Comparative studies on analyses of lipids using LC-NH2 silica gel columns and styrene-divinylbenzene columns from Supelco and Macherey-Nagel.

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- We have previously established a method to isolate lipid classes on aminopropyl-bonded silica gel columns LC-NH2 (Bodennec et al.,JLR, 2000).

- The eluting solvents for each fraction were defined using columns purchased from Supelco.

- We applied the same elution method using LC-NH2 columns from other suppliers such as Macherey-Nagel.

- Various lipid standards were mixed and taken up in chloroform and applied on aminopropyl-bonded silica gel columns (LC-NH2), then eluted into six fractions (neutral lipids, free ceramides, free fatty acids, neutral glycolipids and phospholipids).

- Components - analyzed by HPTLC using specific solvent systems and differential spray reagents for specific visualization.

- Quantification of the spots on HPTLC plates - Scanning densitometry .

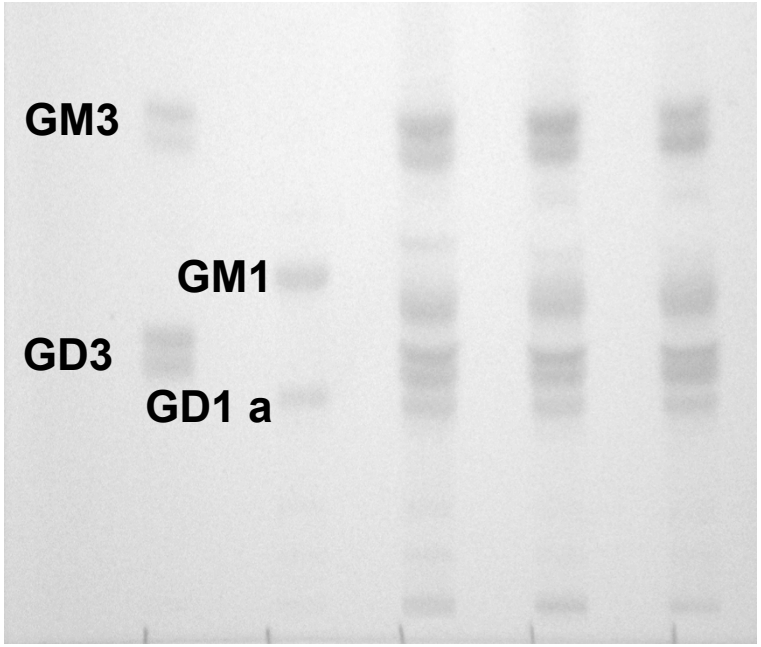
- Standard gangliosides of GM3, GM1, GD3, GD1a and GT1b were mixed and taken in methanol-PBS and applied on Supelco or Macherey-Nagel styrene-divinyl benzene columns (①, ② and ③).

Purification of gangliosides from aqueous phase on ENVI-Chrom P Supelco versus HR-X Macherey-Nagel

Gangliosides standards were applied on 3 columns :
for each column were applied 20µg melanoma gangliosides and 15µg brain gangliosides (evaporate, take up in 1 ml de PBS/methanol 1:1)

	①	②	③
	ENVI-Chrom P	**HR-X**	**HR-X**
Conditioning	1) 3 ml methanol 2) 15 ml PBS / methanol 1:1		1) 5 ml methanol 2) 5 ml water
Applying	~ 1 ml of sample		
washing	12 ml water		5 ml water
Elution	1) 3 ml methanol 2) 3 ml chloroform / methanol 1:1		3 x 2 ml methanol

Gangliosides



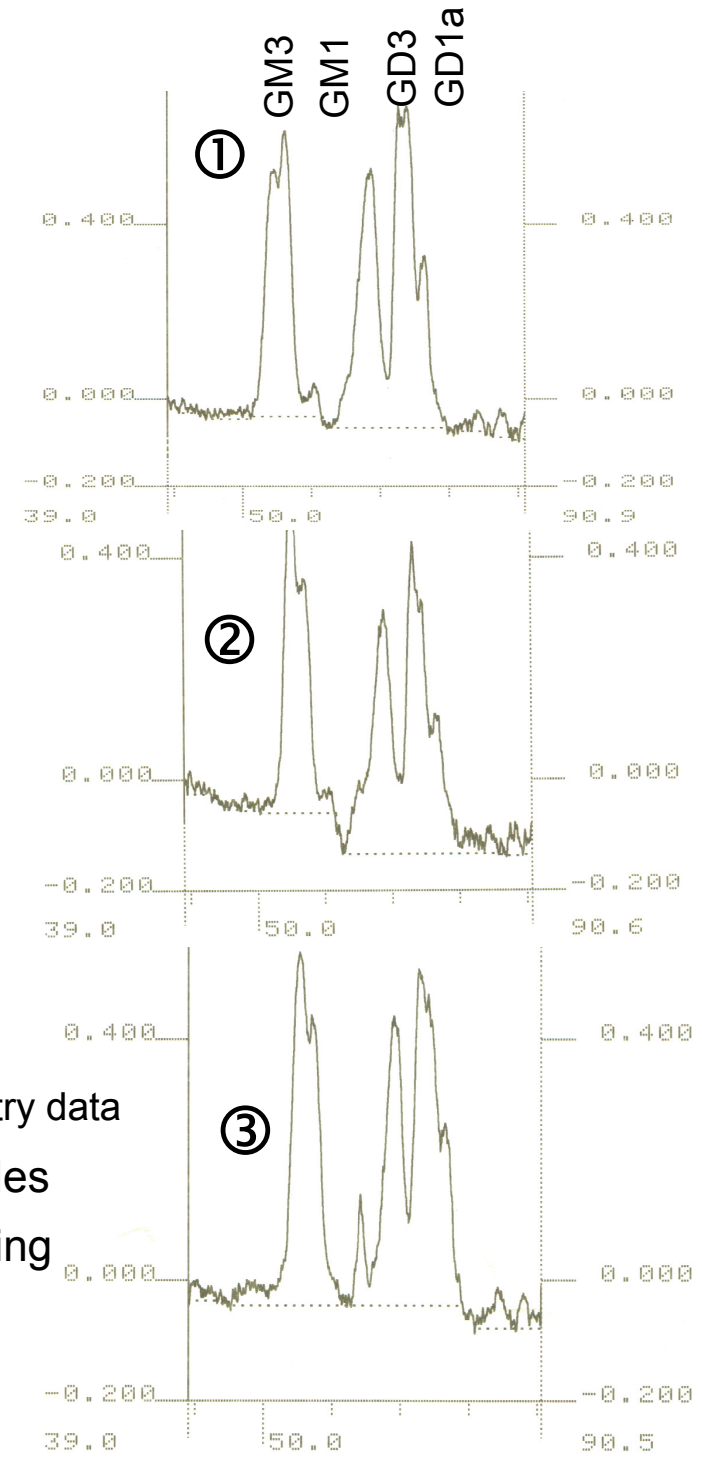
	①	②	③
GM3	33%	40%	40%
GM1	28%	30%	23%
GD3	23%	21%	23%
GD1 a	9%	5%	7%

Standards ① ② ③ ① ② ③

Melanoma Brain

% of total

Scanning densitometry data



On **Macherey-Nagel columns**, a better stability of gangliosides is observed using PBS instead of water for column conditioning before gangliosides solution application. Moreover, a higher recovery is obtained on Supelco columns.

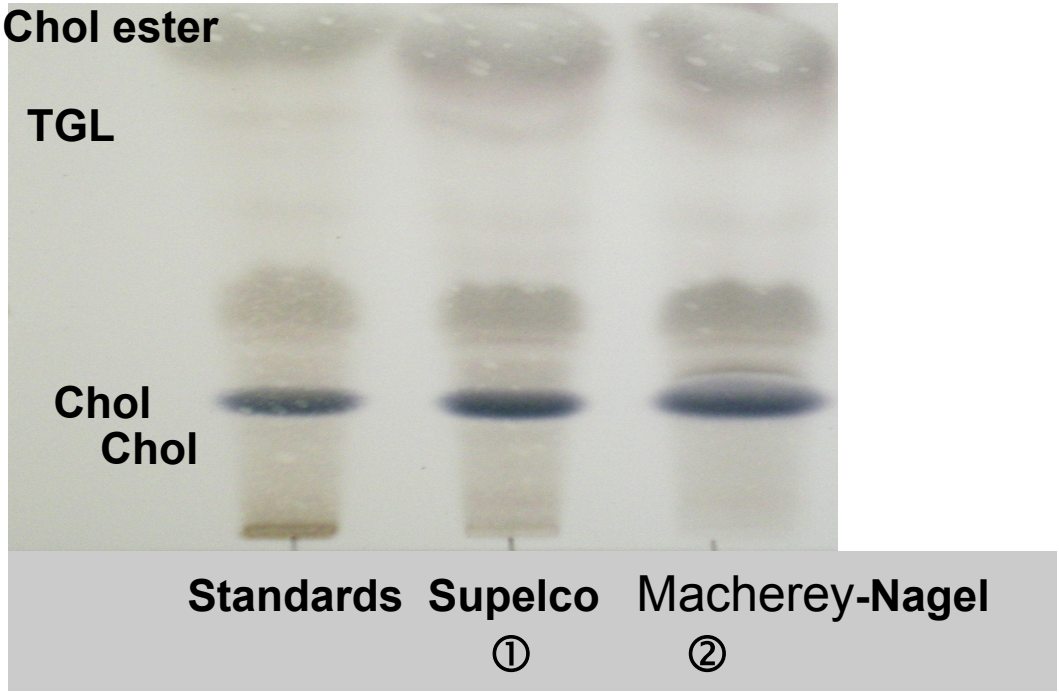
Purification of lipids on columns from LC-NH2 Supelco versus LC-NH2 Macherey-Nagel

Lipids amount
applied on one column:
45 µg Neutral lipids
4µg Ceramide type III
4µg Ceramide type IV
4µg phytoceramide
4 µg palmitic acid
15 µg oleic acid
30 µg CMH-CDH
10 µg Sphingomyelin
15 µg Phosphatidylethanolamine
20 µg DPPC

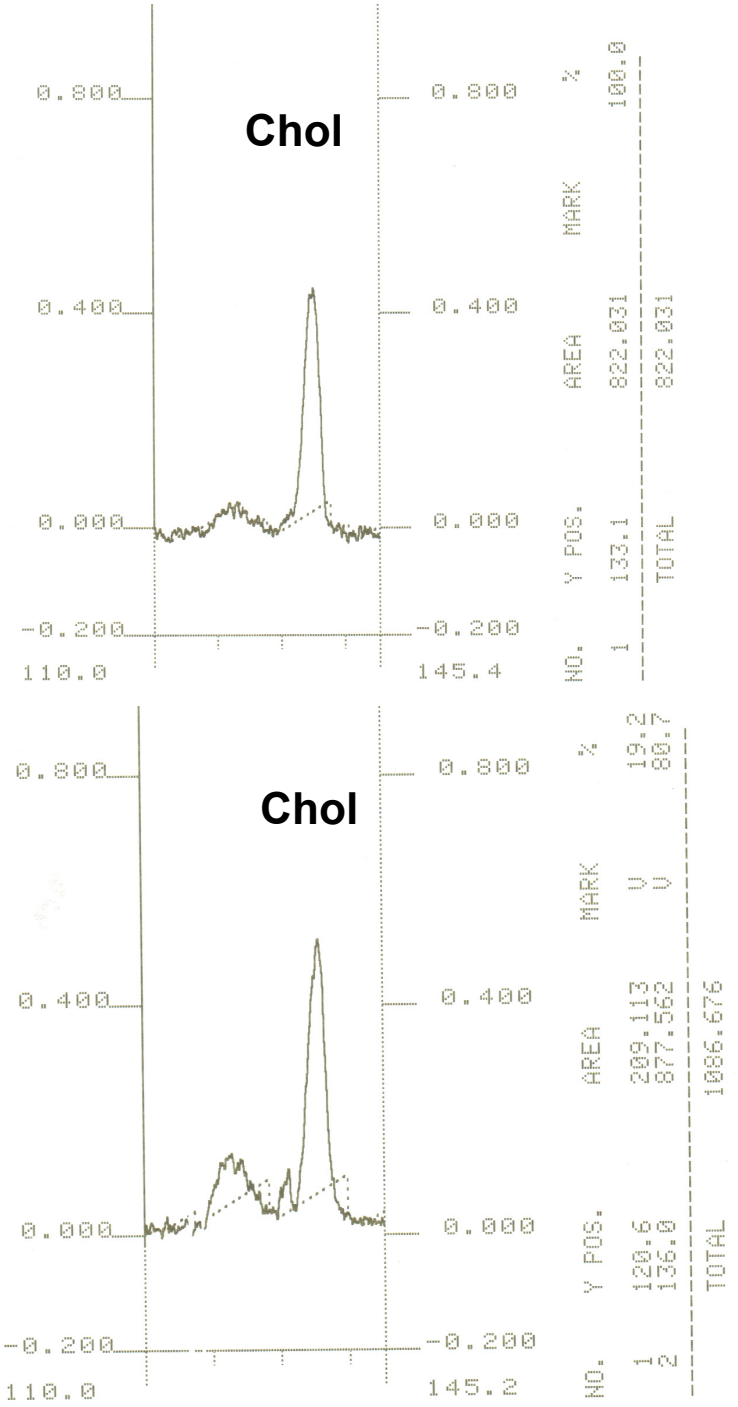
A mixture of Standards were evaporated in order to be applied on two columns: ① /Supelco ②/Macherey-Nagel
The amount of lipids for each column was taken up in 1 ml of chloroform and applied.
The same procedure for both columns was used.

Conditioning of each column	**5 ml hexane**
Lipids applied	**500 µL chloroform**
F1	**4 ml diethyl ether**
F2	**3 ml chloroform / methanol 23:1(v/v)**
F3	**4 ml diisopropylether / acetic acid 98:4(v/v)**
F4	**4 ml acetone / methanol 9:1.2 (v/v)**
F5	**1) 3 ml chloroform / methanol 2:1(v/v)** **2) 4 ml methanol**

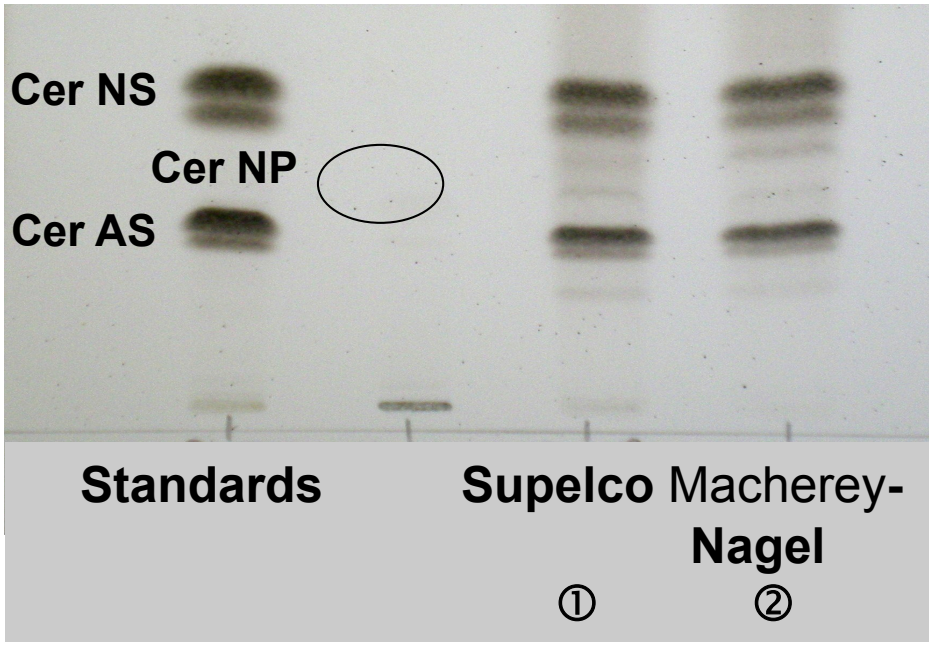
Fraction F1, Neutral lipids

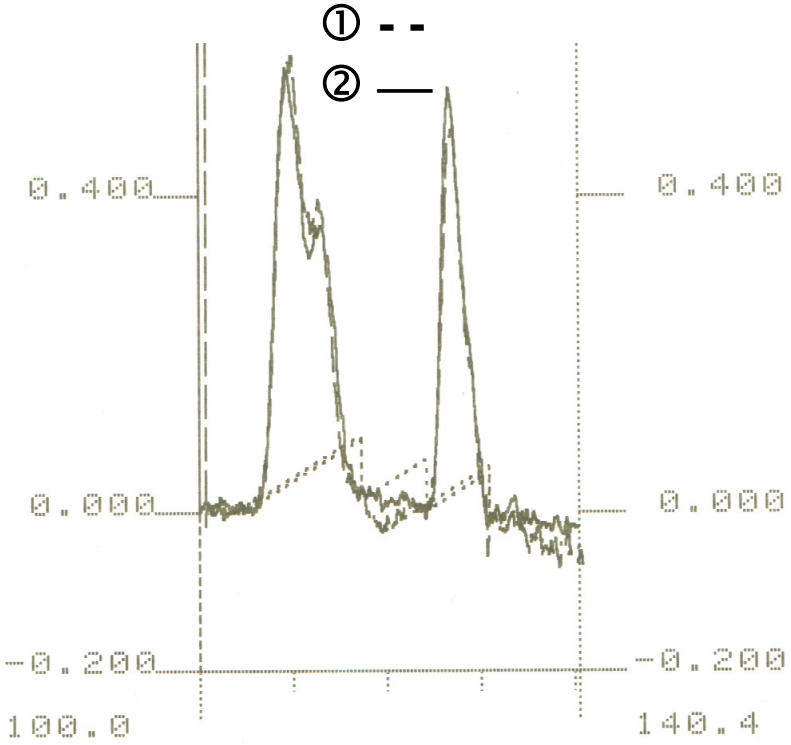


Similar recovery on both columns



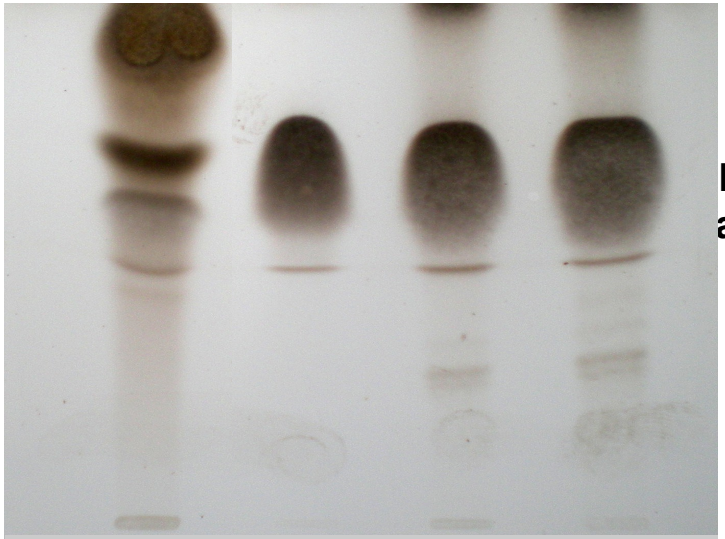
Fraction F2, free ceramides

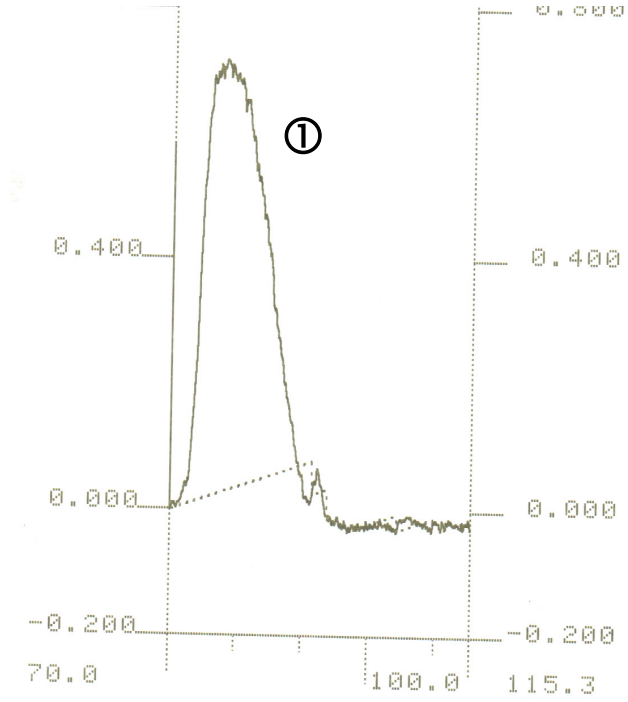


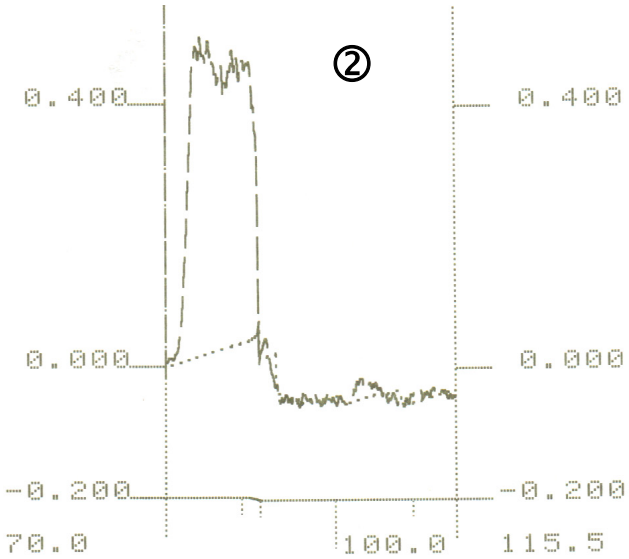


Similar recovery on both columns

Fraction F3,Free fatty acids

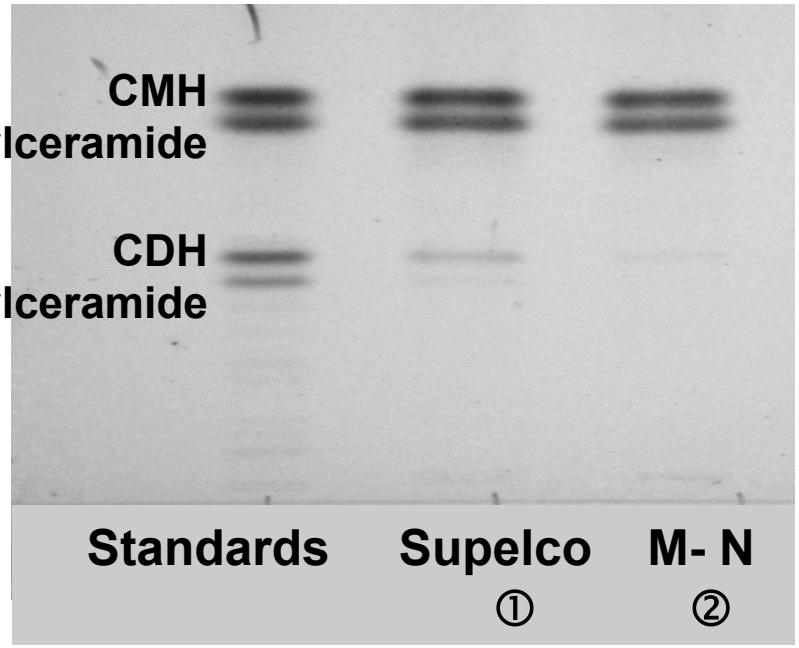


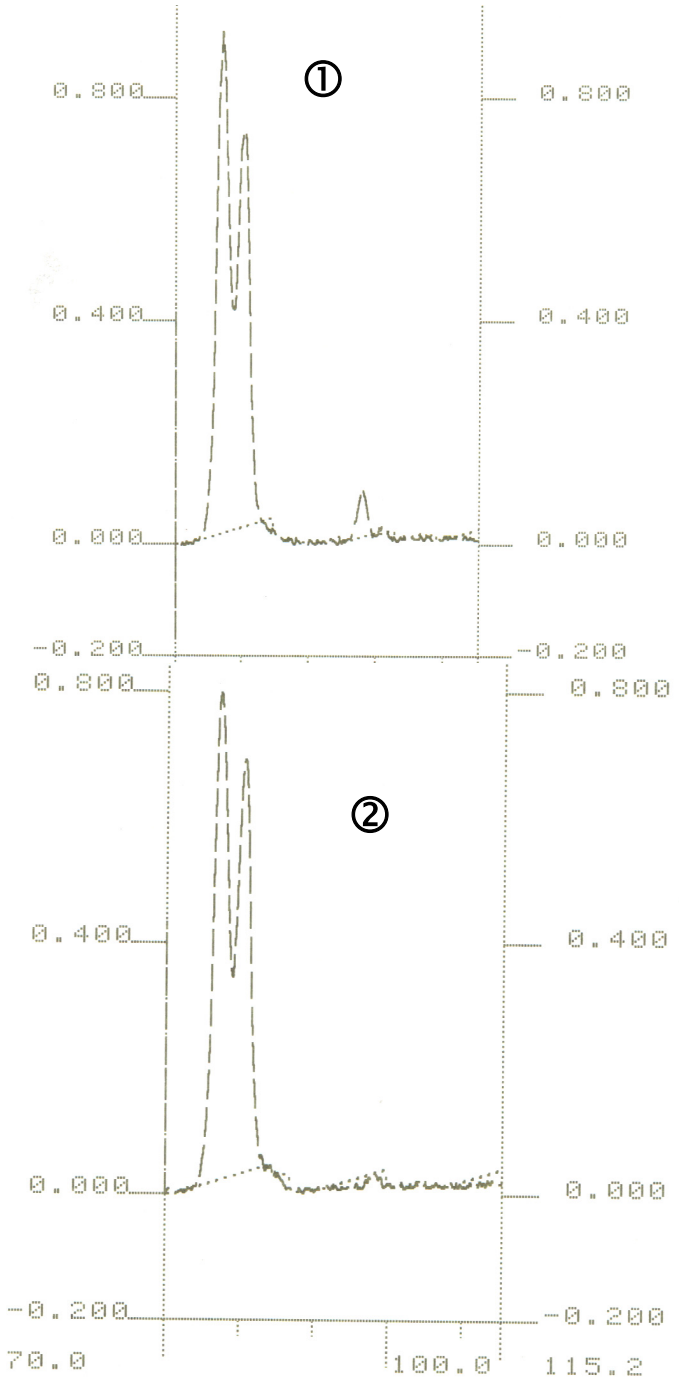




25% better recovery on Supelco column.

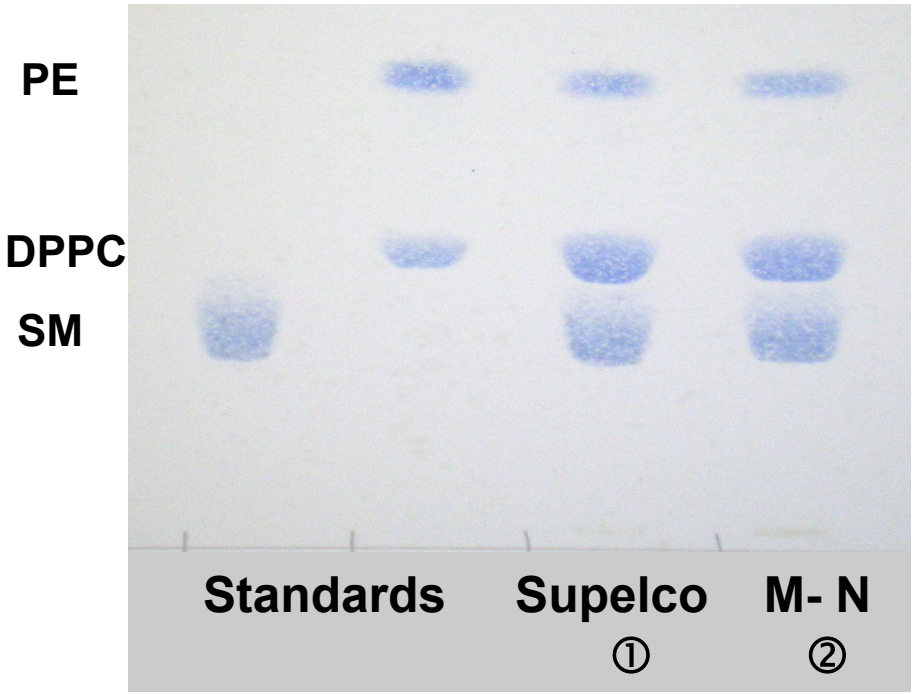
Fraction F4, Neutral Glycolipids

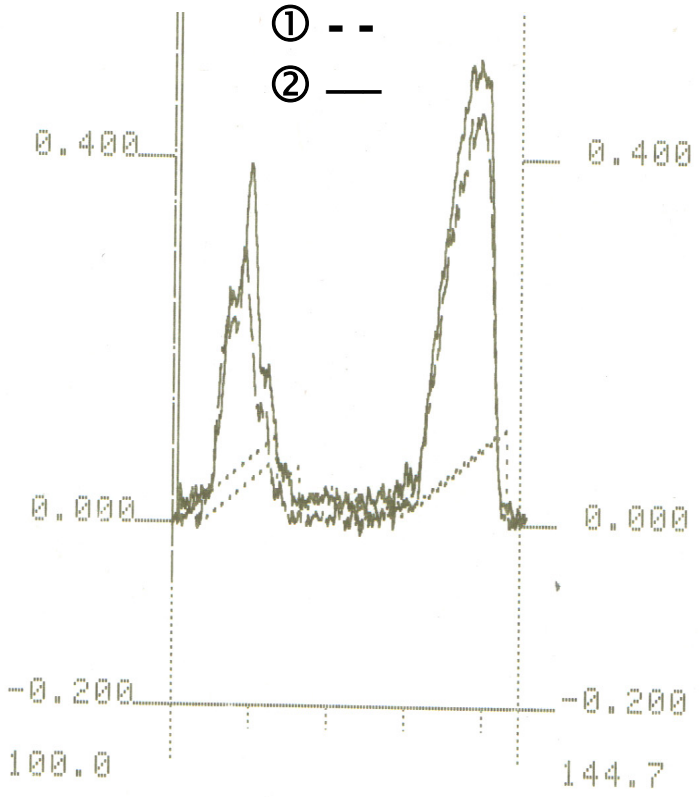




Better recovery of CDH on Supelco column.

Fraction F5, Neutral phospholipids





Slightly better recovery on Supelco column.

Conclusions:

➢The results show the differences between the Supelco and Macherey-Nagel columns.

Concerning gangliosides purification from aqueous phase, we observed:

❖ A difference in adsorption capacity between the columns of these two suppliers

❖ A higher recovery was obtained on Supelco columns.

❖ On Macherey-Nagel columns, a better stability of gangliosides was seen using PBS instead of water for column conditioning before gangliosides solution application. This emphasizes the sensitivity of the sialic acid bond to the pH which should be neutral to ensure the stability of the molecule in aqueous solution.

Concerning the isolation of lipid classes on LC-NH2 columns, we observed:

❖ A globally better recovery on Supelco columns that may be explained by a higher capacity of the silica gel matrix from Supelco to adsorb lipids.