IATROSCAN

INSTRUMENT APPLICATION

NO. 20

EXPERIMENTAL ANALYSIS FOR INFINITESTIMAL COMPONENTS CONTAINED IN THE MAIN INGREDIANTS

(Detection of Infinitestimal Phospholipid in Edible Oils)

Infinitestimal amounts of Impurity and Additive contained in the main ingrediants 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 infinitestimal components contained in Edible Oils.

Experimental Research Infinitestimal Components

There are few analytical methods available to detect infinitestimal phospholipids at each process of refining or purifying edible oils such as the Colorimetory, Lorenz and Acetone methods being applicable. The IATROSCAN MK-5 p-erforms relatively easier to detect phospholipid contents in this particul-ar 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 preparea sample solution.
3. A certain amount of this sample solution, normally in microliter order, to be spotted onto Chromarod-SIII 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 adimitted 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

Volumetric ratio of Lecithin and Cholesterol ester	0.3	0.6	0.9	1.2
25mg Cholesterol ester/5mL	0.50 (mL)	0.50	0.50	0.50
25mg Lecithin/5mL	0.15 (mL)	0.30	0.45	0.60

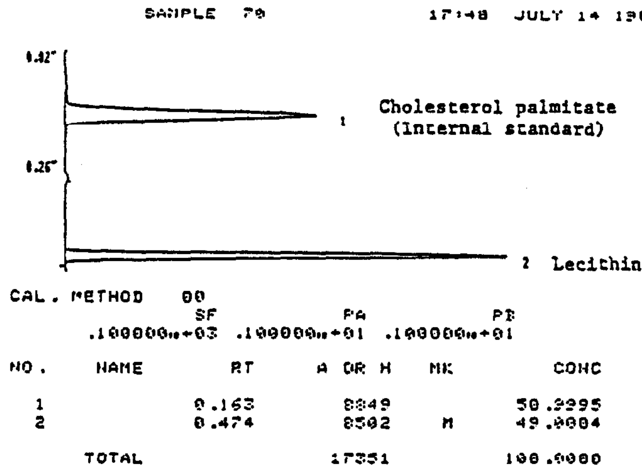
1-2 Analysis of the Standard Mixture by the IATROSCAN

① Perfom blank scanning for Chromarod-SⅢ to remove any unburn component may still remain on the rod.
② 1 μL 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℃ 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



CONDITION:
Stationary phase: CHROMAROD-SⅢ
Mobile phase:
Hexane : Diethyl ether : Formic acid
48 : 12 : 0.1
Gas flow: H_2 160 ml/min, Air 2 l/min
Integrator attenuation: 16

Fig. 2 - Calibration curve of internal stnadard

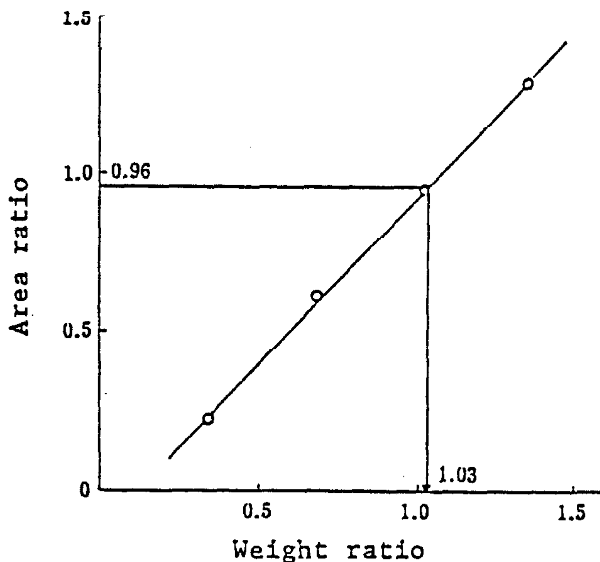


Table 2 - Date of Calibration Curve

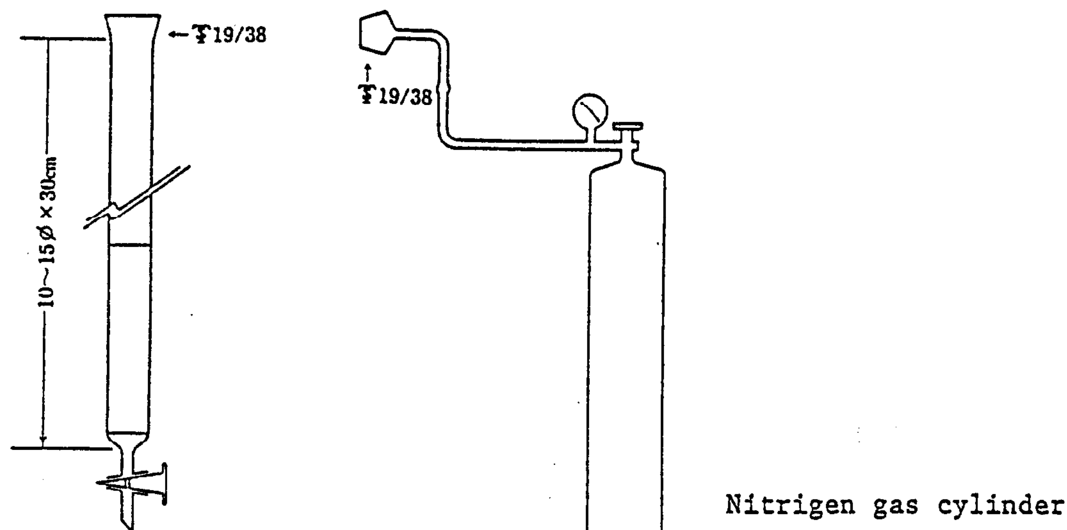
Weight ratio Lecithin/Cholesterol Palmitate	Area ratio Lecithin/Cholesterol Palmitate
0.34	0.23
0.68	0.61
1.02	0.95
1.35	1.29

2. Preparation of Sample Solution by Column Method:

Chromatography Column:

It is recommended to use a Chromatography Column consisting of I.D 10 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.

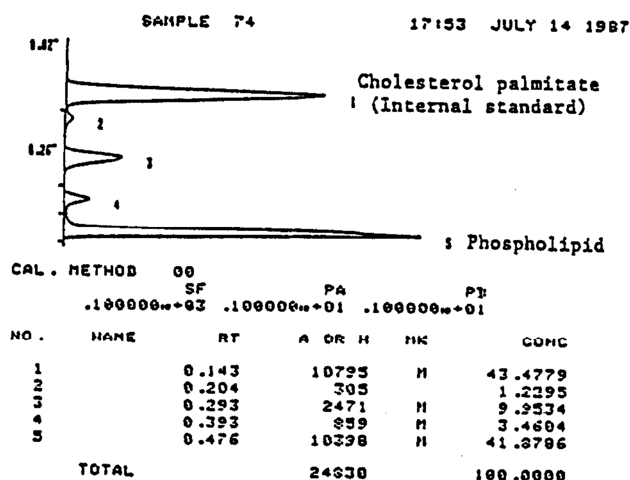
① A 5g of IATROBEADS 6RS-8060 (silica acid 60μm made by Iatron) shall be admitted into a breaker with 50mL and Methanol to mix well. Pourthis mixture into Chromatography Column.
② Open a cock on the column and add Chloroform until Methanol reaches to the top of silica acid in order to wash the column.
③ Weight 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 6mL Chloroform and pour the sample into the column quantitatively.
⑦ With Chloroform between 15 and 20mL, flush flow from the column. Now, Chloroform fractinations obtained by all above procedure have eluted simple lipids.
⑧ Then perform elution with Methanol 60mL. At this stage, connect the end of Nitrigen 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-SIIIs. 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)



NO.	NAME	RT	A OR H	MK	CONC
1		0.143	10795	M	43.4779
2		0.204	305		1.2295
3		0.293	2471	M	9.9534
4		0.393	859	M	3.4604
5		0.476	10398	M	41.8786
	TOTAL		24830		100.0000

CONDITION :

Stationary phase : CHROMAROD-SIII

Mobile phase :

Hexane	:	Diethyl ether	:	Formic acid
48	:	12	:	0.1

Gas flow : H_2 160 ml/min, Air 2 l/min

Integrator attenuation : 16

② 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.

$$\text{Phospholipid content \%} = \frac{\overset{*1}{\text{Weight ratio}} \times \overset{*2}{\text{Weight of internal ST}}}{\underset{*3}{\text{Weight of sample}}} \times 100$$

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

Weight of sample	Weight of internal ST	Area ratio	Weight ratio
510.6	2.02	0.96	1.03

$$\text{Phospholipid cntent \%} = \frac{1.03 \times 2.02}{510.6} \times 100 = 0.41\%$$

*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.

Reference: L.M.Duplessis and H.E. Pretorius, JAOCS, vol 60, 1261 - 1265 (1983)