

IATROSCAN INSTRUMENT APPLICATION

TLC/FID

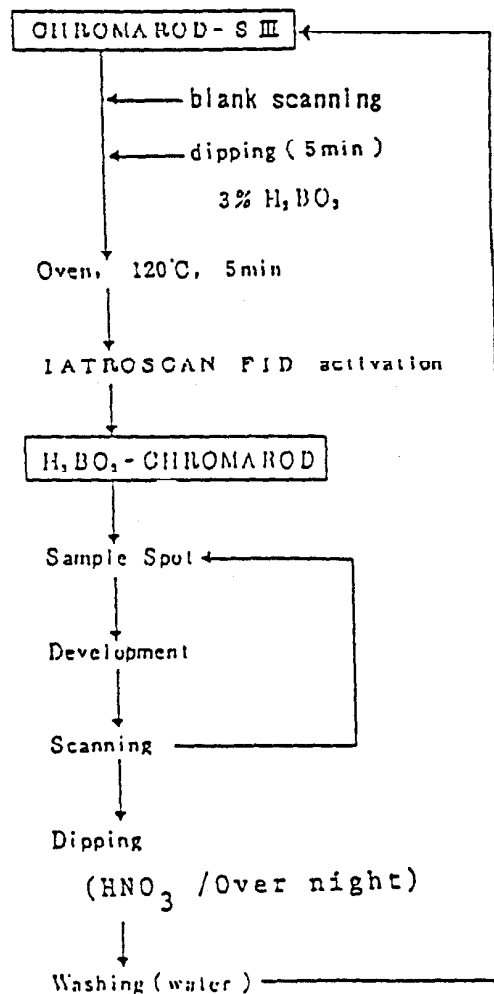
Analysis of Glyceride isomers by
Boric acid-Impregnated CHROMARODS.

(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. 1 μ l 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 - Hydrolyzed compounds of Triglyceride.

(1) Sample

Hydrolysis compounds of olive-oil by lipase

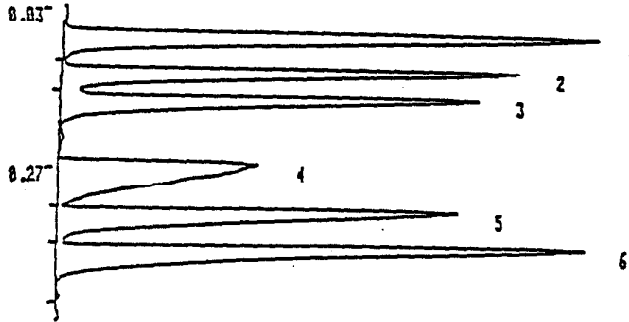
(2) Development method:

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

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NO.	NAME	RT	A OR H	MK	CONC
1	tripalmitin	0.003	10522	M	10.9379
2	1,3-dimyristin	0.136	8845	M	15.9194
3	1,2-dipalmitin	0.178	8123		14.6210
4	palmitic acid	0.276	7688	M	13.8384
5	2-monopalmitin	0.348	9021	M	16.2362
6	1-monostearin	0.403	11360	M	20.4469
TOTAL			55561		100.0000

CONDITIONS:

Satationary phase: 3% boric acid impregnated CHROMAROD-SIII

Mobile phase:

1st Chloroform 100% 8 cm

2nd Chloroform : Methanl/Ammonia (8 : 2)

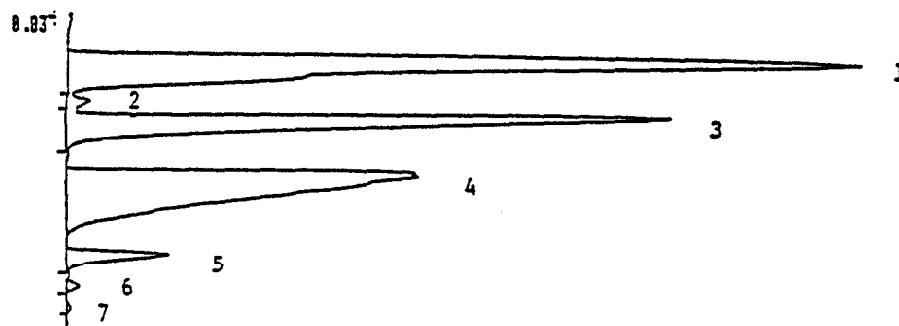
70 : 0.05 10 cm

Gas flow rate: H₂ 160ml/min. Air flow rate: 2.0l/min

Scanning speed: 30 sec/scan

Iatrocoder: Attenuation 16

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



CAL.	METHOD	GC			M	FB
		SF	PA	FB		
		.100000e+03	.100000e+01	.100000e+01		
1	TG	0.106	53517		M	39.5211
2	1,3-DG	0.157	809		M	0.5388
3	1,2-DG	0.183	32462		M	24.0153
4	FA	0.249	43241		M	31.9294
5	2-MG	0.338	4433		M	3.2796
6	1-MG	0.437	559		M	0.4139
7	Origin	0.468	150			0.1116
	TOTAL		135174			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, Air flow rate: 2.0l/min

Scanning speed: 30 sec/scan

Iatrocoder : 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
Lipids, Vol. 15 No. 10, 872-875 (1981)