMAROD-SII
Mobile phase
Chloroform : Methanol : Water  50:20:2

6. Egg yolk lecithin

Component
5. PC
Stationary phase: CHROMAROD-SII
Mobile phase
Chloroform : Methanol : Water  50:20:2.5

7. Glycolipid (1)

Component
2. CMH  3. CDH  4. CDH  5. CTH  6. Globoside 1
Stationary phase: CHROMAROD-SII
Mobile phase
Chloroform : Methanol : Water  50:20:2.5

Glycolipid (2)

Component
3. Ganglioside GD-1a  4. Ganglioside GT-1b
Stationary phase: CHROMAROD-SII
Mobile phase
Chloroform : Methanol : Water (0.2%CaCl$_2$)  
30:30:6
8. Skin surface lipid

Component
6. Anise alcohol (internal standard) 7. Phospholipid

Stationary phase: CHROMAROD-SII
Mobile phase:
1st n-Hexane: Diethyl ether: Formic acid
50:20:0.3 8 cm
2nd n-Hexane: Benzene 1:1 10 cm

9. Lipid of marine product

Stationary phase: CHROMAROD-SII
Mobile phase:
n-Hexane: Diethyl ether: Formic acid 42:28:0.3

10. Lipid of rat liver

Stationary phase: CHROMAROD-SII
Mobile phase:
1st Chloroform: Methanol: Water
50:20:2.5 5 cm
2nd n-Hexane: Diethyl ether: Formic acid
65:5:0.15 10 cm

11. Lipid of rat sciatic nerve

Component
1. Neutral lipid 2. PE 3. PC 4. SM
Stationary phase: CHROMAROD-SII
Mobile phase:
Chloroform: Methanol: 14% Ammonia solution
65:25:2

- 3 -
12. Hepatic microsomal phospholipid of guinea pig

Component
1. Neutral lipid 2. PE 3. PC
Stationary phase: CHROMAROD-S II
Mobile phase
Chloroform : Methanol : Water  40 : 10 : 1

13. Hepatic microsomal neutral lipid of guinea pig

Component
Stationary phase: CHROMAROD-S II
Mobile phase
Benzene : Chloroform : Formic acid  70 : 20 : 0.5

14. Lipid in brain tissue

Stationary phase: CHROMAROD-S II
Mobile phase
Chloroform : Methanol : Water  60 : 20 : 2

15. Lipid of fungi (1)
Lipid in the adult stalk of Fistulinaceae

Stationary phase: CHROMAROD-S II
Mobile phase
Chloroform : Methanol : Water  40 : 10 : 1
Lipid of fungi (2)

Lipid in the pileus of Fistulinaceae

Stationary phase: CHROMAROD-S II
Mobile phase
Chloroform : Methanol : Water  40 : 10 : 1

Lipid of fungi (3)

Lipid in the adult tubular pores of Fistulinaceae

Stationary phase: CHROMAROD-S II
Mobile phase
Chloroform : Methanol : Water  40 : 10 : 1
Analysis of Lipid by Iatroscan
1) Mobile phases
   1.1 Polar lipid standard mixture
   1.2 Internal standard (Cholic acid)

2) Health foods
   2.1 Vitamin E and Wheat germ oil on the market
   2.2 Lecithin on the market

3) Cosmetics and Skin lipids
   3.1 Lipid standard mixture
   3.2 Lipid standard mixture
   3.3 Lipstick (1)
   3.4 Lipstick (2)
   3.5 Glycolipid in a borine brain
   3.6 Oil Soluble Substance

4) Other lipids
   4.1 Lipid in a colon bacillus
   4.2 Lipid in Wakame seaweed
   4.3 Lipid extraction from washing solution of alveoli of the lung
   4.4 Washing solution of alveoli of the lung

Conditions
   Stationary phase: CHROMAROD-SIII
   Gas flow: H₂ 160mL/min, Air 2.0L/min
   Scanning speed: 30sec/scan

Lipid components are expressed in the following abbreviations

<table>
<thead>
<tr>
<th>Component</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>Cho</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>Cho.E.</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td>CL</td>
</tr>
<tr>
<td>Ceramide mono hexoside</td>
<td>CMH</td>
</tr>
<tr>
<td>Fatty acid</td>
<td>FA</td>
</tr>
<tr>
<td>Lysophosphatidyl choline</td>
<td>LPC</td>
</tr>
<tr>
<td>Lysophosphatidyl ethanolamine</td>
<td>LPE</td>
</tr>
<tr>
<td>Phosphatidyl choline</td>
<td>PC</td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine</td>
<td>PE</td>
</tr>
<tr>
<td>Phosphatidyl glycerol</td>
<td>PG</td>
</tr>
<tr>
<td>Phosphatidyl inositol</td>
<td>PI</td>
</tr>
<tr>
<td>Phosphatidyl serine</td>
<td>PS</td>
</tr>
<tr>
<td>Sphingomyeline</td>
<td>SM</td>
</tr>
<tr>
<td>Sulfatide</td>
<td>CSE</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>TG</td>
</tr>
</tbody>
</table>
1) **Mobile phases**

1.1 **Polar lipid standard mixture**

**Component**
2. PE 3. PI 4. PS 5. LPE 6. PC 7. SM 8. LPC

**Mobile phase**
Chloroform : Methanol : Water

45 : 20 : 2 10cm 2times

**Component**
2. PE+PI 3. PS 4. LPE 5. PC 6. SM+LPC

**Mobile phase**
Chloroform : Methanol : Water

40 : 24 : 2 10cm

**Component**
2. PE 3. PC 4. LPE 5. SM 6. LPC

**Mobile phase**
Chloroform : Methanol : Water : conc Ammonia

39 : 24 : 1.5 : 3 10cm

**Component**
1. PE 2. PC 3. LPE+SM 4. LPC

**Mobile phase**
Chloroform : Methanol : Water : conc Ammonia

52 : 20 : 0.8 : 2.5 10cm
1.2 Internal standard (Cholic acid)

Component
2. Cholic acid 3. PE 4. PC 6. LPC
Mobile phase
Chloroform : Methanol : Water
45 : 25 : 2.5 : 1 10 cm

Component
3. PE 5. Cholic acid 6. PC 8. LPC
Mobile phase
Chloroform : Methanol : Water : Ammonia
50 : 25 : 2.5 : 0.05 10 cm 2 times
2) Health foods

Liquid sample are dissolved in chloroform : methanol = 2 : 1.
Extraction of lipids from solid state samples is done as following:

1. Break down a solid state sample.
2. About 200mg of the sample are stirred in 15mL of Chloroform : methanol = 2 : 1
3. After filtration, add 3mL water to the filtrate and stir.
5. Take off the upper layer, and dry up the lower layer (chloroform layer) by Na stream.
6. Finally, the dried sample is dissolved in chloroform : methanol = 2 : 1.

2.1 Vitamine E and Wheat germ oil on the market:

Vitamin E standard sample

![Component chart]

Component:
1. tocot 2. δ-tocopherol 3. β,γ-tocopherol 4. α-tocopherol

Mobile phase:
Benzene : n-Hexane 48 : 2

Vitamin E and Lipid standard mixture

![Component chart]

Component:
1.TG 2.α-tocopherol 3.β,γ-tocopherol 4.δ-tocopherol

Mobile phase:
Benzene : Methanol 60 : 1 10cm

---
Mobile phase
Benzene : Methanol  60 : 1  10cm

B corporation: Vitamine E

Component
1. TG  2. α-tocopherol

E corporation (1): Vitamine E

Component
1. TG  2. α-tocopherol

E corporation (2): Vitamine E

Component
1. α-tocopherol

K corporation: Vitamine E

Component
1. α-tocopherol

N corporation: Vitamine E

Component
1. TG  2. α-tocopherol

S corporation: Vitamine E

Component
1. α-tocopherol
Mobile phase
Benzene : Methanol 60 : 1 10cm

R corporation: Vitamin E

Component
1. TG 2. \( \alpha \)-tocopherol

H corporation: Wheat germ oil

Component
1. TG 2. \( \alpha \)-tocopherol
3. \( \beta, \gamma \)-tocopherol 4. \( \delta \)-tocopherol

2.2 Lecithin on the market

Mobile phase
A. Chloroform : Methanol : Water : conc Ammonia 39 : 24 : 1.5 : 3 10cm
B. Chloroform : Methanol : Water : Formic acid 45 : 20 : 2.5 : 1 10cm

H corporation: Lecithin

Component
2. PE 3. PC 4.5.PI+PS 6.SM 7.LPC
Mobile phase : A

Component
4. PE 5.PI+PS 7.PC 9.LPC
Mobile phase : B
3) Cosmetics and Skin lipids

3.1 Lipid standard

Component

Mobile phase
1st Chloroform : Methanol : Water
57 : 12 : 0.6 2.5 cm

2nd Chloroform : Methanol : Water
57 : 12 : 0.6 2.5 cm

3rd n-Hexane : Ethyl ether : Formic acid
50 : 20 : 0.3 8 cm

4th n-Hexane : Benzene 35 : 35 10 cm

3.2 Lipid standard

Component
2. 2-Octyl dodecanol
6. N-Lauroyl-L'-glutamic acid

Mobile phase
n-Hexane : Ethyl ether : Formic acid
50 : 20 : 0.7 10 cm

3.3 Lipstick (1)

Component
1. Hydrocarbon 2. Wax
3. Ester of fatty acid 8. FA

Mobile phase
1st Benzene 100% 7 cm

2nd n-Hexane : Benzene 1 : 1 10 cm

3.4 Lipstick (2)

Component
1. Hydrocarbon 2. Wax
3. Ester of fatty acid 8. FA

Mobile phase
1st Benzene 100% 7 cm

2nd n-Hexane : Benzene 1 : 1 10 cm
3.5 Glycolipid in a borine brain

Component
Mobile phase
1st Chloroform : Methanol : Water : Acetic acid
70 : 30 : 3 : 0.5 8.5cm
2nd n-Hexane : Ethyl ether : Formic acid
62 : 7 : 0.1 10cm

3.6 Oil Soluble Substance

Component
Mobile phase
Petroleum ether : Ethyl ether : Acetic acid
90 : 10 : 1 10cm

4) Other lipids

4.1 Lipid in a colon bacillus

Component
4. CL 5. PG 6. PE
Mobile phase
1st Chloroform : Methanol : Ammonia : Water
40 : 20 : 2 : 0.25 8cm
2nd n-Hexane : Ethyl ether : Formic acid
60 : 10 : 0.15 10cm

4.2 Lipid in Wakame seaweed

Component
2. FA 3. 4. 5. 6. Cho+ Chlorophyll 7. PE 8. PC
Mobile phase
1st Chloroform : Methanol : Water
50 : 20 : 2 7cm
2nd n-Hexane : Ethyl ether : Formic acid
55 : 15 : 0.15 10cm
4.3 Lipid extraction from washing solution of alveoli of the lung

Washing solution of alveoli of the lung
  | centrifuging (3000rpm)
Upper layer (10mL)
  | 90mL of chloroform:methanol (2:1)
  | mixing for 1 min on a mixer
20mL of water
  | filtration
  | Centrifugation (3000rpm, 3min)
  | taking off the upper layer (water layer)
  | drying up the lower layer (Chloroform layer)
  | dissolving in 300µL chloroform

4.4 Washing solution of alveoli of the lung

Component

Mobile phase
1st Chloroform : Methanol : Ammonia : Water
  38 : 29 : 0.6 : 2.3  8cm
2nd n-Hexane : Ethyl ether  65 : 5  10cm
Analysis of Lipid by Iatroscan (Marine Products)
A) Samples

1) Fish lipid

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1 Standard mixture-1</td>
<td>L. flavobrunneum (muscle)</td>
</tr>
<tr>
<td>1-2 Todarodes pacificus (liver)</td>
<td>L. flavobrunneum (liver)</td>
</tr>
<tr>
<td>1-3 Halocynthia roretzi (muscle)</td>
<td>C. saira (muscle)</td>
</tr>
<tr>
<td>1-4 Cultured Coho Salmon (muscle)</td>
<td>C. saira (viscera)</td>
</tr>
<tr>
<td>1-5 Oncorhynchus Keta (muscle)</td>
<td>S. melanosticta (muscle)</td>
</tr>
<tr>
<td>1-6 Oncorhynchus Keta (muscle)</td>
<td>S. melanosticta (viscera)</td>
</tr>
<tr>
<td>1-7 Oncorhynchus Keta (ovary)</td>
<td>S. japonicus (muscle)</td>
</tr>
<tr>
<td>1-8 Navodon modestus (muscle)</td>
<td>S. japonicus (viscera)</td>
</tr>
<tr>
<td>1-9 Xiphias gladius (muscle)</td>
<td>Standard mixture-II</td>
</tr>
<tr>
<td>1-10 Xiphias gladius (eye)</td>
<td>Kastuwonspelamis (muscle)</td>
</tr>
</tbody>
</table>

2) Plankton lipid

2-1 Copepoda (Neocalanus plumchrus)

2-2 Artemia

3) Lipid oxidation and hydrolysis in dried ancholy products during drying and storage.

B) Lipid components are expressed in the following abbreviations.

<table>
<thead>
<tr>
<th>Component</th>
<th>Abbreviation</th>
</tr>
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<tbody>
<tr>
<td>Polar lipids</td>
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<tr>
<td>Phosphatidyl choline</td>
<td>PC</td>
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<td>Phosphatidyl ethanolamine</td>
<td>PE</td>
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<tr>
<td>Phosphatidyl serine</td>
<td>PS</td>
</tr>
<tr>
<td>Sphingomyelene</td>
<td>SPM</td>
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<tr>
<td>Cholesterol</td>
<td>Cho</td>
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<td>Sterols</td>
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<td>Monoglyceride</td>
<td>MG</td>
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<tr>
<td>Free fatty acid</td>
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<tr>
<td>Triglyceride</td>
<td>TG</td>
</tr>
<tr>
<td>Cholesterol acetate (Internal standard)</td>
<td>CR (IS)</td>
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<tr>
<td>Cholesterol ester</td>
<td>CE</td>
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<tr>
<td>Wax ester</td>
<td>WE</td>
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<tr>
<td>Hydrocarbone</td>
<td>HC</td>
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<tr>
<td>Diglyceride</td>
<td>DG</td>
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</table>
C) Mobile Phase

System-1: Hexane : Diethyl ether : Formic acid = 42 : 28 : 0.3

System-2:
1st Hexane : Diethyl ether = 66 : 4
2nd Hexane : Diethyl ether : Formic acid = 55 : 15 : 0.5
3rd Hexane : Benzene = 30 : 30

System-3:
1st Chloroform : Methanol : Water = 50 : 20 : 2.5
2nd Hexane : Diethyl ether : Formic acid = 65 : 5 : 0.15

System-4:
1st Chloroform : Methanol : Water : Ammonia = 38 : 29 : 2.3 : 0.6
2nd Hexane : Diethyl ether : Formic acid = 60 : 10 : 0.1


D) Reference


1) Fish lipid

1-1 Standard mixture-I

Mobile phase: System-1

0.02

2 FA
3 DG
1 TG
5 Cho
6 MG
7 PL

CAL. METHOD 00
SF PA PB
160000+63 100000+01 160000+21

NO. NAME RT A OR H MK CONC
1 0.104 13758 M 68.9639
2 0.103 1001 M 4.6632
3 0.204 886 M 4.0269
4 0.231 750 M 3.4875
5 0.264 2236 M 10.7151
6 0.404 1014 M 4.7165
7 0.459 1815 M 8.4375

TOTAL 21511 100.0000

1-2 Todarodes pacificus (liver)

Mobile phase: System-1

0.02

2 FA
3 ST
4 PL

CAL. METHOD 06
SF PA PB
100000+63 100000+01 100000+01

NO. NAME RT A OR H MK CONC
1 0.125 46882 M 69.7584
2 0.179 1446 M 2.7701
3 0.308 957 M 1.8226
4 0.484 7545 M 5.6387

TOTAL 52231 100.0000

1-3 Halocynthia roretzi (muscle)

Mobile phase: System-1

0.02

2 FA
3 1,3-DG
4 1,2-DG
5 ST
6 PL

CAL. METHOD 00
SF PA PB
160000+63 100000+01 160000+21

NO. NAME RT A OR H MK CONC
1 0.112 25319 M 27.0972
2 0.166 417 M 9.4416
3 0.225 3886 M 4.2551
4 0.261 8573 M 10.3846
5 0.268 7287 8.0578
6 0.465 4419 M 48.8635

TOTAL 90434 100.0000

1-4 Cultured Coho Salmon (muscle)

Mobile phase: System-1

0.02

2 FA
3 ST
4 PL

CAL. METHOD 06
SF PA PB
100000+63 100000+01 100000+01

NO. NAME RT A OR H MK CONC
1 0.122 61402 M 63.4922
2 0.177 463 M 0.7073
3 0.303 273 M 0.4159
4 0.483 3397 M 5.1835

TOTAL 65536 100.0000

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IATROSCAN ANALYSIS

4A

Chromarod-SⅢ  Treatment Procedure for FPD Analyzing Phosphorous Compound

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IATROSCAN Analysis (4A)

Chromarod-SIII Treatment Procedure for FPD Analyzing Phosphorous Compound

The special treatments (pretreatment, before-analysis treatment, after-analysis washing) for Chromarod®(s) are required for selective measurement of phosphorous by FPD (Flame Photometric Detector) of IATROSCAN® MK-6. The pretreatment of Chromarod-SIII is necessary for keeping the base stability in case of selective measurement of phosphorous by FPD. The before-analysis treatment is necessary for the analytical reproducibility. The after-analysis washing is necessary for removing the remained P compound(s) from the Chromarod. In case of the selective measurement of P by FPD, be sure to do these treatments.

1. **Pretreatment procedure of the Chromarod (When analyzing P by means of FPD)**

   To begin with, the new Chromarod(s) is treated in a following manner. By means of the pre-treatment, the surface of the Chromarod is washed and the base in case of selective measurement of P by FPD becomes stable.

   **Pretreatment procedure for the Chromarod**

   Chromarod-SIII (new)
   ↓
   steep the new Chromarod in 6N HCl solution for 22-24hour
   ↓
   wash the rod with distilled water for 1 minute (repeat twice)
   ↓
   dry it at 120°C for 1hour
   or
   dry 3 minutes at 120°C and conduct Blank Scan twice.
   (Hydrogen 160mL/min, Air 1.5L/min, Scan Speed 40sec//scan)
   ↓
   [go to the before-analysis treatment (or storage)]
   (In case the pretreated Chromarod is stored for future use, keep the Chromarod in a clean vessel with a lid.)

   **Caution:** Chromarod is fragile. Handle with care.
2. **Before-analysis treatment procedure of the Chromarod**

In case of analyzing a sample having radical(s) with dissociation constant affected by pH, such as phospholipid, there are some cases where the separation pattern becomes stable by using acidic or alkaline development solvent. If a sample development is carried out by use of a development solvent containing acid or alkaline, the pH on the Chromarod is memorized. In case of multi-development, the development should be performed by solvents such as a combination of alkaline and alkaline or a combination of alkaline and neutral. When amphoteric-electrolytes are developed by using a combination of acidic and alkaline solvents, the separation pattern may be unstable because the pH on the Chromarod can not be controlled.

The before-analysis treatment for Chromarod is shown on a following manner. MITSUBISHI CHEMICAL MEDIENCE CORPORATION recommends the following treatment methods when phosphorus compounds are analyzed by using alkaline development solvent.

---

**Before-analysis treatment**

The pre-treated Chromarod·SIII

↓

Blank Scan twice (Hydrogen 160mL/min, Air 1.5L/min, Scan Speed 40sec/scan)

↓

soak it in 2.5% NH₄OH solution for 10 minutes.

↓

wash the rod in distilled water for 1 minute (repeat twice)

↓

dry 3 minutes at 120°C and the Blank Scan twice

↓

[for analyzing]
3. **After analysis treatment procedure**

Phosphorous component sometimes remains on the rod even after IATROSCAN analysis due to its interaction with Chromarod. After the analysis, the Chromarod is required to wash in the following manner. Most of the P compound(s) can be eliminated by the washing procedure. The adsorbent of Chromarod·SIII is silica gel. The silica gel is gradually dissolved due to its poor alkali resistance during this washing. Therefore, the expected number of repeat use of the Chromarod is approx. 7 although it depends on how the rod is used.

From the reproducibility point of view, the Chromarod used for the FPD(P) measurement is valid only in a day. If the Chromarod is used the next day, the separation pattern will be different from that of the previous day.

---

**After-analysis washing**

The used Chromarod·SIII

↓

soak it in 2.5% NH₄OH solution for 10 minutes.

↓

wash the rod in distilled water for 1 minute (repeat twice)

↓

dry 3 minutes at 120°C and the Blank Scan twice

↓

[for analyzing]

---

**Caution:**

Do not use treated Chromarod with the special treatments for any analysis other than the phosphorus analysis, otherwise a good reproducibility will not be obtained by unstable pH on the Chromarod.
4. **Analysis of phospholipids (examples of applications)**

A) Application of lipids (standards mixture) analysis using the alkaline development solvent.

Sample:
1. Cholesterol (Nu-Chek-Prep.),
2. Phosphatidyl ethanolamine (egg: Avanti-Polar-Lipids),
3. Phosphatidyl choline (Wako Chemical),
4. Sphingomyelin (Avanti-Polar-Lipids)

Mobile Phase: Chloroform:Methanol:DW:25% Ammonia = 47:20:2.5:0.28 (20°C)

Detectors: FID (Flame ionization detector) and FPD

Interference Filter: The Interference Filter for Phosphorous (526nm)

**Iatroscan MK-6 conditions:**
- $H_2$ 160mL/min, Air 1.5L/min, Scan Speed 40sec/scan

Preparing a sample solution, the four lipids (Cholesterol, Phosphatidyl ethanolamine, Phosphatidyl choline, Sphingomyelin; @3mg/mL) were dissolved by Chloroform:MeOH (2:1). 1μL of the sample solution was spotted on the special treated Chromarod-III by means of micro-dispenser. It was developed by Chloroform:Methanol:DW:25% Ammonia (47:20:2.5:0.28, 20°C) and measured by FID and FPD(P) simultaneously.

![Figure 1](image_url)

**Figure 1**) Lipids analysis

Sample: Standards Mixture

Stationary Phase: Chromarod-III (treated)

Mobile Phase: Chloroform:Methanol:DW:25% Ammonia = 47:20:2.5:0.28 (20°C)

Measurement conditions: Hydrogen 160mL/min, Air 1.5L/min, Scan Speed 40sec/scan
- Dual detections of FID and FPD(P)

The result is shown in figure 1. Every lipid in the sample was detected by FID, and the phospholipids were detected by FPD(P) selectively.
B) Application of lipids (standards mixture) analysis using the alkaline and neutral development solvents.


Mobile Phase:
1st, Chloroform:Methanol:DW:25%Ammonia = 47:20:2.5:0.28 (20°C) 7cm
2nd, n-Hexane:Diethyl ether = 63:7 (20°C) 10cm

Iatroscan MK-6 conditions: H2 160mL/min, Air 1.5L/min, Scan Speed 40s/Scan

---

Sample: Standard Mixture

Stationary Phase: Chromarod-III (treated)

Mobile Phase: 1st, Chloroform:Methanol:Water:25%Ammonia 47:20:2.5:0.28 (20°C)
2nd, n-Hexane:Diethyl ether 63:7 (20°C)

Measurement conditions: Hydrogen 160mL/min, Air 1.5L/min, Scan Speed 40sec/scan
     Dual detections of FID and FPD(P)
1-5 Oncorhynchus Keta (muscle)  
Mobile phase: System-1

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<th>A OR H</th>
<th>MK</th>
<th>CONC</th>
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<td>33.7929</td>
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</table>
TOTAL | | | | | 100.0000 |
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1-6 Oncorhynchus Keta (muscle)  
Mobile phase: System-1

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<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
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<th>A OR H</th>
<th>MK</th>
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TOTAL | | | | | 100.0000 |
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1-7 Oncorhynchus Keta (ovary)  
Mobile phase: System-1

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<th>RT</th>
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<th>MK</th>
<th>CONC</th>
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<tbody>
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TOTAL | | | | | 100.0000 |
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1-8 Navodon modestus (muscle)  
Mobile phase: System-1

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<th>NAME</th>
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<td>1.2721</td>
</tr>
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</table>
TOTAL | | | | | 100.0000 |
```
1-9 *Xiphias gladius (muscle)*

Mobile phase: System-1

0.02
2 FA
0.26
3 ST

TOTAL 1 TG

CAL. METHOD 00
SP PA PB
100000+0 100000+01 100000+01
NO. NAME RT A OR H MK CONC
1 0.128 66863 85.3356
2 0.176 279 0.3602
3 0.302 619 0.6422
4 0.466 7652 M 10.4918
TOTAL 72555 100.0000

1-10 *Xiphias gladius (eye)*

Mobile phase: System-1

0.02
1
0.26
2

TOTAL 2 TG

CAL. METHOD 00
SP PA PB
100000+0 100000+01 100000+01
NO. NAME RT A OR H MK CONC
1 0.075 178 M 0.2462
2 0.128 76114 M 97.3114
3 0.226 149 M 0.2334
4 0.278 211 M 0.2818
5 0.258 104 M 0.2268
6 0.305 453 M 0.6252
7 0.337 177 M 0.2445
8 0.371 226 M 0.3115
9 0.466 559 M 0.5087
TOTAL 72555 100.0000

1-11 *Lepidocybium flavobrunneum (muscle)*

Mobile phase: System-1

0.02
2 FA
0.26
3 ST

TOTAL 1 TG

CAL. METHOD 00
SP PA PB
100000+0 100000+01 100000+01
NO. NAME RT A OR H MK CONC
1 0.124 69369 M 97.2736
2 0.287 816 M 0.6467
3 0.315 394 M 0.5657
4 0.426 134 0.1862
5 0.466 1099 M 1.6418
TOTAL 71313 100.0000

1-12 *Lepidocybium flavobrunneum (liver&testes)*

Mobile phase: System-1

0.02
2 FA
0.25
3 ST

TOTAL 1 TG

CAL. METHOD 00
SP PA PB
100000+0 100000+01 100000+01
NO. NAME RT A OR H MK CONC
1 0.109 30540 M 76.3577
2 0.161 4743 M 10.3612
3 0.216 115 M 0.2036
4 0.260 256 M 0.6839
5 0.293 799 M 1.7390
6 0.326 239 M 0.5479
7 0.466 6579 M 16.0063
TOTAL 43672 100.0000
### 1-13 Cololabis saira (muscle)

**Mobile phase:** System-1

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<th>OR (%)</th>
<th>H (%)</th>
<th>MK (%)</th>
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**Cal. Method:** 80% SF, 20% PA, 100000 μg/mL PB

### 1-14 Cololabis saira (viscera)

**Mobile phase:** System-1

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<th>OR (%)</th>
<th>H (%)</th>
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**Cal. Method:** 80% SF, 20% PA, 100000 μg/mL PB

### 1-15 Sardinops melanosticta melanosticta (muscle)

**Mobile phase:** System-1

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<tr>
<th>NO.</th>
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<th>RT (min)</th>
<th>A (%)</th>
<th>OR (%)</th>
<th>H (%)</th>
<th>MK (%)</th>
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**Cal. Method:** 80% SF, 20% PA, 100000 μg/mL PB

### 1-16 Sardinops melanosticta melanosticta (viscera)

**Mobile phase:** System-1

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<th>OR (%)</th>
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**Cal. Method:** 80% SF, 20% PA, 100000 μg/mL PB

### 1-17 Scomber japonicus (muscle)

**Mobile phase:** System-1

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**Cal. Method:** 80% SF, 20% PA, 100000 μg/mL PB

### 1-18 Scomber japonicus (viscera)

**Mobile phase:** System-1

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I-19 Standard mixture-II
Mobile phase: System-3

CAL. METHOD 06 SF PA PB
.100000a+03 .100000b+01 .100000c+01

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I-20 Kastuconus pelamis (muscle)
Mobile phase: System-4

CAL. METHOD 06 SF PA PB
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2) Plankton lipid

2-1 Copepoda (Neocalanus plumchrus)

Mobile phase: System-2

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CAL. METHOD SF PA PB
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TOTAL 10994 100.0000

2-2 Artemia

Mobile phase: System-3

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CAL. METHOD SF PA PB
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TOTAL 37531 100.0000

3) Lipid oxidation and hydrolysis in dried anchovy products during drying and storage.

Mobile phase: System-5

Reference 6)

1. LPC
2. PC
3. PE

Maruhoshi

Hirakiboshi

Niboshi

Raw material After drying for 20h After storage for 50days

Iatroscan chromatograms of polar lipid fractions of anchovy products after drying and storage.
Analysis of Glyceride isomers using Boric acid-Impregnated Chromarods
ANALYSIS OF GLYCERIDE ISOMERS BY BORIC ACID-IMPREGNATED CHROMAROD

Using IATROSCAN analytical methods, triglyceride (TG), diglyceride (DG) isomers, mono-glyceride (MG) isomers, and fatty acids (FA) can be separated and analyzed by means of borice acid-treatment of the Chromarod.

1. Boric acid-treated Chromarod

The use of Chromarod-SIII (silica gel) enables the separation of TG, 1,3-DG, 1,2-DG, MG to occur in the analysis of glyceride, but difficulties are experienced in separation 1-MG and 2-MG (see Fig. 1). However, when a boric acid-treated Chromarod is used, prepared by treatment with a boric acid aqueous solution, all the components, including 1-MG and 2-MG isomers can be separated.

Fig. 1 - Glyceride separated by the Chromarod-SIII

CONDITIONS:

Stationary phase: CHROMAROD-SIII
Mobile phase: Benzene : Chloroform : Acetic acid (50:20:0.7)
Gas flow : $\text{H}_2$ 160ml/min, Air 2.0 L/min
Scanning speed: 30sec/scan Integrator: Attenuation 16
(1) Preparation of boric acid-treated Chromarod

The preparation of a boric acid-treated Chromarod is carried out according to the following procedure (also refer to Fig. 2):

1. Chromarod-SIII is first subjected to a blank-scan.
2. The Chromarod-SIII is then dipped into a 3% boric acid aqueous solution for 5 minutes.
3. The Chromarod-SIII is dried out in a drying chamber for 5 minutes at 120°C.
4. Finally, the Chromarod-SIII is subjected to a single blank-scan immediately.

Now, the Chromarod-SIII is fully treated with a boric acid.

Fig. 2 Preparation and use of Boric acid impregnated CHROMAROD.
2. Example of first analysis - A mixture of the standard compounds

(1) Sample:

A chloroform solution of a mixture of tri-palmitin; 1,2-di-palmitin; 1,3-di-palmitin; palmitic acid; 1-mono-stearin; 2-mono-palmitin. The concentration of each component in the solution is 1-2mg/ml.

(2) Development method:

1. The boric acid-treated Chromarod is subjected to a blank-scan.
2. 1μl of sample is spotted onto the Chromarod.
3. The spotted sample is developed for 8cm using 100% chloroform.
4. The developed sample is then dried at room temperature for 2 - 3 minutes.
5. Prepare a solvent mixture of 8 : 2 methanol (MeOH) and concentrated aqueous ammonia (NH₄OH).
6. The Chromarod is then again developed for 10cm with developing solvent of Chloroform : MeOH/NH₄OH, 70 : 0.05.
7. The developed sample is dried in an oven at 120°C for 1 - 2 minutes.
8. Finally, scanning is performed to produce a chromatographic recorder trace (Fig. 3).

3. Example of second analysis - Hyrolyzed compounds of Triglyceride.

(1) Sample

Hydrosis compounds of olive-oil by lipase

(2) Development method:

The development method is same manner as that of the first Example (Fig. 4).
Fig. 3 - Separation performed on a boric acid-impregnated CHROMAROD with mixtures of glyceride and fatty acid

SAMPLE 49  14:42  JUNE 24  1947

CAL. METHOD  00
SF  PA  PB
1.00000x+03  1.00000x+01  1.00000x+01

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TOTAL  55561  100.0000

CONDITIONS:
Stationary phase: 3% boric acid impregnated CHROMAROD-SIII
Mobile phase:
1st Chloroform 100%  8 cm
2nd Chloroform : Methanol/Ammonia (8 : 2)  70 : 0.05  10 cm
Gas flow rate: H₂ 160ml/min.  Air flow rate: 2.0%/min
Scanning speed: 30 sec/scan
Intercorder: Attenuation 16

- 4 -
Fig. 4 - Separation performed on a boric acid-impregnated CHROMAROD with hydrolyzed compounds of olive oil

<table>
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<th>PB</th>
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CONDITIONS:

Stationary phase: 3% boric acid impregnated CHROMAROD-SII

Mobile phase:

1st Chloroform 100% 8 cm
2nd Chloroform: Methanol/Ammonia (8 : 2)
20 : 0.05 10 cm

Gas flow rate: H₂ 160ml, Air flow rate: 2.0l/min
Scanning speed: 30 sec/scan
Integrator: Attenuation 16
4. Regeneration of boric acid-impregnated CHROMAROD

The repeated use of the impregnated CHROMAROD often causes separating conditions to deteriorate. However, the process of washing and regenerating treatment to repeatedly-used boric acid-impregnated CHROMAROD make them reusable. It is recommended that a one impregnation can be used for 5 - 6 runs.

1) Method of washing and regenerating boric acid-impregnated CHROMAROD.

1) The CHROMAROD is immersed overnight in nitric acid.

2) The CHROMAROD is then removed from the acid and washed with pure water. In this case, the CHROMAROD may be put in a container such as a test tube and then washed with pure water in batches, ensuring that 5 to 6 water changes take place.

3) The CHROMAROD is dried in an oven at 120°C for one hour.

As stated, now the CHROMAROD can be washed.

Regeneration of a boric acid-impregnated CHROMAROD can be performed same manner as that of stated in (1) - 1) through.

A CHROMAROD which is subjected to washing and regeneration often exhibits a separation capability slightly different from its original capability, a phenomenon which is caused by variation in the adsorptive activity of the regenerated CHROMAROD. However, its original separation performance can be restored by slightly changing the composition of the developing solvent used.

Reference:

1. Masamichi Tanaka, Toshiro Itoh, and Hiroshi Kaneko
Analysis of Triglyceride Molecular Species using Silver nitrate-Impregnated Chromarods
Analysis of Triglyceride Molecular Species using Silver Nitrate impregnated Chromarods (new type SIII)

Triglyceride molecular species can easily be separated and analysed quantitatively on the basis of different degrees of unsaturation of the constituent fatty acids by the IATROSCAN TLC/FID using the new type CHROMAROD SIII treated with silver nitrate. This analysis can be used as a simple method of detection for oils and fats.

1. Chromarods treated with silver nitrate.

   A. Preparation of the CHROMARODS:

   Make up a 3% silver nitrate Acetonitrile solution:

   The silver nitrate (0.9g), fully dissolved in 30 ml of acetonitrile, is placed in a test tube. Cover the test tube with aluminium foil to exclude the light.

   The preparation of a silver nitrate-treated CHROMAROD is then carried out in the following manner:

   [1] CHROMAROD-SIII is first subjected to a blank-scan in the MK IV IATROSCAN to remove any absorbed organic substances.

   [2] Immerse the CHROMAROD into 3% silver nitrate Acetonitrile solution for 5 minutes.

   [3] The CHROMAROD is then removed from the solution and dried in an oven at 120 degrees C for 10 minutes.

   In this way the CHROMAROD is properly treated.

   Fig 1. The preparation sequence for CHROMARODS treated with silver nitrate

   ![Diagram of CHROMAROD preparation sequence]

-1-
B. Washing and regeneration of the CHROMARODS treated with silver nitrate:

Once CHROMARODS treated with silver nitrate are used, the effectiveness of the silver nitrate is reduced. The following procedure for washing and regeneration is, therefore, recommended.

1. Immerse CHROMARODS in concentrated nitric acid overnight.
2. Prepare two test tubes filled with pure water.
3. Remove CHROMARODS from the nitric acid and place them in the test tubes in order to wash them.
4. The CHROMARODS are then dried in an oven at 120 degrees C for one hour.
5. Treat CHROMARODS with silver nitrate in same manner as stated in A, through 2.

The RODS can be repeatedly used after being washed and reactivated according to the above procedures (repeated use for up to ten times or more does not adversely affect the separation performance).

2. Analytical Method

1. Spot a chloroform solution (1 µl) containing 10 to 20 µg of lipid onto the silver nitrate-treated CHROMARODS prepared according to Section 1.

2. Develop the spot using

Benzene : Ethylether : Formic acid
68  2  0.1  ...... (solvent mixture A)

Benzene : Ethylether : Formic acid
65  5  0.1  ...... (solvent mixture B)

Benzene : Chloroform : Acetic acid
63  7  0.7  ...... (solvent mixture C)

The solvent mixture A is suitable for the analysis of samples containing constituents having up to about 5 double combinations with a lesser degree of unsaturation. Use solvent mixtures B and C for relatively higher degrees of unsaturation.

Remarks: While developing the spot, the development chamber is required to be wrapped in aluminium foil to prevent decomposition of silver nitrate by exposure to light.

3. Dry the CHROMARODS after development at 120 degrees C in a dryer for 5 minutes to remove all solvent.
4. Measure the CHROMARODS in the IATROSCAN analyser after solvent removal.

Conforming to the analytical procedures explained above, a standard mixture of triglyceride (triolein, trilinolein and triolein) developed in solvent mixtures A, B and C exhibit chromatographic separations shown in Fig.2. Identification of oils and fats is also shown in Figs. 3 and 4 respectively.
Fig. 2 Chromatograms obtained with a mixture of standard Triglycerides by using silver nitrate-treated CHROMARODS

Fig 2-1. Solvent mixture A

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>RT</th>
<th>A OR H</th>
<th>MK</th>
<th>COHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P3</td>
<td>0.172</td>
<td>12264</td>
<td>35.2200</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>03</td>
<td>0.200</td>
<td>12522</td>
<td>36.0621</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>L3</td>
<td>0.141</td>
<td>.9118</td>
<td>26.0820</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>32234</td>
<td></td>
<td>100.0000</td>
<td></td>
</tr>
</tbody>
</table>

Fig 2-2. Solvent mixture B

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>RT</th>
<th>A OR H</th>
<th>MK</th>
<th>COHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P3</td>
<td>0.162</td>
<td>26739</td>
<td>34.5836</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>03</td>
<td>0.204</td>
<td>20808</td>
<td>35.3630</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>L3</td>
<td>0.176</td>
<td>.9379</td>
<td>28.1211</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>75992</td>
<td></td>
<td>100.0000</td>
<td></td>
</tr>
</tbody>
</table>

Fig 2-3. Solvent mixture C

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>RT</th>
<th>A OR H</th>
<th>MK</th>
<th>COHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P3</td>
<td>0.187</td>
<td>204520</td>
<td>35.7239</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>03</td>
<td>0.258</td>
<td>202936</td>
<td>34.7292</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>L3</td>
<td>0.104</td>
<td>427</td>
<td>20.5144</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.457</td>
<td>193438</td>
<td>20.3142</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.452</td>
<td>46</td>
<td>0.0273</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>6548</td>
<td></td>
<td>100.0000</td>
<td></td>
</tr>
</tbody>
</table>

Identifications:
- P3: Tripalmitin
- 03: Triolein
- L3: Trilinolein

Mobile phase:
- Solvent mixture A
  Benzene: Ethyl ether: Formic acid 68:2:0.1
- Solvent mixture B
  Benzene: Ethyl ether: Formic acid 65:5:0.1
- Solvent mixture C
  Benzene: Chloroform: Acetic acid 63:7:0.7

CONDITIONS:
- Stationary phase: CHROMAROD-SII (3% silver nitrate impregnated)
- Gas flow: H₂ 160ml/min, Air 2.0 l/min
- Scanning speed: 30sec/scan
- Integrator TC-11
- Playback attenuation: 16-32mv f.s
3. Application for identification of oils and fats. Figs. 3 and 4 show chromatograms obtained with samples of fats, palm oil, cocoa butter, coconut oil and beef tallow.

Fig. 3 Patterns of separated components of fats using CHROMARODS treated with silver nitrate.

**Fig 3-1. Palm oil**

**Solvent mixture A**

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>RT (min)</th>
<th>A or H</th>
<th>NC</th>
<th>CONC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.216</td>
<td>17552</td>
<td>H</td>
<td>33.34%</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.261</td>
<td>12152</td>
<td>H</td>
<td>27.22%</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.311</td>
<td>6005</td>
<td>H</td>
<td>16.72%</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.384</td>
<td>3473</td>
<td>H</td>
<td>11.23%</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.446</td>
<td>4224</td>
<td>H</td>
<td>7.30%</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>48031</td>
<td></td>
<td></td>
<td>100.00%</td>
</tr>
</tbody>
</table>

**Fig 3-2. Cacao oil**

**Solvent mixture A**

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>RT (min)</th>
<th>A or H</th>
<th>NC</th>
<th>CONC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.218</td>
<td>46571</td>
<td>H</td>
<td>79.57%</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.242</td>
<td>8042</td>
<td>H</td>
<td>10.14%</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.324</td>
<td>1744</td>
<td>H</td>
<td>3.03%</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.416</td>
<td>1951</td>
<td>H</td>
<td>3.09%</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>56929</td>
<td></td>
<td></td>
<td>100.00%</td>
</tr>
</tbody>
</table>

**Fig 3-3. Coconut oil**

**Solvent mixture A**

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>RT (min)</th>
<th>A or H</th>
<th>NC</th>
<th>CONC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.205</td>
<td>21237</td>
<td>H</td>
<td>30.94%</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.274</td>
<td>1372</td>
<td>H</td>
<td>3.50%</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.322</td>
<td>5376</td>
<td>H</td>
<td>4.09%</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.355</td>
<td>2157</td>
<td>H</td>
<td>2.70%</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.376</td>
<td>4911</td>
<td>H</td>
<td>1.51%</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>34526</td>
<td></td>
<td></td>
<td>100.00%</td>
</tr>
</tbody>
</table>

**Fig 3-4. Beef tallow**

**Solvent mixture A**

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>RT (min)</th>
<th>A or H</th>
<th>NC</th>
<th>CONC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.135</td>
<td>440</td>
<td>H</td>
<td>46.6%</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.217</td>
<td>25003</td>
<td>H</td>
<td>15.7%</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.266</td>
<td>10932</td>
<td>H</td>
<td>10.8%</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.312</td>
<td>2419</td>
<td>H</td>
<td>4.18%</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.370</td>
<td>5991</td>
<td>H</td>
<td>6.04%</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>0.447</td>
<td>5393</td>
<td>H</td>
<td>6.05%</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>0.456</td>
<td>6057</td>
<td>H</td>
<td>10.23%</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>56575</td>
<td></td>
<td></td>
<td>100.00%</td>
</tr>
</tbody>
</table>

- 4 -
Fig. 4. Patterns of separated components of fats using CHROMAROIDS treated with silver nitrate.

**Fig 4-1. Olive oil**

Solvent mixture A

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>15130 JUNE 30 1987</th>
</tr>
</thead>
</table>

**Fig 4-2. Soybean oil**

Solvent mixture B

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>14113 JULY 01 1987</th>
</tr>
</thead>
</table>

**Fig 4-3. Sunflower oil**

Solvent mixture B

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>14117 JULY 01 1987</th>
</tr>
</thead>
</table>

**Fig 4-4. Safflower oil**

Solvent mixture C

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>13125 JULY 02 1987</th>
</tr>
</thead>
</table>
Fig 4-5. Linseed oil
Solvent mixture B

SAMPLE 16 14120 JULY 01 1967

Fig 4-6. Tung oil
Solvent mixture B

SAMPLE 16 14125 JULY 01 1967

Fig 4-7. Rape seed oil
Solvent mixture B

SAMPLE 6 15182 JUNE 30 1967

CONDITIONS:

Stationary phase:
CHROMAROD-SIII (3% AgNO₃ impregnated)

Mobile phase:
Solvent mixture A
Benzene : Ethyl ether : Formic acid
68 : 2 : 0.1

Solvent mixture B
Benzene : Ethyl ether : Formic acid
65 : 5 : 0.1

Solvent mixture C
Benzene : Chloroform : Acetic acid
63 : 7 : 0.7

Scanning speed : 30sec/min
Integrator TC-11
Playback attenuation : 16

Reference:
As shown in Figs. 3 and 4, solid lipids contain numbers of triglyceride molecular species of a relatively lesser degree of unsaturation. On the other hand, liquid lipids contain numbers of triglyceride molecular species of a higher degree of unsaturation which reflect the composition ratio of the constituents of fatty acids respectively.

Depending on the type of oils and fats, specific chromatograms of triglyceride constituents can be obtained and are applicable for the identification of oils and fats.

4. Analytical results

Table 1 shows reproducibility relating to a standard triglyceride analysed using a CHROMAROD-SIII treated with 3% silver nitrate, and the area percentage obtained. When the total amount spotted varies from 4 to 20 µg in the case of olive oil, the relationship between total weight and response can be seen (Fig. 5).

**Table 1 - Reproducibility of percentage of standard triglycerides.**

<table>
<thead>
<tr>
<th>Rod No.</th>
<th>Tripalmitin %</th>
<th>Triolein %</th>
<th>Trilinolein %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.6</td>
<td>37.6</td>
<td>27.8</td>
</tr>
<tr>
<td>2</td>
<td>34.9</td>
<td>36.8</td>
<td>28.3</td>
</tr>
<tr>
<td>3</td>
<td>33.7</td>
<td>36.8</td>
<td>29.5</td>
</tr>
<tr>
<td>4</td>
<td>34.9</td>
<td>36.4</td>
<td>28.7</td>
</tr>
<tr>
<td>5</td>
<td>34.9</td>
<td>36.9</td>
<td>28.2</td>
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<tr>
<td>6</td>
<td>34.6</td>
<td>36.7</td>
<td>28.7</td>
</tr>
<tr>
<td>7</td>
<td>34.9</td>
<td>37.1</td>
<td>28.0</td>
</tr>
<tr>
<td>8</td>
<td>35.4</td>
<td>37.5</td>
<td>27.1</td>
</tr>
<tr>
<td>9</td>
<td>36.3</td>
<td>36.9</td>
<td>26.8</td>
</tr>
<tr>
<td>10</td>
<td>35.0</td>
<td>36.7</td>
<td>28.3</td>
</tr>
<tr>
<td>X</td>
<td>34.9</td>
<td>36.9</td>
<td>28.2</td>
</tr>
<tr>
<td>CV %</td>
<td>1.9</td>
<td>0.8</td>
<td>2.8</td>
</tr>
</tbody>
</table>
Fig. 5 - Relationships between the amount of olive oil spotted and the response

Stationary phase:
CHROMAROD-S III (3% AgNO₃ impregnated)

Mobile phase:
Solvent mixture A
Benzene: Ethyl ether : Formica acid
68  2  0.1

Total olive oil weight (µg)
IATROSCAN ANALYSIS

22

Lipid Analysis using Copper Sulphate-Impregnated Chromarods
Lipid Analysis by Copper Sulphate Impregnated Chromarod-SIII

When a lipid sample to be analyzed by using untreated, normal CHROMAROD-SIII, the sensitivity in results may vary depending on components of the lipid such as a high degree of Cholesterol contained in it. It is therefore recommended that copper sulphate impregnated CHROMAROD-SIII to be used to minimize the type of variations involved.

1. Preparations of Copper Sulphate Impregnated CHROMAROD-SIII.
   Treat CHROMAROD-SIII with Copper Sulphate as follows:—
   1. Immerse the rod in concentrated sulfuric acid solution.
   2. Draw the rod from the solution and wash with running water.
      Rinse the rod with distilled water.
   3. Dry the rod at 120°C for 30 minutes.
   4. Immerse the rod in a 5 per cent Copper Sulphate solution.
   5. Draw the rod from the solution and dry at 120°C for 30 minutes.
   6. Blank scanning to be taken.

2. Analytical Practise
   1. Take blank scanning on the Copper Sulphate Impregnated CHROMAROD-SIII.
   2. Spot a sample onto the rod.
   3. Develop the rod with solvents.
   4. Dry the rod in a rod dryer, TK-5 at 120°C for 1 to 2 minutes.
   5. Detect the sample.
   6. Repeat the practise 1 to 5 if required to detect the sample.
3. Results

1. Standard Mixtures of Lipid-1

Sample: Cholesterol palmitate (Cho.E), Olive oil (TG), Cholesterol (Cho.)
Mobile phase: Hexane : Diethyle ether 63:7
Stationary phase: 5% Copper(II) sulphate impregnated CHROMAROD-SIII

![Graph showing the separation of Cho.E, TG, and Cho. with retention times and peak areas.]

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>RT</th>
<th>A (%)</th>
<th>H (%)</th>
<th>MK</th>
<th>CONC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cho.E</td>
<td>0.121</td>
<td>15884</td>
<td>T</td>
<td>27.8558</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>TG</td>
<td>0.241</td>
<td>29940</td>
<td>H</td>
<td>52.5130</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cho.</td>
<td>0.434</td>
<td>11189</td>
<td></td>
<td>19.6261</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>57914</td>
<td></td>
<td>100.0000</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 - Relationship between Peak Area % and Weight %

<table>
<thead>
<tr>
<th>Copper Sulphate Impregnated CHROMAROD-SIII</th>
<th>CHROMAROD-SIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>27.6</td>
</tr>
<tr>
<td>s.d.</td>
<td>0.35</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>1.3</td>
</tr>
<tr>
<td>wt (%)</td>
<td>26.0</td>
</tr>
<tr>
<td></td>
<td>X-wt</td>
</tr>
</tbody>
</table>

As it has been seen in the Table 1, when Copper Sulphate impregnated CHROMAROD-SIII was used, variations between Peak Area per cent and Weight per cent are narrowed and enhanced separation and reproducibility performances resulting it is appropriate to use for the lipid analysis.
2. Standard Mixtures of Lipid-2

Sample: Cholesterol palmitate (Cho.E), Cholesterol acetate (Cho.A), Cholesterol (Cho), Phosphatidyl ethanolamine (PE), Phosphatidyl choline (PC), Sphingomyeline (SPM)

Mobile phase:
1st Chloroform: Methanol: Water: Formic acid 50:25:2:0.5 5cm
2nd Hexane: Diethyle ether: Formic acid 65:5:2:0.15 10cm

Stationary phase: 5% Copper(II) Sulphate impregnated CHROMAROD-S III

---

Stationary phase: CHROMAROD-S III

---
Table 2 - Relationship between Peak Area per cent and Weight per cent for various Cholesterol Acetates and the reproducibility.

CHROMAROD-SIII

<table>
<thead>
<tr>
<th></th>
<th>CE/CA</th>
<th>CA</th>
<th>C/CA</th>
<th>PE/CA</th>
<th>PC/CA</th>
<th>SPM/CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>0.94</td>
<td>1.00</td>
<td>1.47</td>
<td>0.26</td>
<td>0.60</td>
<td>0.37</td>
</tr>
<tr>
<td>s.d.</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>1.0</td>
<td>0.7</td>
<td>5.1</td>
<td>2.9</td>
<td>7.1</td>
<td></td>
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<tr>
<td>wt ratio</td>
<td>1.20</td>
<td>1.00</td>
<td>1.15</td>
<td>0.53</td>
<td>0.86</td>
<td></td>
</tr>
</tbody>
</table>

\( n=5 \)

Copper Sulphate treated CHROMAROD-SIII

<table>
<thead>
<tr>
<th></th>
<th>CE/CA</th>
<th>CA</th>
<th>C/CA</th>
<th>PE/CA</th>
<th>PC/CA</th>
<th>SPM/CA</th>
</tr>
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<tr>
<td>X</td>
<td>0.98</td>
<td>1.00</td>
<td>1.33</td>
<td>0.47</td>
<td>0.78</td>
<td>0.43</td>
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<td>s.d.</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td></td>
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<tr>
<td>C.V. (%)</td>
<td>1.0</td>
<td>2.4</td>
<td>1.7</td>
<td>3.8</td>
<td>6.3</td>
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<tr>
<td>wt ratio</td>
<td>1.20</td>
<td>1.00</td>
<td>1.15</td>
<td>0.53</td>
<td>0.86</td>
<td></td>
</tr>
</tbody>
</table>

\( n=5 \)

Abbreviations:

CE; cholesterol palmitate, C; cholesterol, PE; phosphatidyl ethanolamine
PC; phosphatidyl choline, SPM; sphingomyeline,
CA; cholesterol acetate (internal standard)
Table 3 - Calibration Curve

Stationary phase: 5% Copper(II) sulphate impregnated CHROMAROD-S III

Stationary phase: CHROMAROD-S III
Experimental Analysis for Infinitesimal Components Contained in Main Ingredients
(Detection of Infinitesimal Phospholipid in Edible Oils)

Infinitesimal amounts of impurity and additive contained in the main ingredients may raise difficulties to analyze sometime. The data collected in this report describes applications by combined with the Iatroscan and Column chromatography to be used for detection of infinitesimal components contained in Edible oils.
Experimental Research Infinitesimal Components

There are few analytical methods available to detect infinitesimal phospholipids at each process of refining or purifying edible oils such as the Colorimetry, Lorenz and Acetone methods being applicable. The IATROSCAN MK-5 performs relatively easier to detect phospholipid contents in this particular application.

Method:

Prior to detection, it is recommended that an edible oil needs to be prepared with the following procedure in order to results be converted into amounts of Lecithin or obtained amount of phospholipid directly.

1. Provide a calibration curve.
2. Extract all phospholipids from edible oils by the Column Method and add internal standard substance in it to prepare a sample solution.
3. A certain amount of this sample solution, normally in microliter order, to be spotted onto Chromarod-SWW to run into the IATROSCAN in order to obtain amount of phospholipid from the calibration curve. Details are as follows:--

1. Preparation of Calibration Curve:

Correlative sensitivities by the IATROSCAN for lecithin against the internal standard substance (Cholesterol ester) shall be determined to provide a calibration curve between lecithin and Cholesterol ester.

1-1 Preparation of the Standard Mixture:

As indicated in the table below, Lecithin solution (25mg/5mL) and Cholesterol solution (25mg/5mL) shall be admitted into 4 vials in aliquote by using a female pipette in order to prepare the standard mixture at each volumetric ratio.

Table 1 - Composition of the Standard Mixture

<table>
<thead>
<tr>
<th>Volumetric ratio of Lecithin and Cholesterol ester</th>
<th>0.3</th>
<th>0.6</th>
<th>0.9</th>
<th>1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>25mg Cholesterol ester/5mL</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>(mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25mg Lecithin/5mL</td>
<td>0.15</td>
<td>0.30</td>
<td>0.45</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>(mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1-2 Analysis of the Standard Mixture by the IATROSCAN

1. Perform blank scanning for Chromarod-SIII to remove any unburn component may still remain on the rod.
2. 1μL of the standard mixture shall be spotted in aliquot onto the rod(s)
3. Development solvent, Hexane:Ethyl ether:Formic acid in proportions of 48:12:0.1 shall be developed to 10cm.
4. Dry up the Chromarod in a Rod Dryer at 120°C for 5 to 10 minutes.
5. Detection

1-3 Preparation of the Calibration Curve:

From the Chromatogram, obtain area ratios of Cholesterol ester (internal standard) against Lecithin in order to plot relationship between area and volume ratios in a graph paper.

Fig. 1 - Chromatogram of standard mixture

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>76</th>
<th>17:40</th>
<th>JULY 14 1987</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL. METHOD</td>
<td>D0</td>
<td>EF</td>
<td>PA</td>
</tr>
<tr>
<td>.100000a+03</td>
<td>.100000a+01</td>
<td>.100000a+01</td>
<td></td>
</tr>
<tr>
<td>NO.</td>
<td>NAME</td>
<td>RT</td>
<td>A OR H</td>
</tr>
<tr>
<td>1</td>
<td>0.143</td>
<td>6649</td>
<td>50.2995</td>
</tr>
<tr>
<td>2</td>
<td>0.474</td>
<td>6602</td>
<td>49.0644</td>
</tr>
<tr>
<td>TOTAL</td>
<td>17251</td>
<td>100.0000</td>
<td></td>
</tr>
</tbody>
</table>

CONDITION:
Stationary phase: CHROMAROD-SIII
Mobile phase:
Hexane: Diethyl ether: Formic acid
48 : 12 : 0.1
Gas flow: H₂ 160 ml/min, Air 21/min
Integrator attenuation: 16

Fig. 2 - Calibration curve of internal standard

Area ratio

Weight ratio

- 2 -
Table 2 - Date of Calibration Curve

<table>
<thead>
<tr>
<th>Weight ratio Lecithin/Cholesterol Palmitate</th>
<th>Area ratio Lecithin/Cholesterol Palmitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.34</td>
<td>0.23</td>
</tr>
<tr>
<td>0.68</td>
<td>0.61</td>
</tr>
<tr>
<td>1.02</td>
<td>0.95</td>
</tr>
<tr>
<td>1.35</td>
<td>1.29</td>
</tr>
</tbody>
</table>

2. Preparation of Sample Solution by Column Method:

Chromatography Column:

It is recommended to use a Chromatography Column consisting of 1.0 to 15mm, Length 300mm with a P.T.F.E cock attached and upper part provides conical shape ¥19/38 joint in common and bottom part provides a small vent plugged with quartz glass wool as shown in Fig. 3 below.

1. A 5g of IATROBEADS 6RS-8060 (silica acid 60µm made by latron) shall be admitted into a breaker with 50mL and Methanol to mix well. Pour this mixture into Chromatography Column.
2. Open a cock on the column and add Chloroform until Methanol reaches to the top of silica acid in order to wash the column.
3. Weigh the actual fatty acid amounts by balance weight precisely to 0.5g that was weighted in advance with a volumetric vial.
4. Add Chloroform for 20mL to resolve this sample fatty acid.
5. Pour the sample into the column while keeping the column to flow at velocity between 0.5 and 1mL.
6. Furthermore, wash the volumetric vial with 8mL Chloroform and pour the sample into the column quantitatively.
7. With Chloroform between 15 and 20mL, flush flow from the column. Now, Chloroform fractations obtained by all above procedure have eluted simple lipids.
8. Then perform elution with Methanol 60mL. At this stage, connect the end of Nitrogen gas pipe(rubber) and a connection(90° bent) attached on the ¥19/38 joint on the column. Apply gas pressure at rate of 2mL/min. onto the column in order to shorten time in elution of Methanol to be completely flown from the column for about 30 minutes. Please note that fractinations of Methanol consist of part of simple lipids and all amounts of Phospholipids.
9. After a certain amount of Cholesterol ester (internal standard) added into Methanol fractinations, condense until it has almost solidified (the internal standard should be added corresponding to amounts of phospholipid contained in the sample).
10. Add 1mL of solvent so called 'Forch' solvent (Chloroform:Methanol mixture, proportions of 2:1) to resolve. Now, sample preparation has been completed by all above procedure.
3. Measurement of the Sample Solution and Calculation of Phospholipid contents:

1) Spot the sample solution onto Chromarod-SIII. Care must be taken into consideration that amounts of this spot need to be adjusted equivalent to the spot size that of peaks in the internal standard calculated into the Calibration Curve. The same conditions shall be applied to a Calibration Curve. An example Chromatogram is shown in Fig. 4.

Fig 4 - Chromatogram obtained on Edible oil (Methanol Fraction)

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>RT (min)</th>
<th>A OR H</th>
<th>HK</th>
<th>CONC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.143</td>
<td>10775</td>
<td>M</td>
<td>42.4778</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.204</td>
<td>397</td>
<td>M</td>
<td>1.2975</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.293</td>
<td>2471</td>
<td>M</td>
<td>5.5574</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.322</td>
<td>622</td>
<td>M</td>
<td>3.4604</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.476</td>
<td>1028</td>
<td>M</td>
<td>41.0786</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>24638</td>
<td>100.0000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONDITION:
Stationary phase: CHROMAROD-SIII
Mobile phase:
Hexane : Diethyl ether : Formic acid
48 : 12 : 0.1
Gas flow: H₂ 160 ml/min, Air 2 l/min
Integrator attenuation: 16
Calculate area ratio for Cholesterol ester from the Chromatogram obtained on phospholipid in order to convert weight ratio from the Calibration Curve. Obtain amount of Phospholipid by the following equation.

\[ \text{Phospholipid content} \times = \frac{\text{Weight ratio} \times \text{Weight of internal ST}}{\text{Weight of sample}} \times 100 \]

*1

Example of Analysis:
Sample: edible oil in process of refining

<table>
<thead>
<tr>
<th>Weight of sample</th>
<th>Weight of internal ST</th>
<th>Area ratio</th>
<th>Weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>510.6</td>
<td>2.02</td>
<td>0.96</td>
<td>1.03</td>
</tr>
</tbody>
</table>

Phospholipid content \( \times = \frac{1.03 \times 2.02}{510.6} \times 100 = 0.41\% \)

*1 Refer to the conversion made from Calibration Curve to weight ratio.

*2 Weight of the internal standard added on wash components from the Column.

*3 Sample weight used for Chromatography.

As it has been seen in the Table 2 and 3, when Copper Sulphate impregnated CHROMAROD-SIII was used where more linear calibration curves are delineated reflecting to Area ratios against Weight ratios for Cholesterol Acetate contents in various lipids as well as resulting excellent separation and reproducibility performances. It is appropriate to use for the lipid analysis.

Reference

Tracing of Reaction with Enzymatic Experimental Reaction by iatroscan
Tracing of Reaction with Enzymatic Experimental Reaction by
"IATROSCAN"

Ever since there has been GLC, TLC and LC, often incorporating to
analyze reaction synthesis in organic reaction testing. However,
the GLC comes across difficulty to analyze those highboiling-point
compounds and thermal-unstable substance, the TLC requires color
producing after development, time consuming by LC and the HPLC
deteriorates its solid phase having inherent drawbacks and problems
involved particularly, agility and readily detection required in
tracing of reaction testing.

The IATROSCAN has overcome all these drawbacks to analyze synthesis
in the course of reaction with ease and readily.

1. Example Analysis:
   After an olive oil was used as a substrate and then hydrolytic
   reaction performed with lipase, synthesis in the course of reac-
tion was detected by the IATROSCAN to study reaction phenomenon.

(1) Reaction and Extraction Method:
   A rotator was admitted into a Erlenmeyer flask with a volume of
   50ml. A 2.5ml of lipase solution containing 1.0g of olive oil
   and 6mg of lipase were added into the flask and agitated to react
   at room temperature (at about 25°C). A 0.5ml of sample in aliquot
   was taken out from reaction solution at a constant interval and 1ml
   of water was added in it. An extraction was performed for
twice with 4ml of Chloroform:Methanol (proportions of 2:1) and 2ml
of Chloroform also added to obtain lipid as a sample.

(2) Detection Method:
The sample was diluted for two-fold. A 1µl of this diluted
solution was spotted onto Chromarod-III and developed with Hexane:
Ethyl ether:Acetic acid in proportions of 55:15:0.5.
After development, Chromarod were dried out at 120°C for 5 minutes
to remove solvent and then detected by the IATROSCAN.

(3) Detection Conditions:
   Stationary phase: CHROMAROD-III
   Gas flow: N₂ 160ml/min, Air 2.0l/min,
   Scanning speed: 30sec/scan
   IATROCORDER TC-11: Playback attenuation: 8 - 16mv f.s

(4) Results:
   Results in each reaction time course are shown in Fig.1 and 2 were
clearly revealing a substrate of triglyceride decreased and fatty-
acid in synthesis increased.
Fig. 1 Tracing of enzymatic reaction by 1atroscan.

Reaction time:  0 min

Reaction time:  10 min

Reaction time:  30 min

Reaction time:  60 min

Reaction time:  120 min

Peak 1:  Triglyceride
Peak 2:  Fatty acid
Peak 3:  1,2-Diglyceride
Peak 4:  Monoglyceride

- 2 -
2. Applications:

(1) It is recommended that when a calibration curve prepared in advance, reaction compounds and synthesis can be quantitative and determined reaction speed.

(2) This is the most efficient analytical system in particular, when a side reaction of synthesis to be known in synthetic reaction.

(3) Detection and Quantitative of Impurity Compounds:
For a single separation in distillation, sublimation, re-crystallization, extraction and LC Methods, this is the most efficient system to detect synthesis as a single substance.

As seen in above, the IATROSCAN performs excellent detection when a rapid or convenient detection is required.
IATROSCAN ANALYSIS

9

Separation of Isomers and Derivatives
IATROSCAN ANALYSIS (9)

Separation of the isomers and derivatives

--- INDEX ---

<table>
<thead>
<tr>
<th>Substance</th>
<th>Page</th>
</tr>
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<tbody>
<tr>
<td>Androsterone</td>
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<tr>
<td>Bromvalerylurea</td>
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<td>Caffeine</td>
<td>5</td>
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<td>Chlorpheniramine maleate</td>
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<td>m-Coumaric acid</td>
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<td>p-Coumaric acid</td>
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<td>Dodecanedioic acid</td>
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<td>Quinidine sulphate</td>
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<td>Quinine hydrochloride</td>
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<td>Tetrahydrocortisol</td>
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<td>α-Tocopherol</td>
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<td>8</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>8</td>
</tr>
<tr>
<td>σ-Tocopherol</td>
<td>8</td>
</tr>
<tr>
<td>Trigonelline</td>
<td>7</td>
</tr>
</tbody>
</table>
Stationary phase: Chromarod-SII
Mobile Phase:
1. Ethylacetate : Acetone = 45 : 15
2. Chloroform : Methanol : Water = 63 : 7 : 0.7
3. n-Hexane : Ethylether (twice) = 35 : 35

Component:
1. Digitoxigenin
2. Digitoxin
3. Gitoxigenin

Component:
1. Dioxyprostaglandin Me
2. Quinidine sulphate
3. Quinine hydrochloride
Stationary phase: Chromarod-SII
Mobile Phase: Benzene : Acetone
70 : 1.4

Component:
1. OCH₃
   O
   OBu BuO
   OCH₃

2. OCH₂
   O
   OH BuO
   OCH₃

3. OCH₂
   O
   O Bu HO
   OCH₃

4. OCH₂
   O
   O OH HO OCH₃
   Bu : CH₂Ph

Component:
1. Epicholesterol

2. Cholesterol

Component:
1. Androsterone

1. Dehydroepiandrosterone

2. Etiocholanolone
Stationary phase  Chromarod-SII  
Mobile Phase  
Chloroform : Acetone : Methanol : 25% Ammonia  
30 : 30 : 1.5 : 1.2

Component:  
1. Noscapine

\[
\text{Component:}  
1. \text{Noscapine}
\]

2. Chlorpheniramine maleate

\[
\text{Component:}  
2. \text{Chlorpheniramine maleate}
\]

3. d-Methylephedrine hydrochloride

\[
\text{Component:}  
3. d-Methylephedrine hydrochloride
\]

4. Caffeine

\[
\text{Component:}  
4. \text{Caffeine}
\]

Stationary phase  Chromarod-SII  
Mobile Phase  
Benzene : Ethylether : Acetic acid  
42 : 16 : 1

Component:  
1. Bromvalerylurea

\[
\text{Component:}  
1. \text{Bromvalerylurea}
\]

2. Ethenzamide

\[
\text{Component:}  
2. \text{Ethenzamide}
\]

3. Phenacetin

\[
\text{Component:}  
3. \text{Phenacetin}
\]

4. Disyston

\[
\text{Component:}  
4. \text{Disyston}
\]

Stationary phase  Chromarod-SII  
Mobile Phase  n-Hexane : Acetone  
45 : 15

Component:  
1. Disyston sulfone

\[
\text{Component:}  
1. \text{Disyston sulfone}
\]

2. Disyston sulfoxide

\[
\text{Component:}  
2. \text{Disyston sulfoxide}
\]

3. Disyston sulfoxide

\[
\text{Component:}  
3. \text{Disyston sulfoxide}
\]
Stationary phase: Chromarod·SII
Mobile Phase:
- n-Hexane : Toluene
  50 : 10
- Benzene
- Chloroform : Methanol : Formic acid
  70 : 2.8 : 0.42
- n-Hexane : Ethylether
  66 : 9

Component:
1. [Chemical structure]
2. [Chemical structure]
3. Unknown

Component:
1. m-Phenoxy benzaldehyde
2. Cyano·m-Phenoxyl benzyl alcohol

Component:
1. Coumarin
2. Coumalic acid
3. p-Coumalic acid

Component:
1. [Chemical structure]
Component:
1. d-Methionine ethylate
\[
\text{CH}_3\text{S(CH}_2)_2\text{CH(NH}_2\text{)COOC}_2\text{H}_5
\]
2. d-Methionine
\[
\text{CH}_3\text{S(CH}_2)_2\text{CH(NH}_2\text{)COOH}
\]
Component:
1. Nicotinamide
2. Nicotinic acid
\[
\text{CONH}_2 \quad \text{COOH}
\]
3. Trigonelline
4. Quinolinic acid
\[
\text{COO}^- \quad \text{COOH}
\]
Component:
1. Estrone
2. Estradiol
3. Estriol
Component:
1. Hydrocortisone
2. Tetrahydrocortisol
3. β-Cortolone
Stationary phase: Chromarod-SII
Mobile Phase: Benzene : n-Hexane
72 : 3

Component:
1. α-Tocopherol
2. γ-Tocopherol
3. δ-Tocopherol
4. Tocol

Component:
1. Dioctyl phthalate
2. Diethyl phthalate
3. Dimethyl phthalate

Component:
1. Dodecanedioic acid
2. 12-Hydroxy dodecanoic acid

Component:
1. OCH₃
2. (Ph₅P)₄N⁺
3. Ph₅P⁻
4. OCH₃

Stationary phase: Chromarod-S
Mobile Phase: Butylether : n-Hexane
60 : 15

Component:
1. Dioctyl phthalate

Component:

Stationary phase: Chromarod-SII
Mobile Phase: Benzene : Dioxane : Formic acid
54 : 9 : 1

Component:
1. OCH₃

Stationary phase: Chromarod-SII
Mobile Phase: Benzene : Methanol
70 : 5
Analysis of Surface Active Agents by Iatroscan
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   2 – 2. α-Olefine sulfonate  
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   2 – 4. Sodium N-lauroyl methyltaurate  
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   3 – 11. POE(9) Secondary alkyl ether  
   3 – 12. POE(12) Secondary alkyl ether  
   3 – 13. POE(2) Cetyl ether  
   3 – 14. POE(7) Cetyl ether  
   3 – 15. POE(15) Cetyl ether  
   3 – 16. POE(1) POP(4) Cetyl ether  
   3 – 17. POE(20) POP(6) Cetyl ether  
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   3 – 19. Glyceryl monostearate
4) Amphoteric surface active agents
   4 - 1. Lauryl dimethyl aminoacetic acid betaine
   4 - 2. Cocoyl amidopropyl dimethyl aminoacetic acid betaine

5) Other surface active agents
   5 - 1. Sugar ester

6) Mixture
   6 - 1. Sodium alkyl benzene sulfate + EO(4) Alkyl ether

B) Conditions
   Spotting: ca. 1 μl of the sample solution containing 10-20 μg of surface active agents
   Stationary phase: CHROMAROD-SIII
   Gas flow: H₂ 160 ml/min, Air 2.01/min
   Scanning speed: 30sec/scan
   Integrator TC-11
   Playback attenuation 16-32mV f.s.
1) Cationic surface active agents

Fig 1-1. Monoalkyl ammonium chloride

Mobile phase:

Acetone : Water : conc Ammonia (63 : 6 : 1)

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Fig 1-2. Dialkyl ammonium chloride

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Fig 1-3. Stearoyl amidioethyl diethyl amine

Mobile phase:

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2) Anionic surface active agents

Fig 2-1. Sodium alkyl benzene sulfate

Mobile phase:

Chloroform : Methanol : Formic acid (30 : 30 : 1)

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Fig 2-2. a-Olefine sulfonate

Mobile phase:

Chloroform : Methanol : Formic acid (30 : 30 : 1)

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Fig 2-3. Sodium N-palmitoyl sarcosinate
Mobile phase:
Chloroform : Methanol : Formic acid (50 : 10 : 1)

Fig 2-4. Sodium N-lauroyl methytaurate
Mobile phase:
Chloroform : Methanol : Formic acid (50 : 10 : 1)

Fig 2-5. Sodium lauryle sulfate
Mobile phase:
Chloroform : Methanol : Formic acid (50 : 10 : 1)

3) Nonionic surface active agents
Fig 3-1. POE(2) Monostearate
Mobile phase:
Ethyl acetate : Acetone : Water (45 : 20 : 3)

Fig 3-2. POE(10) Monostearate
Mobile phase:
Ethyl acetate : Acetone : Water (45 : 20 : 3)
Fig 3-3. Diglyceryl monooleate

Mobile phase:

Ethyl acetate : Acetone : Water (62 : 15 : 3)

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Fig 3-4. Diglyceryl dioleate

Mobile phase:

Ethyl acetate : Acetone : Water (62 : 15 : 3)

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Fig 3-5. Solbitan monooleate

Mobile phase:

Chloroform : Methanol : Formic acid (65 : 2 : 1)

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Fig 3-6. Solbitan trioleate

Mobile phase:

Chloroform : Methanol : Formic acid (65 : 2 : 1)

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Fig 3-7. ED(4) Alkyl ether

Mobile phase:

Ethyl acetate : Acetone : Water (52 : 15 : 3)

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Fig 3-8. ED(10) Nonyl phenyl ether

Mobile phase:

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Fig. 3-9. POE(9) Lauryl ether
Mobile phase:
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**TOTAL**
- 31474 - 100.0000

Fig 3-10. POE(15) Oleyl ether
Mobile phase:
Ethyl acetate : Acetone : Water (52 : 15 : 3)

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<th>M (mM)</th>
<th>CONC. (mg/ml)</th>
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**TOTAL**
- 44210 - 100.0000

Fig 3-11. POE(9) Secondary alkyl ether
Mobile phase:
Ethyl acetate : Acetone : Water (52 : 15 : 3)

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<th>M (mM)</th>
<th>CONC. (mg/ml)</th>
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**TOTAL**
- 31474 - 100.0000

Fig 3-12. POE(12) Secondary alkyl ether
Mobile phase:
Ethyl acetate : Acetone : Water (52 : 15 : 3)

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<tr>
<th>No.</th>
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<th>RT (min)</th>
<th>A (a.u.)</th>
<th>M (mM)</th>
<th>CONC. (mg/ml)</th>
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**TOTAL**
- 1303 - 100.0000

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### Fig 3-13. POE(2) Cetyl ether

**Mobile phase:**

Ethyl acetate : Acetone : Water \(52 : 15 : 3\)

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### Fig 3-14. POE(7) Cetyl ether

**Mobile phase:**

Ethyl acetate : Acetone : Water \(52 : 15 : 3\)

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### Fig 3-15 POE(15) Cetyl ether

**Mobile phase:**

Ethyl acetate : Acetone : Water \(52 : 15 : 3\)

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<td>109</td>
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<td>109</td>
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<td>109</td>
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### Fig 3-16 POE(4) POP(4) Cetyl ether

**Mobile phase:**

Ethyl acetate : Acetone : Water \(52 : 15 : 3\)

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</table>
Fig 3-17. POE(20) POP(6) Cetyl ether
Mobile phase:
Ethyl acetate : Acetone : Water : Formic acid
(30 : 30 : 3 : 1)

Fig 3-18. Sodium POE(3) alkyl ether sulfate
Mobile phase:
Chloroform : Methanol : Formic acid (50 : 10 : 1)

Fig 3-19. Glycerol monostearate
Mobile phase:
Chloroform : Methanol : Formic acid (70 : 1 : 0.3)
4) Amphoteric surface active agents

Fig 4-1. Lauryl dimethyl aminoacetic acid betaine

Mobile phase:

Chloroform : Methanol : Formic acid (50 : 20 : 1)

---

5) Other surface active agents

Fig 5-1. Sugar ester

Mobile phase:

Chloroform : Methanol : Formic acid (50 : 10 : 1)

---

6) Mixture

Fig 6-1. Sodium alkyl benzene sulfate + EO(4) Alkyl ether

Mobile phase:

Chloroform : Methanol : Formic acid (60 : 10 : 1)
IATROSCAN ANALYSIS

24

Analysis of Polymer Additives by Iatrosan

MITSUBISHI CHEMICAL MEDIENCE CORPORATION
A) 試料 Samples

1. Phthalic acid, Monocholyl phthalate, Diethyl phthalate

2. 可塑剤 Plasticizer
   DBP, DOP

3. 酸化防止剤1) Antioxidant(1)
   BHT

4. 酸化防止剤2) Antioxidant(2)
   BHT (Yoshinox BHT)
   Dimyristyl thiodipropionate (DMTP Yoshitomi)
   Pentaerythritol tetrakis 3-(3,5-di-tert-butyl-4-hydroxyphenyl)
   propionate (Tominox TT)
   4,4'-Butyldiethenebis-(6-tert-butyl-3-methylphenol) (Yoshinox BB)

5. 加硫促進剤 Rubber accelerator
   MBT, MBTS

6. 滑剤 Slip additive
   Parafin, n-Butyl stearate, Stearic acid, Stearamide

7. 帯電防止剤 Antistatic agent
   Sodium N-myristoyl sarcosinate (anion)
   PO(5) Oleyl amine (nonion)
   Monoalkyl ammonium chloride (cation)

8. プラスチック, ゴム等の添加剤 Additive reagents from plastics or rubber

9. フィルム添加剤 標品 Standard mixture of film additives
   BHT, Irganox 1076, Erucic amid

10. フィルム添加剤 Film additives
11. 紫外線吸収剤  Ultraviolet absorbent
   2(2'-Hydroxy-3'-tert-butyl-5'-methylphenyl)-5-chlorobenzotriazole (Tomisap 600)
   2-Hydroxy-4-n-octoxybezophenone (Tomisap 800)

12. 老化防止剤  Antioxidant (Age resistor)
   BHT (BHT Swanox)
   Octylated diphenylamine (Nonflex OD-3)
   Pentaerythritol tetrakis [3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate]
   (Tominox TT)
   N,N,-Di-β-Naphthyl-p-phenylendiamino (DNPD, Nonflex F)

13. 添加剤  標品  Standard mixture of additive reagents
   BHT, Mark 2112, Denon-331P, DMTDP, Irganox 1010,
   Cadenax GS-90, Erucyl amide

14. ポリエチレンペレットからの抽出物  Extract from polyethylene pellet
   BHT, Denon 331P, DMTDP, Irganox 1010, Cadenax GS-90.

B) 下記の成分名は略語を用います。

Components are expressed in the following abbreviations.

<table>
<thead>
<tr>
<th>成分名</th>
<th>Component</th>
<th>略語</th>
<th>Abbreviation</th>
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<tr>
<td>Dioctyl Phthalate</td>
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<td>Dibutyl Phthalate</td>
<td>DBP</td>
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<td></td>
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<td>Butylated hydroxytoluene</td>
<td>BHT</td>
<td></td>
<td></td>
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<tr>
<td>Mercaptobenzothiazole</td>
<td>MBT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dibenzothizylisulfide</td>
<td>MBTS</td>
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<td></td>
</tr>
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</table>
1. Phthalic acid, Monoethyl phthalate, Diethyl phthalate

Mobile phase
n-Hexane: Ethyl ether: Formic acid  20: 30: 1  10cm
Component

2. 可塑剂 Plasticizer

Mobile phase
n-Hexane: Ethyl ether  58: 12  10cm
Component
1. DOP  2. DBP
3. Antioxidant(1)

Mobile phase
n-Hexane : Ethyl ether  36 : 24  10 cm

Component
1. BHT

4. Antioxidant(2)

Mobile phase
1st  n-Hexane : Chloroform  45 : 15  10 cm
2nd  n-Hexane : Chloroform  40 : 20  10 cm

Component
1. BHT (Yoshinox BHT)  2. Dimyristyl thiodipropionate (DMTP Yoshitomi)
3. Pentaerythritol tetraakis 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Tominox TT)
4. 4,4'-Butylidenebis-(6-tert-butyl-3-methylphenol) (Yoshinox BB)
5. Rubber accelerator

<table>
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Mobile phase

n-Hexane : Ethyl ether : Formic acid  50 : 20 : 1  10cm

Component

1. MBT  2. MBTS

6. Slip additive

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Mobile phase

n-Hexane : Ethyl ether : Formic acid  65 : 5 : 0.15  10cm

Component

7. 帯電防止剤  Antistatic agent

CAL. METHOD  90  SP  PA  PB
0.10000ug +03 .10000ug +01 .10000ug +01

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<td>0.351</td>
<td>7665</td>
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Mobile phase
Chloroform : Methanol : Formic acid  50 : 20 : 1  10cm

Component
1. Sodium N-myristoyl acescinate (anion)  2-4.POE(5) Oleyl amine (nonion)
5. Monoalkyl ammonium chloride (cation)

8. プラスチック、ゴム等の添加剤  Additive reagents from plastics or rubber

CAL. METHOD  90  SP  PA  PB
0.10000ug +03 .10000ug +01 .10000ug +01

<table>
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<th>NAME</th>
<th>RT</th>
<th>A OR H</th>
<th>MK</th>
<th>CONC</th>
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<td>7</td>
<td>0.454</td>
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Mobile phase
1st  Chloroform : Methanol : Water  65 : 5 : 0.5  2.0cm
2nd  n-Hexane : Ethyl ether : Formic acid  60 : 10 : 0.2  8.5cm
3rd  n-Hexane  100%  10.0cm
9. Film Additives

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>RT</th>
<th>A or H</th>
<th>MK</th>
<th>CONC</th>
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<tr>
<td>1</td>
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TOTAL 47758 100.0000

Mobile phase

1st n-Hexane : Ethyl ether : Formic acid 60 : 10 : 0.1 8cm
2nd n-Hexane : Ethyl ether : Formic acid 66.5 : 3.5 : 0.1 11cm

Component

1. BHT 2. Irganox 1076 3. Erucic acid

10. Film Additives

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
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<th>A or H</th>
<th>MK</th>
<th>CONC</th>
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TOTAL 39455 100.0000

Mobile phase

1st n-Hexane : Ethyl ether : Formic acid 60 : 10 : 0.1 8cm
2nd n-Hexane : Ethyl ether : Formic acid 66.5 : 3.5 : 0.1 11cm
11. 紫外線吸収剤  Ultraviolet absorbent

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Mobile phase
n-Hexane : Chloroform  50 : 10  10cm

Component
1. 2(2'-Hydroxy-3'-tert-butyl-5'-methylphenyl)-5-chlorobenzotriazole (Tomisoap 600)
2. 2-Hydroxy-4-n-octoxybezophenone (Tomisoap 800)

12. 老化防止剤  Antioxidant (Age resistor)

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</table>

Mobile phase
1st  n-Hexane : Ethyl ether  42 : 28  5cm
2nd  n-Hexane  100%  10cm
3rd  n-Hexane  100%  10cm

Component
1. BHT (BHT Swanox)  2.Octylated diphenylamine (Nonflex OD-3)
3. Pentaerythritol tetraakis [3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate] (Tominox TT)
4. N,N-Di-β-Naphthyl-p-phenylenediamino (DNPD, Nonflex P)
13. **Standard mixture of additive reagents**

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**TOTAL** 25748 100.0000

**Mobile phase**

1st: n-Hexane : Ethyl ether  36 : 27  5cm

2nd: n-Hexane : Ethyl ether : Formic acid  60 : 10 : 0.2  8cm

3rd: n-Hexane  100%  10cm

**Component**

2. BHT + Mark2112  3. Denon-331P + DMTDP  4. Irganox 1010

5. Cadex GS-90 + Brucyl amide

14. **Extract from polyethylene pellet**

<table>
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**TOTAL** 64958 100.0000

**Mobile phase**

1st: n-Hexane : Ethyl ether  36 : 27  5cm

2nd: n-Hexane : Ethyl ether : Formic acid  60 : 10 : 0.1  8cm

3rd: n-Hexane  100%  10cm

**Component**

Environment and Health Friendly Developing Solvents for Iatroscan
最近、有機溶剤による環境や健康への影響が注目され、その使用が世界的に規制を受けています。日本でも有機溶剤は、“有機溶剤中毒予防規定”、“特定化学物質等設定予防規定”、“大気汚染防止法”等で規制されており、イアトロスキャンによく用いられる展開溶媒のなかで規制の対象となるのは、ベンゼン・クロロホルム・ジクロルメタン・トリクロルエチレン・四塩化炭素・トルエン等です。

ここでは、これらの規制溶媒の使用を避け、より低毒性溶媒の使用へ切り替えることを目的に中性脂質・極性脂質・重質油分離用溶媒の検討を行いました。有用と思われる溶媒系の例を示しますので、溶媒選択の参考にしていたければ幸いです。

The effects of organic solvents on our environment and health have been spotlighted lately and their use have been restricted worldwide. In Japan, organic solvents are restricted under such regulations as “Regulations on prevention of organic solvents toxication”, “Regulations on prevention of problems caused by specified chemical substances” or “Air pollution prevention law.” Among the development solvents used frequently for Iatroscan, the following solvents are subject to restrictions: benzene, chloroform, dichloromethane, trichloroethylene, tetrachloride and toluene.

In order to avoid the use of these restricted solvents and switch to the use of less-toxic solvents, we have looked into the solvents for separation of neutral lipid, polar lipid and bitumen. The following are examples of solvents deemed useful. Please use them for your reference when selecting solvents.
1. 中性脂質 Neutral lipids

1.1 Hexane : Ether 系

n-Hexane : Diethylether = 60 : 10

n-Hexane : Diethylether : Formic acid = 65 : 35 : 0.04
1.2 Heptane : Ether 系

Heptane: Diethyl ether = 60 : 10

2. 極性脂質  Polar lipids

2.1 Hexane : Diisopropyl ether 系

n-Hexane : Diisopropyl ether : Acetone : Methanol : Formic acid : Water
= 6 : 32 : 12 : 6 : 6 : 3

(3)
3. 重質油  Bitumen

3.1 Hexane : Diisopropyl ether 系

<table>
<thead>
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<th>番目</th>
<th>成分</th>
<th>溶媒(1)</th>
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<td>n-Hexane : Diisopropyl ether = 50 : 10</td>
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</tr>
<tr>
<td>3rd</td>
<td>n-Hexane : Ethylacetate = 30 : 30</td>
<td>2 cm</td>
</tr>
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</table>

Developing solvent(1)

1. Saturate
2. Aromatics
3. Resin
4. Asphaltene

3.2 Heptane : Diisopropyl ether 系

<table>
<thead>
<tr>
<th>番目</th>
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<td>Heptane : Diisopropyl ether = 40 : 20</td>
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<tr>
<td>3rd</td>
<td>Heptane : Acetonitrile = 40 : 20</td>
<td>2 cm</td>
</tr>
</tbody>
</table>

Developing solvent(2)

1. Saturate
2. Aromatics
3. Resin
4. Asphaltene

(4)
3.3 Hexane: Acetone 系

1st n-Hexane 10 cm
2nd Acetone 5 cm
3rd n-Hexane: Acetone = 30:30 2 cm

(5)
3.4 Heptane: Acetone 系

1st Heptane                      10 cm
2nd Acetone                      5 cm
3rd Heptane: Acetone = 30:30     2 cm

Developing solvent⑤

4. 従来法との比較 Comparison with the usual developing solvent

常圧残油をサンプルとし、従来法との比較を行ないました。
従来法として次の展開溶媒を選びました。The usual developing solvent

1st n-Hexane                      10 cm
2nd Toluene                       5 cm
3rd Dichromomethane: Methanol = 95:5 2 cm

溶媒①～⑤のクロマトグラムは、このクロマトグラムとよく似ています。
The chromatograms (developing solvents ①～⑤) look like this chromatogram.

(6)
Heavy oil is a mixture composed of many chemical compounds. Therefore, area percent (%) data of bitumen vary with developing solvents.

<table>
<thead>
<tr>
<th></th>
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<th>Aromatics(%)</th>
<th>Resin(%)</th>
<th>Asphaltene(%)</th>
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<td>18</td>
<td>59</td>
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<td>④</td>
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<td>61</td>
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<td>⑤</td>
<td>17</td>
<td>59</td>
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<td>8</td>
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</tbody>
</table>

Analysis results are shown below.

Area %

Heavy oil is a mixture composed of many chemical compounds. Therefore, area percent (%) data of bitumen vary with developing solvents.
Chromatogram

Dye (Food dye, Naphthol quinone, Azo dye)
Hormons (Pregnandiol)
Ginseng Saponin
Liquid Crystal
Capsaicine
Cosmetic Cream
Rubber Antioxidant
Polymer
2.2.1

• Water soluble food dyes

SAMPLE:
1. Tartrazine 10.7μg
2. Acid Red 9.0μg
3. Phloxine B 9.1μg

CONDITIONS:
Stationary phase: CHROMAROD - S
Mobile phase:
Ethyl Acetate : MeOH :
70 : 20 :
28% NH₄OH : H₂O 10 : 10
Gas flow: H₂ 160ml/min
Air 2000ml/min
Scanning speed: 32sec/scan
Chart speed: 240mm/min

• Water soluble food dyes

SAMPLE:
1. New Cocaine 7.0μg
2. Acid Red 9.1μg
3. Rose Bengal 10.0μg

CONDITIONS:
Stationary phase: CHROMAROD - S
Mobile phase:
Ethyl Acetate : MeOH :
70 : 20 :
28% NH₄OH : H₂O 10 : 10
Gas flow: H₂ 160ml/min
Air 2000ml/min
Scanning speed: 32sec/scan
Chart speed: 240mm/min
2.2.1

● Water soluble Food Dye

SAMPLE:
1. Fast Green FCF 9.6 µg
2. Acid Violet 6B 8.9 µg

CONDITIONS:
Stationary phase: CHROMAROD-S
Mobile phase:
Ethyl Acetate : MeOH : 
70 : 20 :
28% NH₄OH : H₂O
10 : 10
Gas flow: H₂ 160 ml/min
Air 2000 ml/min
Scanning speed: 32 sec/scan
Chart speed: 240 mm/min

● Water soluble Food Dye

SAMPLE:
1. Amaranth 7.8 µg
2. Acid Red 7.4 µg
3. Erythrosine 9.5 µg

CONDITIONS:
Stationary phase: CHROMAROD-S
Mobile phase:
Ethyl Acetate : MeOH :
70 : 20 :
28% NH₄OH : H₂O
10 : 10
Gas flow: H₂ 160 ml/min
Air 2000 ml/min
Scanning speed: 32 sec/scan
Chart speed: 240 mm/min
Dye

Sample: Naphthol quinone

CONDITIONS:
Stationary phase: CHROMAROD
Mobile phase:
Chloroform:Benzene 7:3
Gas flow: H₂ 160ml/min
            Air 2000ml/min
Scanning speed: 30sec/scan

Sample: Azo dye

CONDITIONS:
Stationary phase: CHROMAROD
Mobile phase:
Ethyl acetate:Methanol:Chloroform
        15:  6 :  30
Gas flow: H₂ 160ml/min
            Air 2000ml/min
Scanning speed: 30sec/scan
**HORMONES**

**Adult urine**

Components: 1. Pregnandiol 2. Free cholesterol (internal standard)

**Pregnant woman's urine**

Conditions:
- Stationary phase: CHROMAROD-SII
- Mobile phase: CHCl₃:CH₃OH 50:1
- Gas flow: H₂ 160ml/min
  - Air 2.0 l/min
- Scanning speed: 30sec/scan

**Components:**
- 1. Esteriol
- 2. Estradiol
- 3. Estrone

**Conditions:**
- Stationary phase: CHROMAROD-S
- Mobile phase: Toluene:Methanol 90:10
- Gas flow: H₂ 160ml/min
  - Air 2.0 l/min
- Scanning speed: 30sec/scan
**GINSENG SAPONIN**

CONDITIONS: Stationary phase: CHROMAROD-S

Mobile phase: CHCl₃:CH₃OH:H₂O (65:35:10, lower)

Gas flow: H₂ 160ml/min  Air 2.0 l/min

Scanning speed: 30sec/scan

The values indicate the average percentage of ten experiments of peak-area given by individual saponins.

**IDENTIFICATION**

![Diagram of saponin structures]

- Ginsenoside Rα: R₁ = glucose
- Ginsenoside Rβ: R₂ = glucose
- Ginsenoside Rb: R₃ = glucose
- Ginsenoside Rc: R₄ = glucose
- Ginsenoside Rd: R₅ = glucose
- Ginsenoside Ro: R₆ = glucose

- Ginsenoside Rg₁: R₇ = glucose
- Ginsenoside Rg₂: R₈ = glucose
- Ginsenoside Rg₃: R₉ = glucose
- Ginsenoside Rg₄: R₁₀ = glucose

- Ginsenoside Rb₁: R₄ = glucose
- Ginsenoside Rb₂: R₅ = glucose

- Chikusetsu saponin III: R₁₀ = glucose
- Chikusetsu saponin IV: R₁₁ = glucose

IATRON LABORATORIES, INC.
Sample: Liquid Crystal
Stationary phase: CHROMAROD-S11
Mobile phase:
  Cyclohexane:Toluene
  1 : 1
Peak 1. Cholesteryl acetate
2. Cholesteryl propionate
3. Cholesteryl nonanoate
4. Cholesteryl chloride

Sample: Capsaicine Extract
An ingredient found in chill peppers, red peppers and green paprika.

Stationary phase: CHROMAROD-S11
Mobile phase:
  Benzene:Ethyl ether:Acetic acid
  90 : 10 : 1
Separation of Cosmetic Cream

Preparation of Test Solution.

Cream

<table>
<thead>
<tr>
<th>H₂O Added</th>
<th>CHCl₃ Added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Soluble Substance. Evaporation</td>
<td>Oil Soluble Substance. Evaporation</td>
</tr>
<tr>
<td>H₂O Added</td>
<td>CHCl₃ Added</td>
</tr>
<tr>
<td>Test Solution</td>
<td>Test Solution</td>
</tr>
</tbody>
</table>

Mobile phase:
CHCl₃:MeOH:HCOOH
80 : 20 : 0.5

Peak 1. Glycerin, 2. Propylene glycol

Mobile phase:
Pet.ether:Ethyl ether:AcON
90 : 10 : 1

Peak 1. Propylene glycol
Tri-Ethanol amine
2. Cethanol, 3. Stearic acid
4. 2-Ethyl hexyl TG
5. Wool Wax, Liquid Parafin and Squalan

Stationary phase: CHROMAROD-S11
RUBBER ANTIOXIDANT

Separation on Chromarod SII

3.32 mg/ml  1. Epoxidised Soya Oil
0.46 mg/ml  2. Tin mercaptan stabiliser complex
2.16 mg/ml  3. Butylated reaction product of p-cresol and dicyclopentadiene
0.2 mg/ml   4. 2,5-Di-t amylhydroquinone
0.66 mg/ml  5. Tris(nonylphenyl)phosphate

Two stage development: n-Hexane:Acetone 4 cm
                      n-Hex:Ethyl Ether 10 cm
ANALYSIS OF ACRYLIC RESIN COATINGS

Samples:

Acrylic resin for coating (No. A and No. B)
Molecular weight: 10,000 - 20,000

The pigment dispersibility of Sample A is larger than that of Sample B, but no appreciable difference in IR- and NMR- spectra and the like can be observed between either sample.

Procedure and Results of Analysis:

Both Samples No. A and No. B were developed with the same solvents and were investigated in order to determine whether any distinction in their chromatographic profile could be observed or not.

When both samples are developed in a solvent system of ethyl acetate-formic acid on Chromarod-A, components which move with the solvent front are naturally increased within a greater concentration of formic acid but, as can be seen from the charts, components which move at a lower concentration of formic acid, are more in evidence in Sample No. A than in Sample No. B (see Charts).

This illustrates that samples which cannot be distinguished from each other by optical methods can be distinguished by the IATROSCAN method relatively easily.

Further, the presented data may be helpful in the selection of eluates for separation analyses of these samples such as by column chromatography methods and the like.

Conditions:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Acrylic Resin</th>
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<tr>
<td>Sample Size:</td>
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<table>
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<tr>
<th>Chart No:</th>
<th>1.</th>
<th>2.</th>
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<tbody>
<tr>
<td>Sample No:</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
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<td>Mobile Phase:</td>
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</tbody>
</table>

Stationary Phase: CHROMAROD-A
Gas Flow: H₂- 160 ml/min Air 2.0 l/min
Scanning Speed: 30 sec/scan
Chart Speed: 240 mm/min
Recorder Range (Chromatogram): 100 mV
Recorder Range (Integrator): 200 mV
SAMPLE: Styrene + MMA and Butylmethyacrylate

copolymer 10 mg/ml
chloroform

1st. ether 10 cm, 2nd. acetone 5 cm

TAMAROCH 11

-160 min
-30 min
-240 min

IATROSCAN TH 10

21292.