

IATROSCAN TLC/FID
INSTRUMENT APPLICATION

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Lipid Analysis by Copper Sulphate
Impregnated Chromarod-SIII

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.

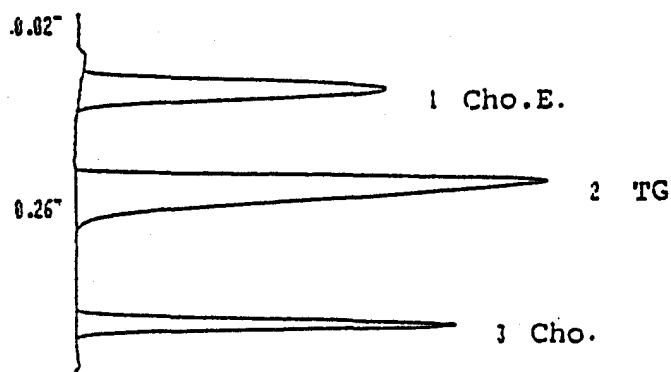
3. Results

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



NO.	NAME	RT	A OR H	MK	CONC
1	Cho.E	0.121	15004	T	27.0600
2	TG	0.245	29940	M	52.5130
3	Cho.	0.434	11189		19.6261
TOTAL			57014		100.0000

Table 1 - Relationship between Peak Area % and Weight %

Copper Sulphate Impregnated
CHROMAROD-SIII

	Cho.E	TG	Cho.
X (%)	27.6	52.5	19.9
s.d.	0.35	0.14	0.44
C.V.(%)	1.3	0.3	2.2
wt (%)	26.0	53.7	20.4
X-wt	1.6	1.2	0.5

n=5

CHROMAROD-SIII

	Cho.E	TG	Cho.
X (%)	21.2	47.6	28.1
s.d.	0.34	0.19	0.33
C.V.(%)	1.4	0.4	1.2
wt (%)	26.0	53.7	20.4
X-wt	1.8	6.1	7.7

n=5

As it has 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

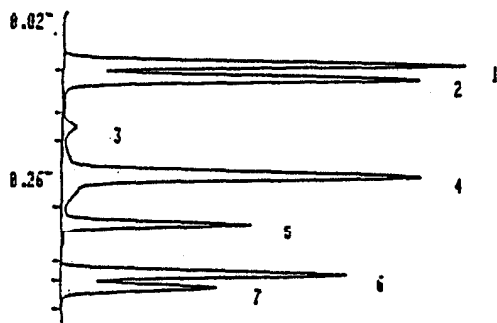
Sample: Cholesterol palmitate (Cho.E), Cholesterol acetate (Cho.A), Cholesterol (Cho),
Phosphatidyl ethanolamine (PE), Phosphatidyl choline (PC), Sphingomyeline (SPM)

Mobile phase:

1st Chloroform:Methanol:Water:Formic acid 50:25:2:0.5 5cm

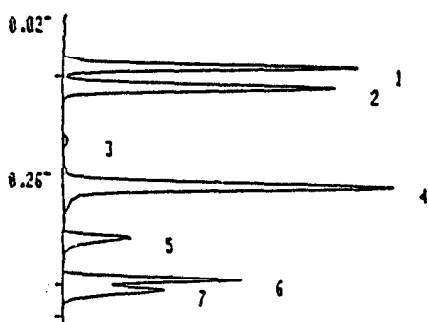
2nd Hexane:Diethyle ether:Formic acid 65:5:2:0.15 10cm

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



NO.	NAME	RT	CAL. METHOD			CONC
			SF	PA	PI	
1	Cho.E	0.098	10170	M	19.1094	
2	Cho.A	0.120	10497	M	19.7250	
3		0.198	651	M	1.2247	
4	Cho.	0.272	14312	M	26.8925	
5	PE	0.347	5038	M	9.4662	
6	PC	0.427	8068	M	15.1603	
7	SPM	0.448	4482	M	8.4215	
TOTAL			53221		100.0000	

Stationary phase: CHROMAROD-SIII



NO.	NAME	RT	CAL. METHOD			CONC
			SF	PA	PI	
1	Cho.E	0.103	7048	M	19.6744	
2	Cho.A	0.133	7543		21.0542	
3		0.215	141		0.3249	
4	Cho.	0.289	11041		30.8199	
5	PE	0.368	2022		5.8417	
6	PC	0.435	4767	M	13.3069	
7	SPM	0.452	3191	M	8.9077	
TOTAL			35227		100.0000	

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

CHROMAROD-SIII

	CE/CA	CA	C/CA	PE/CA	PC/CA	SPM/CA
X	0.94	1.00	1.47	0.26	0.60	0.37
s.d.	0.01		0.01	0.01	0.02	0.03
C.V.(%)	1.0		0.7	5.1	2.9	7.1
wt ratio	1.20	1.00	1.15	0.53	0.86	0.57

n=5

Copper Sulphate treated CHROMAROD-SIII

	CE/CA	CA	C/CA	PE/CA	PC/CA	SPM/CA
X	0.98	1.00	1.33	0.47	0.78	0.43
s.d.	0.01		0.03	0.01	0.03	0.03
C.V.(%)	1.0		2.4	1.7	3.8	6.3
wt ratio	1.20	1.00	1.15	0.53	0.86	0.57

n=5

Abbreviations :

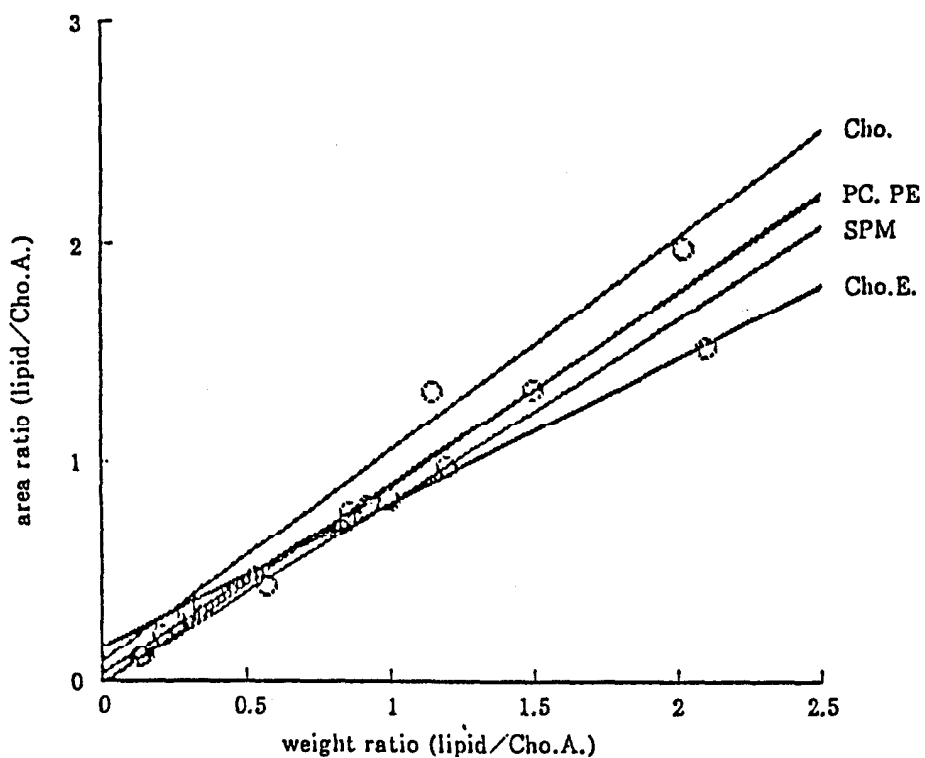
CE; cholesterol palmitate, C; cholesterol, PE; phosphatidyl ethanolamine

PC; phosphatidyl choline, SPM; sphingomyeline,

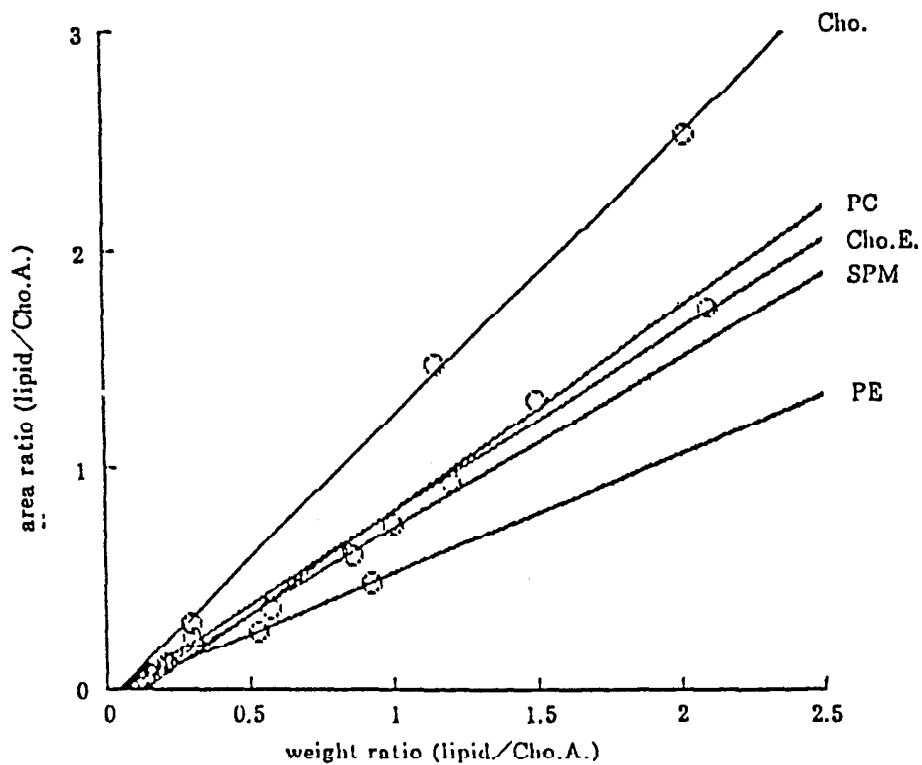
CA; cholesterol acetate (internal standard)

Table 3 - Calibration Curve

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



Stationary phase : CHROMAROD-SIII



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

T.N.B.Kaimal, N.C.Shantha : J.Chromatogr., 288, 177-186, 1984