

IATROSCAN TLC/FID
INSTRUMENT APPLICATION

5 - II

Analysis of Lipids by the Iatroscan

1) Mobile phases

- 1.1 Polar lipid standard mixture
- 1.2 Internal standard (Cholic acid)

2) Health foods

- 2.1 Vitamine E and Wheat germ oil on the market
- 2.2 Lecithin on the market

3) Cosmetics and Skin lipids

- 3.1 Lipid standard mixture
- 3.2 Lipid standard mixture
- 3.3 Lipstick (1)
- 3.4 Lipstick (2)
- 3.5 Glycolipid in a bovine brain
- 3.6 Oil Soluble Substance

4) Other lipids

- 4.1 lipid in a colon bacillus
- 4.2 lipid in Wakame seaweed
- 4.3 lipid extraction from washing solution of alveoli of the lung
- 4.4 Washing solution of alveoli of the lung

Conditions

Stationary phase : CHROMAROD-SIII

Gas flow : H₂ 160mL/min , Air 2.0L/min

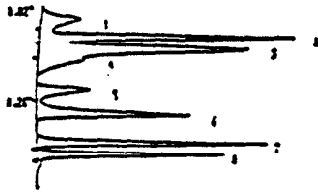
Scanning speed : 30sec/scan

Lipid components are expressed in the following abbreviations

Component	Abbreviation
Cholesterol	Cho
Cholesterol ester	Cho.E.
Cardiolipin	CL
Ceramide mono hexoside	CMH
Fatty acid	FA
Lysophosphatidyl choline	LPC
Lysophosphatidyl ethanolamine	LPE
Phosphatidyl choline	PC
Phosphatidyl ethanolamine	PE
Phosphatidyl glycerol	PG
Phosphatidyl inositol	PI
Phosphatidyl serine	PS
Sphingomyeline	SM
Sulfatide	CSE
Triglyceride	TG

1) Mobile phases

1.1 Polar lipid standard mixture



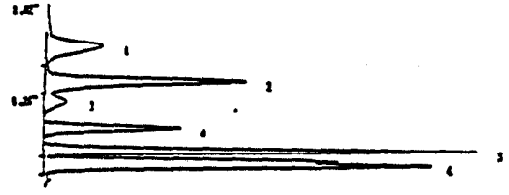
Component

2.PE 3.PI 4.PS 5.LPE 6.PC 7.SM 8.LPC

Mobile phase

Chloroform : Methanol : Water

45 : 20 : 2 10cm 2times



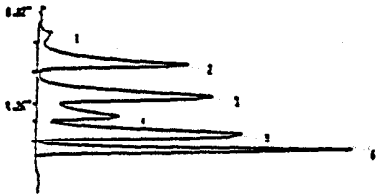
Component

2.PE+PI 3.PS 4.LPE 5.PC : 6.SM+LPC

Mobile phase

Chloroform : Methanol : Water-

40 : 24 : 2 10cm



Component

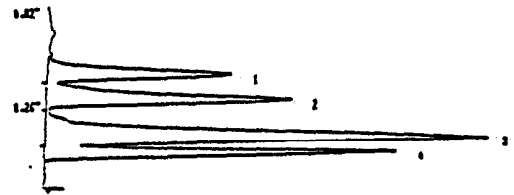
2.PE 3.PC 4.LPE 5.SM 6.LPC

Mobile phase

Chloroform : Methanol : Water : conc

Ammonia

39 : 24 : 1.5 : 3 10cm



Component

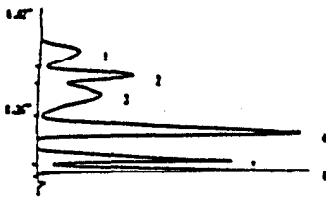
1.PE 2.PC 3.LPE+SM 4.LPC

Mobile phase

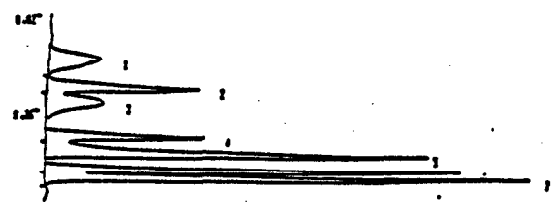
Chloroform : Methanol : Water : conc

Ammonia

52 : 20 : 0.8 : 2.5 10cm

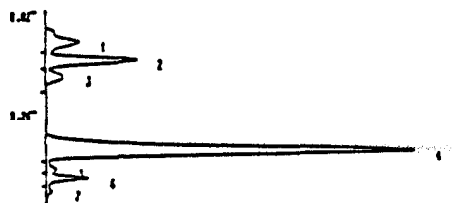


Component
 2.PE 3.PI+PS 4.LPE+PC 5.SM 6.LPC
 Mobile phase
 Chloroform : Methanol : Water : Formic acid
 45 : 20 : 2.5 : 1 10cm

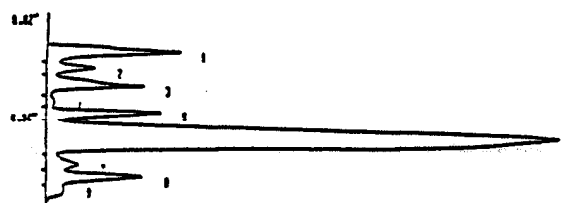


Component
 2.PE 3.PI+PS 4.LPE 5.PC 6.SM 7.LPC
 Mobile phase
 Chloroform : Methanol : Water : Formic acid
 45 : 25 : 2.5 : 1 10cm

1.2 Internal standard (Cholic acid)



Component
 2.Cholic acid 3.PE 4.PC 6.LPC
 Mobile phase
 Chloroform : Methanol : Water
 50 : 25 : 2.5 10cm



Component
 3.PE 5.Cholic acid 6.PC 8.LPC
 Mobile phase
 Chloroform : Methanol : Water : conc
 Ammonia
 50 : 25 : 2.5 : 0.05 10cm 2times

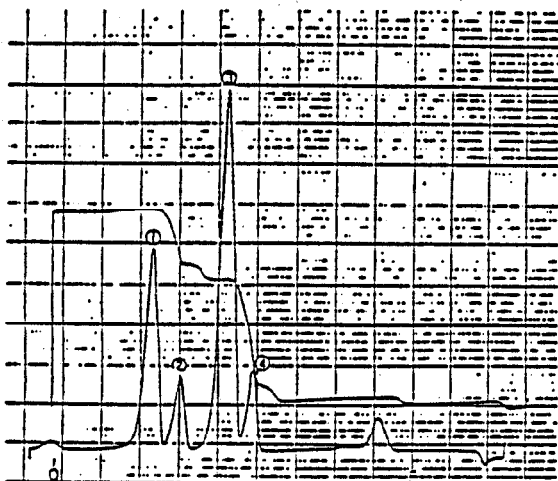
2) Health foods

Liquid sample are dissolved in chloroform : methanol = 2 : 1.
Extraction of lipids from solid state samples is done as following :

1. Break down a solid state sample.
2. About 200mg of the sample are stirred in 15mL of Chloroform : methanol = 2 : 1
3. After filtration, add 3mL water to the filtrate and stir.
4. Centrifuge for 10min at 2000rpm.
5. Tke off the upper layer, and dry up the lower layer (chloroform layer) by N₂ stream.
6. Finally, the dried sample is dissolved in chloroform : methanol = 2 : 1.

2.1 Vitamine E and Wheat germ oil on the market

Vitamine E standard sample



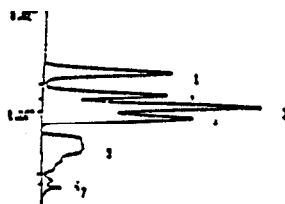
Component

1. tocol 2. δ -tocopherol 3. β, γ -tocopherol 4. α -tocopherol

Mobile phase

Benzene : n-Hexane 48 : 2

Vimarine E and
Lipid standard mixture



Component

1. TG 2. α -tocopherol 3. β, γ -tocopherol 4. δ -tocopherol

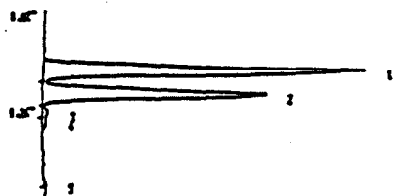
Mobile phase

Benzene : Methanol 60 : 1 10cm

Mobile phase

Benzene : Methanol 60 : 1 10cm

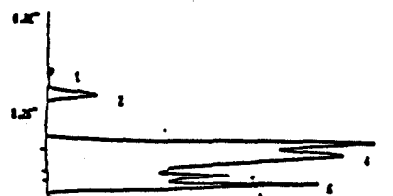
B corporation: Vitamine E



Component

LTG 2 α -tocopherol

E corporation (1): Vitamine E



Component

LTG 2 α -tocopherol

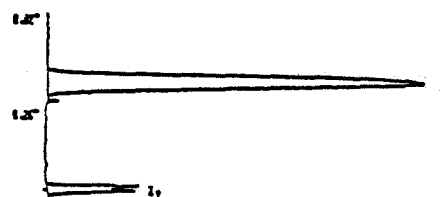
E corporation (2): Vitamine E



Component

1 α -tocopherol

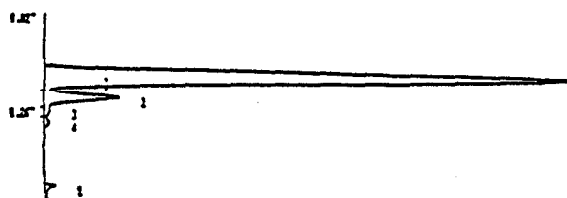
K corporation: Vitamine E



Component

1 α -tocopherol

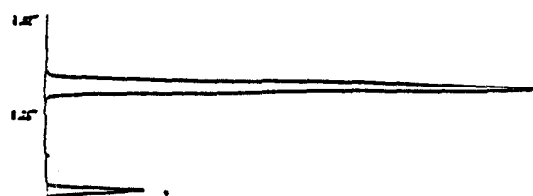
N corporation: Vitamine E



Component

LTG 2 α -tocopherol

S corporation: Vitamine E



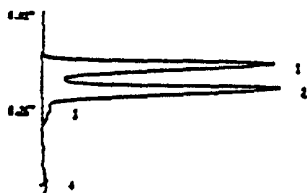
Component

1 α -tocopherol

Mobile phase

Benzene : Methanol 60 : 1 10cm

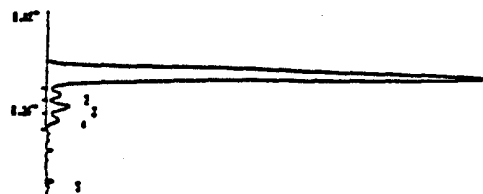
R corporation: Vitamine E



Component

1.TG 2. α -tocopherol

H corporation: Wheat germ oil



Component

1.TG 2. α -tocopherol
3. β, γ -tocopherol 4. δ -tocopherol

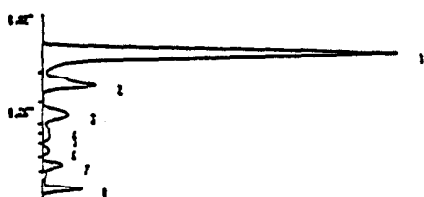
2.2 Lecithin on the market

Mobile phase

A. Chloroform : Methanol : Water : conc Ammonia 39 : 24 : 1.5 : 3 10cm

B. Chloroform : Methanol : Water : Formic acid 45 : 20 : 2.5 : 1 10cm

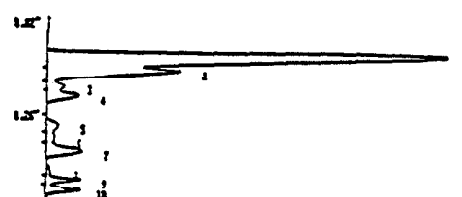
H corporation: Lecithin



Component

2.PE 3.PC 4.5.PI+PS 6.SM 7.LPC

Mobile phase : A

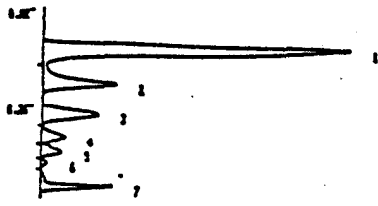


Component

4.PE 5.PI+PS 7.PC 9.LPC

Mobile phase : B

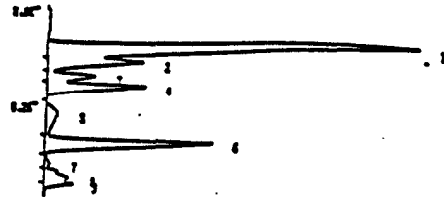
K corporation: Lecithin



Component

2.PE 3.PC 4.PI+PS 5.SM 6.LPC

Mobile phase : A

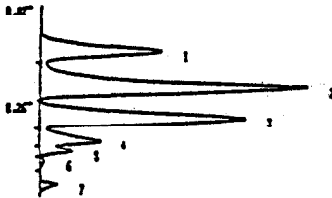


Component

4.PE 5.PI+PS 6.PC

Mobile phase : B

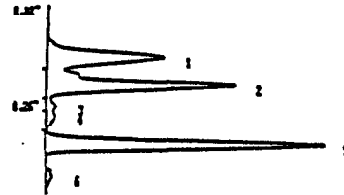
N corporation: Lecithin



Component

2.PE 3.PC 4.PI+PS 5.SM 6.LPC

Mobile phase : A

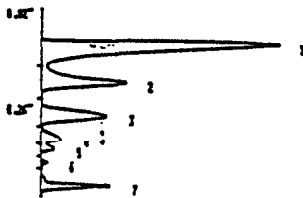


Component

2.PE 3.PI+PS 5.PC

Mobile phase : B

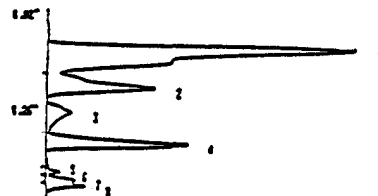
W corporation: Lecithin



Component

2.PE 3.PC 4.PI+PS 5.SM 6.LPC

Mobile phase : A



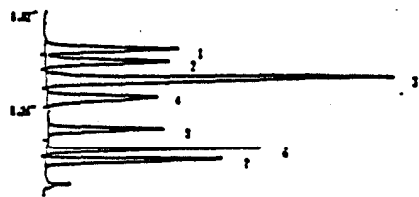
Component

2.PE 3.PI+PS 4.PC 6.SM 7.LPC

Mobile phase : B

3) Cosmetics and Skin lipids

3.1 Lipid standard



Component

1.Squalane 2.Wax 3.TG 4.FA 5.Cho
6.Ceramide 7.Cho-sulphate

Mobile phase

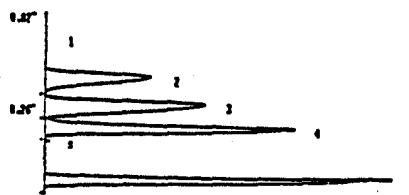
1st Chloroform : Methanol : Water
57 : 12 : 0.6 2.5cm

2nd Chloroform : Methanol : Water
57 : 12 : 0.6 2.5cm

3rd n-Hexane : Ethyl ether : Formic acid
50 : 20 : 0.3 8cm

4th n-Hexane : Benzene 35 : 35 10cm

3.2 Lipid standard



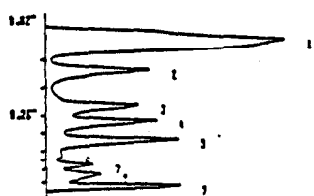
Component

2. 2-Octyl dodecanol
3. Behenyl alcohol 4.Cho
6. N-Lauroyl-L'-glutamic acid

Mobile phase

n-Hexane : Ethyl ether : Formic acid
50 : 20 : 0.7 10cm

3.3 Lipstick (1)



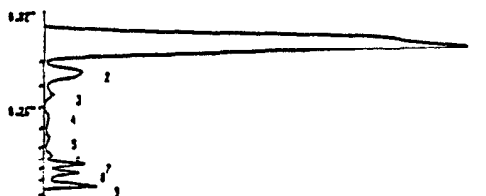
Component

1.Hydrocarbon 2.Wax
3.Ester of fatty acid 8.FA

Mobile phase

1st Benzene 100% 7cm
2nd n-Hexane : Benzene 1 : 1 10cm

3.4 Lipstick (2)



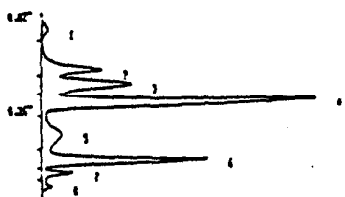
Component

1.Hydrocarbon 2.Wax
3.Ester of fatty acid 8.FA

Mobile phase

1st Benzene 100% 7cm
2nd n-Hexane : Benzene 1 : 1 10cm

3.5 Glycolipid in a bovine brain



Component

2.Cho 3.CMH+CSE 4.PE 5.PS 6.PC
7.SM

Mobile phase

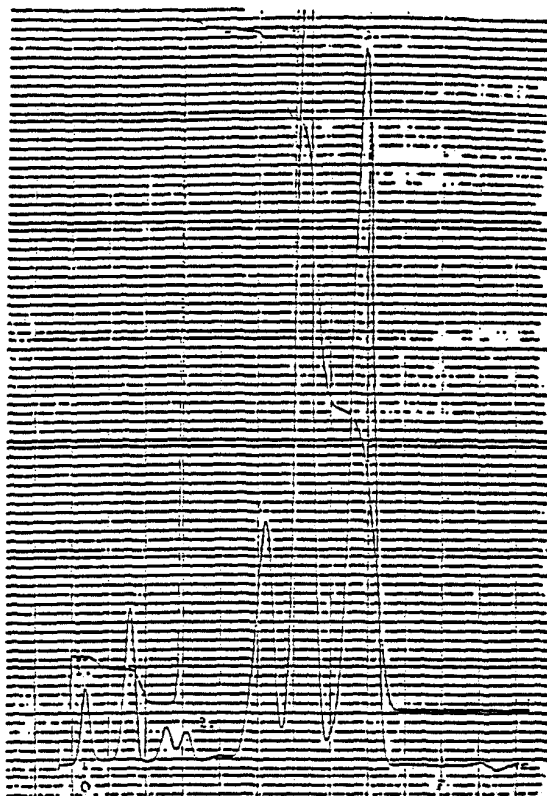
1st Chloroform : Methanol : Water : Acetic acid

70 : 30 : 3 : 0.5 8.5cm

2nd n-Hexane : Ethyl ether : Formic acid

63 : 7 : 0.1 10cm

3.6 Oil Soluble Substance



Component

1.Propylene glycol 2.Cetanol
3.Stearic acid 4. 2-Ethyl hexyl TG
5.Wool Wax, Liquid Paraffin, Squalane

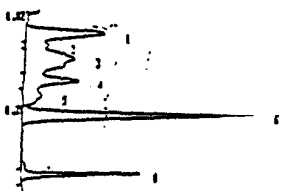
Mobile phase

Petroleum ether : Ethyl ether : Acetic acid

90 : 10 : 1 10cm

4) Other lipids

4.1 lipid in a colon bacillus



Component

4.CL 5.PG 6.PE

Mobile phase

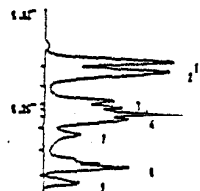
1st Chloroform : Methanol : Ammonia : Water

40 : 20 : 2 : 0.25 8cm

2nd n-Hexane : Ethyl ether : Formic acid

60 : 10 : 0.15 10cm

4.2 lipid in Wakame seaweed



Component

2.FA 3.4.5.6.Cho+Chlorophyll 7.PE 8.PC

Mobile phase

1st Chloroform : Methanol : Water

50 : 20 : 2 7cm

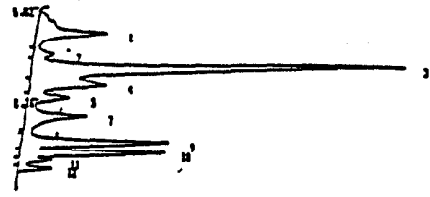
2nd n-Hexane : Ethyl ether : Formic acid

55 : 15 : 0.15 10cm

4.3 Lipid extraction from washing solution of alveoli of the lung

Washing solution of alveoli of the lung
 ↓ centrifuging (3000rpm)
 Upper layer (10mL)
 ↓
 90mL of chloroform:methanol (2:1)
 ↓ mixing for 1 min on a mixer
 20mL of water
 ↓ mixing
 filtration
 ↓
 Centrifugation (3000rpm, 3min)
 ↓
 taking off the upper layer (water layer)
 ↓
 drying up the lower layer
 (Chloroform layer)
 ↓
 dissolving in 300μL chloroform

4.4 Washing solution of alveoli of the lung



Component

1.Cho.E.	3.Cho	5.PE	7.PI,PS	9.PC	10.SM
11.LPC					

Mobile phase

1st Chloroform : Methanol : Ammonia : Water	
	38 : 29 : 0.6 : 2.3 8cm
2nd n-Hexane : Ethyl ether	65 : 5 10cm