

I A T R O S C A N T L C / F I D
I N S T R U M E N T A P P L I C A T I O N

N o. 13

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 testings. 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 testings.

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 reaction 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 1 μ l of this diluted solution was spotted onto Chromarod-SIII 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-SIII

Gas flow: H₂ 160ml/min, Air 2.0l/min,

Scanning speed: 30sec/scan

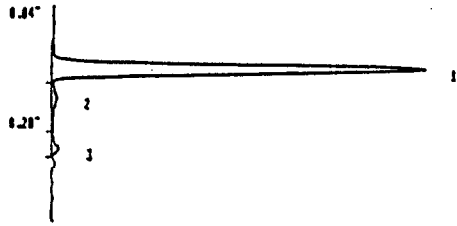
IATROCORDER TC-11: Playback attenuation: 8 - 16mv f.s

(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.

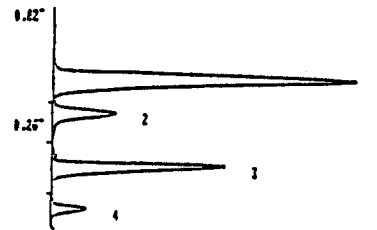
Fig.1 Tracing of enzymatic reaction by latroscan.

Reaction time : 0 min



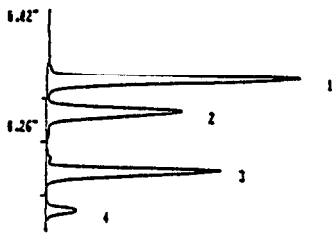
CAL. METHOD 00					
SF .100000e+03 PA .100000e+01 PB .100000e+01					
NO.	NAME	RT	A OR H	NK	CONC
1		0.179	12542	N	94.2185
2		0.239	322	N	2.2713
3		0.348	278	N	2.1100
TOTAL			13214		100.0000

Reaction time : 10 min



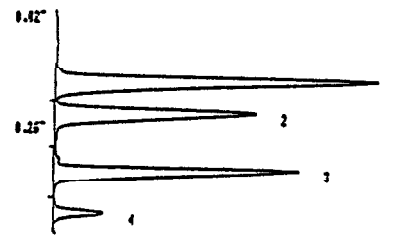
CAL. METHOD 00					
SF .100000e+03 PA .100000e+01 PB .100000e+01					
NO.	NAME	RT	A OR H	NK	CONC
1		0.180	12216	N	52.8981
2		0.233	2155	N	10.3758
3		0.375	5127	N	25.3328
4		0.466	827	N	4.7831
TOTAL			20467		100.0000

Reaction time : 30 min



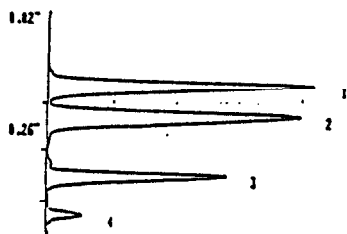
CAL. METHOD 00					
SF .100000e+03 PA .100000e+01 PB .100000e+01					
NO.	NAME	RT	A OR H	NK	CONC
1		0.178	7228	N	42.7246
2		0.248	4732	N	23.4383
3		0.374	5877	N	27.3501
4		0.466	819	N	4.4167
TOTAL			18558		100.0000

Reaction time : 60 min



CAL. METHOD 00					
SF .100000e+03 PA .100000e+01 PB .100000e+01					
NO.	NAME	RT	A OR H	NK	CONC
1		0.181	11829	N	41.4361
2		0.249	7729	N	27.1096
3		0.372	7723	N	25.2273
4		0.466	1261	N	4.2245
TOTAL			28694		100.0000

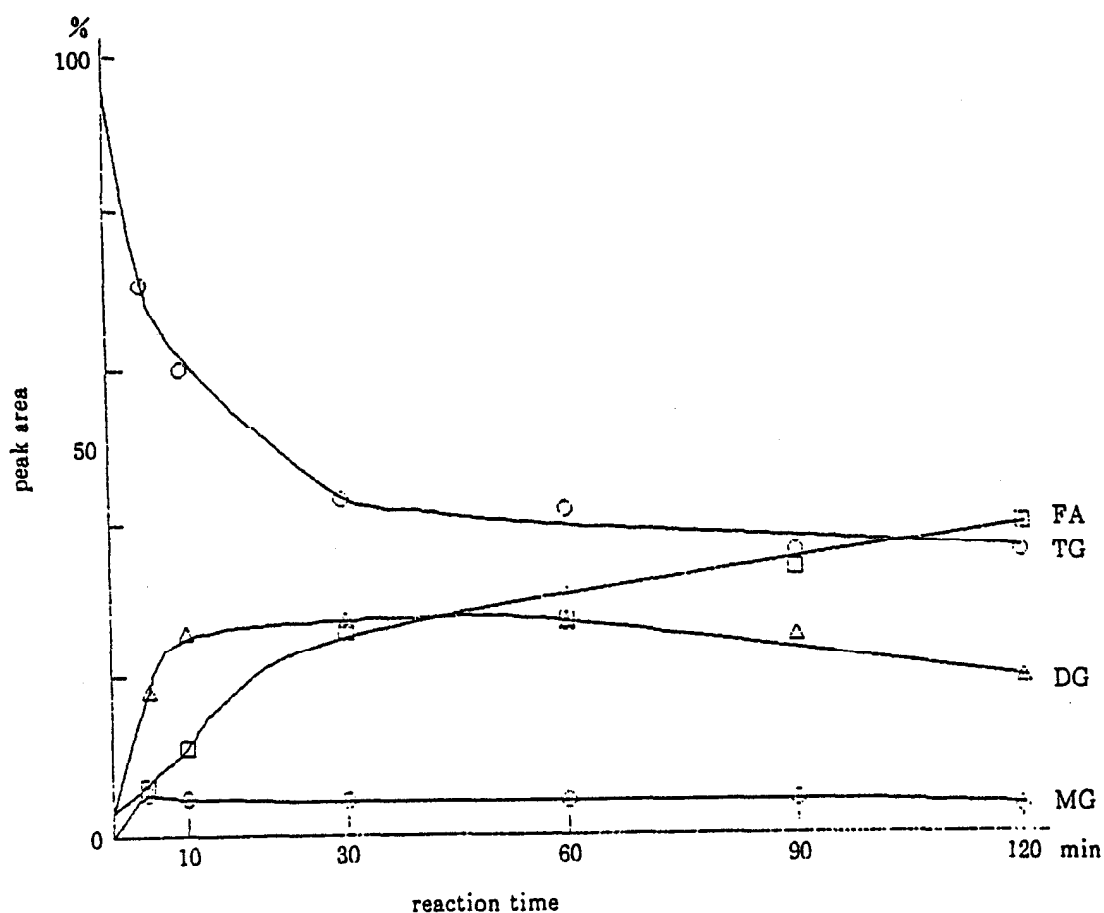
Reaction time : 120 min



CAL. METHOD 00					
SF .100000e+03 PA .100000e+01 PB .100000e+01					
NO.	NAME	RT	A OR H	NK	CONC
1		0.181	9873	N	36.3727
2		0.250	10737	N	39.7653
3		0.377	5500	N	20.3698
4		0.466	898	N	3.2218
TOTAL			27001		100.0000

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

Fig.2 Temporal change of reaction rate.



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.