IATROSCAN INSTRUMENT APPLICATION

No. 16

Analysis of Triglyceride Molecular Species using the New SIII CHROMARODS (silver nitrate impregnated)

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:

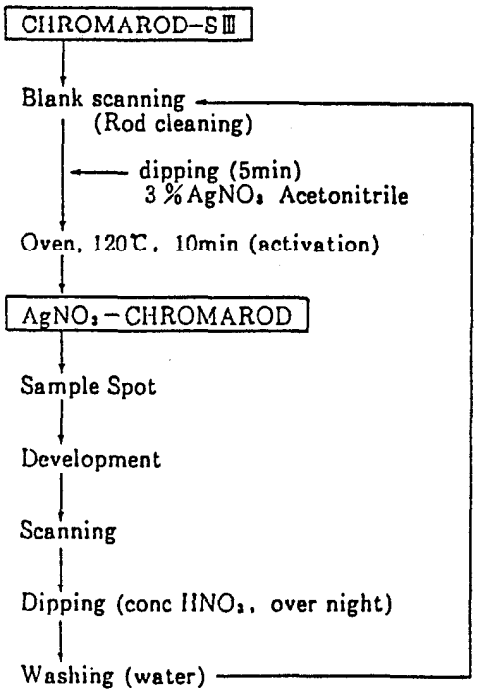
[1] CHROMAROD-SIII is first subjected to a blank-scan in the MK IV IATROSCAN to remove any absorbed organic substances.

[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



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 [2] 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, [1] through [3]

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.

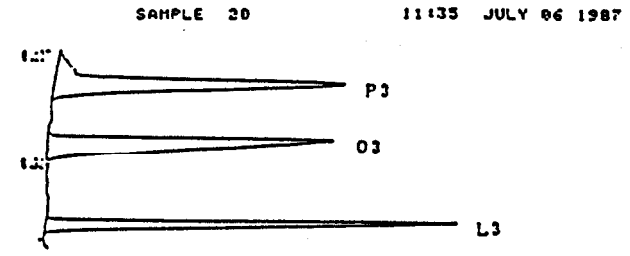
[3] Dry the CHROMARODS after development at 120 degrees C in a dryer for 5 minutes to remove all solvent.

[4] Measure the CHROMARODS in the IATROSCAN analyser after solvent removal.

Conforming to the analytical procedures explained above, a standard mixture of triglyceride (tripalmitin, triolein and trilinolein) 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

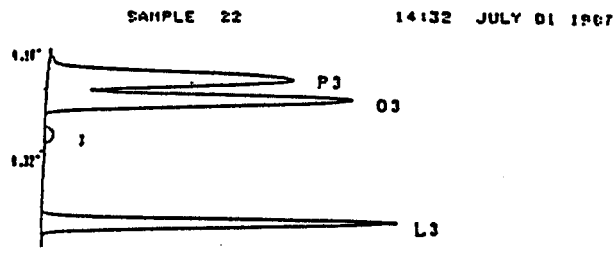
Fig 2-1. Solvent mixture A



CAL. METHOD 00

		SF .100000e+03	PA .100000e+01	PB .100000e+01		
NO.	NAME	RT	A OR H	MK		CONC
1	P3	0.173	12064			36.2938
2	O3	0.288	12254			36.8691
3	L3	0.461	8918		H	26.8320
	TOTAL		33236			100.0000

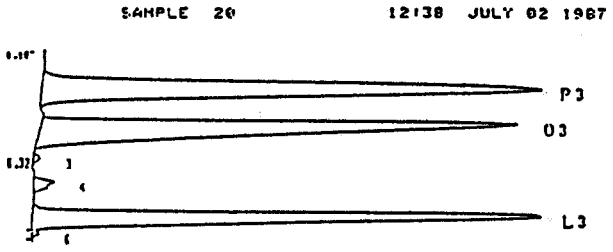
Fig 2-2. Solvent mixture B



CAL. METHOD 00

		SF .100000e+03	PA .100000e+01	PB .100000e+01		
NO.	NAME	RT	A OR H	MK		CONC
1	P3	0.162	26339	H		34.6656
2	O3	0.204	26869			35.3630
3		0.276	959	H		1.1311
4	L3	0.459	21913			28.8402
	TOTAL		75982			100.0000

Fig 2-3. Solvent mixture C



CAL. METHOD 00

		SF .100000e+03	PA .100000e+01	PB .100000e+01		
NO.	NAME	RT	A OR H	MK		CONC
1	P3	0.187	24530			35.7330
2	O3	0.258	23836			34.7222
3		0.328	148			0.2166
4		0.384	627			0.9144
5	L3	0.457	19438		H	28.3163
6		0.492	66		H	0.0973
	TOTAL		68648			100.0000

Identifications :

P 3 : Tripalmitin

O 3 : Triolein

L 3 : 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-SIII (3% silver nitrate impregnated)

Gas flow : H_2 160ml/min, Air 2.0 l/min

Scanning speed : 30sec/scan

Iatrocorder TC-11

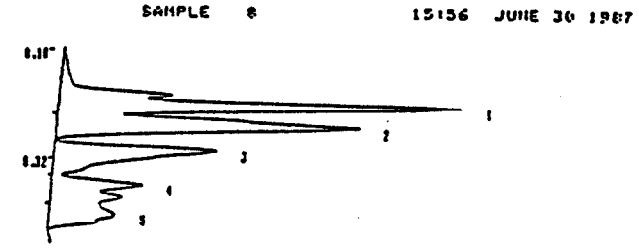
Playback attenuation : 16-32mv f.s

3. Application for identification of oils and fats. Figs. 3 and 4 show chromatograms obtained with samples of fats, palmoil, 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

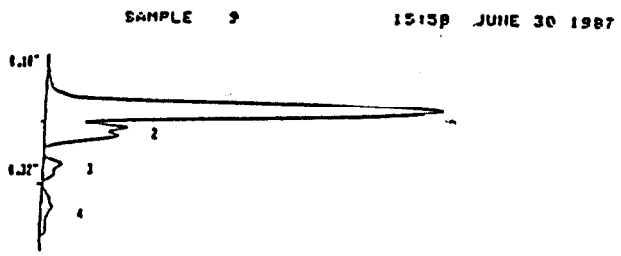


CAL. METHOD 00

NO.	NAME	RT	A OR H	MK	CONC
		SF .100000E+03	PA .100000E+01	PB .100000E+01	
1		0.216	17363	H	35.9475
2		0.261	13152		27.2291
3		0.311	8085	H	16.7402
4		0.384	5425	H	11.2306
5		0.446	4224		8.7459
	TOTAL		48301		100.0000

Fig 3-2. Cacao oil

Solvent mixture A

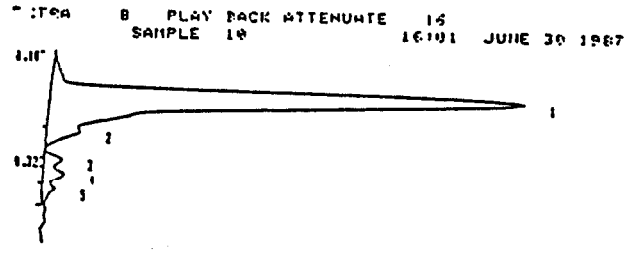


CAL. METHOD 00

NO.	NAME	RT	A OR H	MK	CONC
		SF .100000E+03	PA .100000E+01	PB .100000E+01	
1		0.210	46371	H	78.5560
2		0.248	8922		15.1149
3		0.324	1794	H	3.0223
4		0.416	1751		3.3067
	TOTAL		59029		100.0000

Fig 3-3. Coconut oil

Solvent mixture A

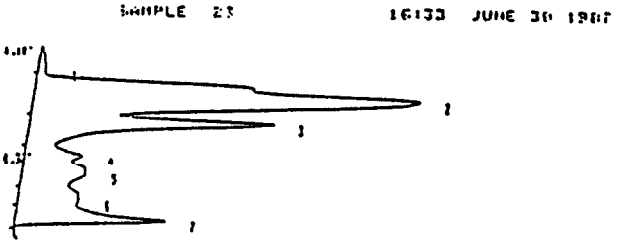


CAL. METHOD 00

NO.	NAME	RT	A OR H	MK	CONC
		SF .100000E+03	PA .100000E+01	PB .100000E+01	
1		0.205	31367	H	90.8494
2		0.274	1242		3.5980
3		0.332	576	H	1.6710
4		0.363	825	H	2.3903
5		0.396	514	H	1.4911
	TOTAL		34526		100.0000

Fig 3-4. Beef tallow

Solvent mixture A



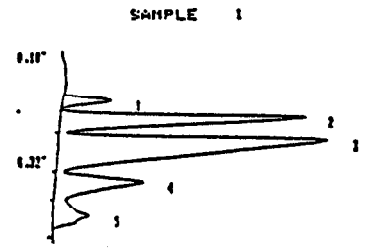
CAL. METHOD 00

NO.	NAME	RT	A OR H	MK	CONC
		SF .100000E+03	PA .100000E+01	PB .100000E+01	
1		0.155	448		853
2		0.213	25893		51.9428
3		0.261	10293	H	18.7478
4		0.331	2420	H	4.1327
5		0.370	5491	H	8.4324
6		0.427	3593	H	6.3055
7		0.468	6929	H	10.2934
	TOTAL		56575		100.0000

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

Fig 4-1. Olive oil

Solvent mixture A



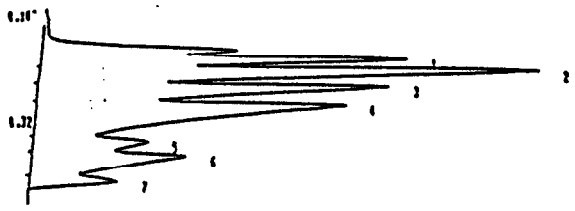
SAMPLE 1 15:38 JUNE 30 1987

C-_. METHOD 00

		SF .100000E+03	PA .100000E+01	PB .100000E+01	
NO.	NAME	RT	A OR H	MK	CONC
1		0.207	2661		3.8898
2		0.247	18369	H	26.8474
3		0.296	34707	H	50.7258
4		0.378	8455	H	12.3580
5		0.451	4227	H	6.1788
	TOTAL		68421		100.0000

Fig 4-2. Soybean oil

Solvent mixture B



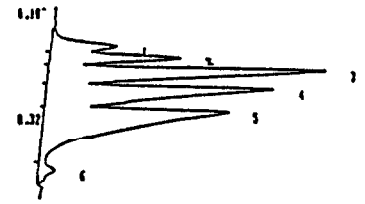
SAMPLE 13 14:13 JULY 01 1987

CAL. METHOD 00

		SF .100000E+03	PA .100000E+01	PB .100000E+01	
NO.	NAME	RT	A OR H	MK	CONC
1		0.213	17666	H	20.3136
2		0.238	18640	H	21.4340
3		0.2[illegible]	14445	H	16.6108
4		0.310	20207	H	23.2335
5		0.382	4665	H	5.3645
6		0.411	8537	H	9.8168
7		0.465	2804		3.2246
	TOTAL		86967		100.0000

Fig 4-3. Sunflower oil

Solvent mixture B



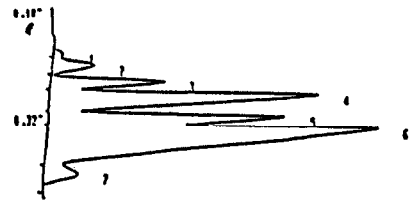
SAMPLE 15 14:17 JULY 01 1987

CAL. METHOD 00

		SF .100000E+03	PA .100000E+01	PB .100000E+01	
NO.	NAME	RT	A OR H	MK	CONC
1		0.186	2235	H	5.5456
2		0.211	3782	H	9.3844
3		0.238	10006	H	24.8248
4		0.276	10182	H	25.2627
5		0.324	13412	H	33.2764
6		0.449	687		1.7058
	TOTAL		40306		100.0000

Fig 4-4. Safflower oil

Solvent mixture C



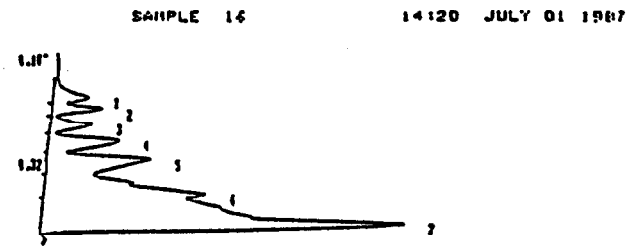
SAMPLE 14 12:25 JULY 02 1987

CAL. METHOD 00

		SF .100000E+03	PA .100000E+01	PB .100000E+01	
NO.	NAME	RT	A OR H	MK	CONC
1		0.200	220	H	0.4282
2		0.224	1432	H	2.7805
3		0.258	3267	H	6.3430
4		0.288	11332	H	21.9969
5		0.333	8132	H	15.8024
6		0.361	25505	H	49.5060
7		0.457	1577	H	3.0627
	TOTAL		51519		100.0000

Fig 4-5. Linseed oil

Solvent mixture B

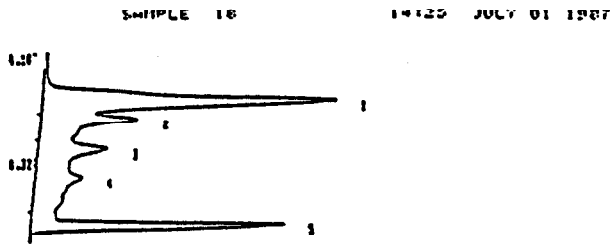


CAL. METHOD 00

NO.	NAME	RT	A OR H	HK	CONC
		SF .100000E+03	PA .100000E+01		PB .100000E+01
1		0.191	1405	H	3.0537
2		0.216	1306	H	2.8385
3		0.253	1453	H	3.1581
4		0.287	2981	H	6.2625
5		0.326	5264	H	11.4414
6		0.398	8364	H	18.1799
7		0.462	25336		55.0658
	TOTAL		46010		100.0000

Fig 4-6. Tung oil

Solvent mixture B

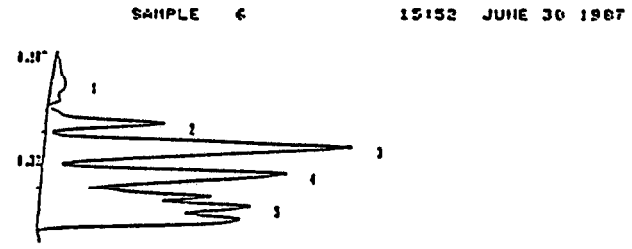


CAL. METHOD 00

NO.	NAME	RT	A OR H	HK	CONC
		SF .100000E+03	PA .100000E+01		PB .100000E+01
1		0.203	13548	H	38.5773
2		0.241	5436	H	15.4810
3		0.306	3900	H	11.1072
4		0.369	5222	H	14.8712
5		0.460	7011		19.9631
	TOTAL		35119		100.0000

Fig 4-7. Rape seed oil

Solvent mixture B



CAL. METHOD 00

NO.	NAME	RT	A OR H	HK	CONC
		SF .100000E+03	PA .100000E+01		PB .100000E+01
1		0.172	1221		2.2224
2		0.258	3876	H	7.2380
3		0.308	15697	H	29.5119
4		0.362	11402	H	21.5984
5		0.434	20541		38.6191
	TOTAL		53109		100.0000

CONDITIONS :

Stationary phase

CHROMAROD-SIII (3% $AgNO_3$ 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

Iatrocorder TC-11

Playback attenuation : 16

Reference :

1) M. Tanaka, T. Itoh and H. Kaneko : Yukagaku 28(2):96, 1979.

2) Sebedio, J.L., Ackman, R.G. : *J. Chromatogr. Sci.* 19 : 552, 1981.

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.

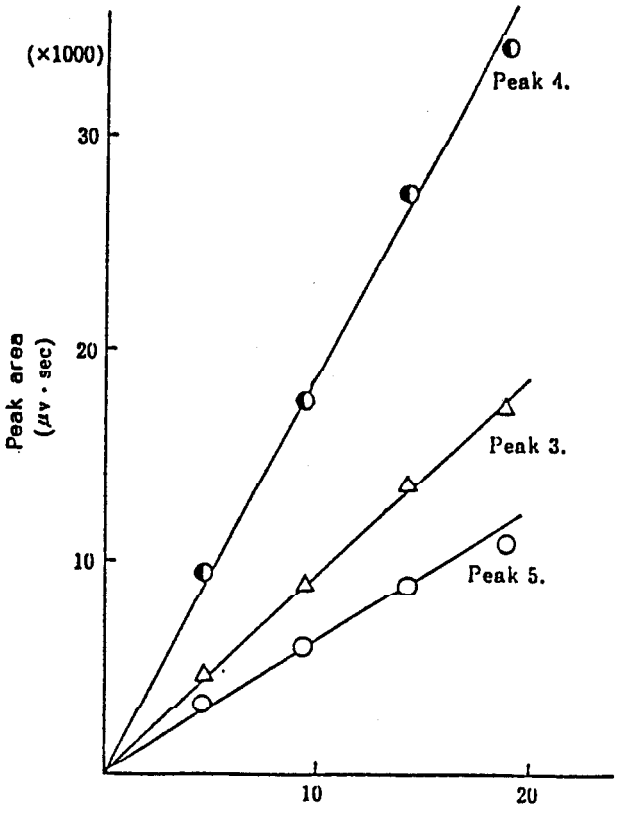
4. Analytical results

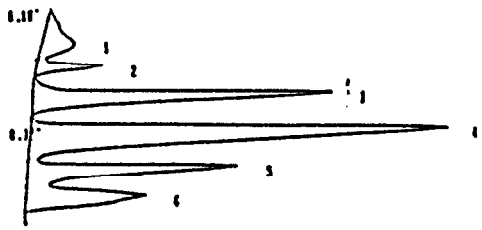
Table 1 shows reproducibility relating to a standard triglyceride analysed using a CHROMAROD-SIII treated with 3% silver nitrate, and the area percentage obtained. When the total amount spotted varies from 4 to 20 μg in the case of olive oil, the relationship between total weight and response can be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

Table 1 - Reproducibility of percentage of standard triglycerides.

Rod No.	Tripalmitin %	Triolein %	Trilinolein %
1	34.6	37.6	27.8
2	34.9	36.8	28.3
3	33.7	36.8	29.5
4	34.9	36.4	28.7
5	34.9	36.9	28.2
6	34.6	36.7	28.7
7	34.9	37.1	28.0
8	35.4	37.5	27.1
9	36.3	36.9	26.8
10	35.0	36.7	28.3
$\bar{X}$	34.9	36.9	28.2
CV%	1.9	0.8	2.8

Fig. 5 - Relationships between the amount of olive oil spotted and the response





Stationary phase:

CHROMAROD-SIII (3% $AgNO_3$ impregnated)

Mobile phase:

Solvent mixture A

Benzene	Ethyl ether	Formica acid
68	2	0.1