Overview on the identification of different classes of lipids by HPTLC (High Performance Thin Layer Chromatography) and ITLC (Immuno Thin Layer Chromatography)

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Gangliosides isolated on copolymer styrene divinylbenzen

Popa et al., J. Lipid Res. 2002, 43:1335-1340

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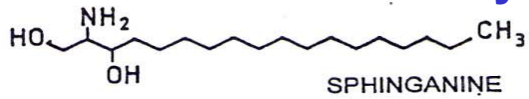
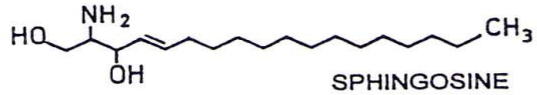
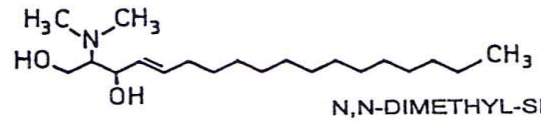
Chemical structures of some sphingoid molecules naturally occurring in mammalian cells



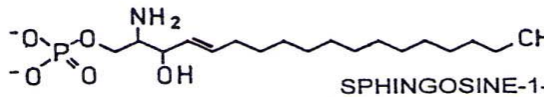
SPHINGANINE



SPHINGOSINE

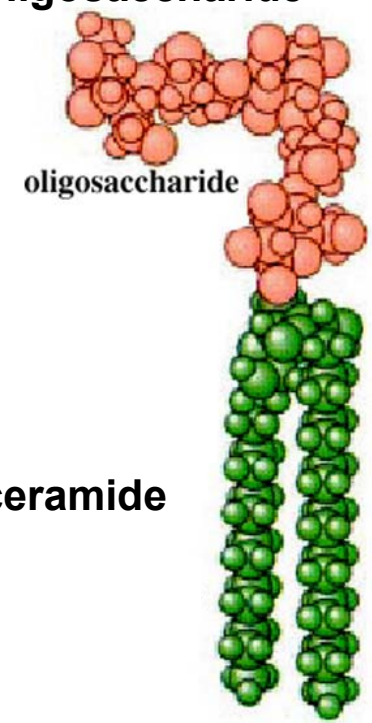


N,N-DIMETHYL-SPHINGOSINE

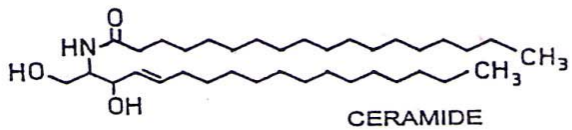


SPHINGOSINE-1-PHOSPHATE

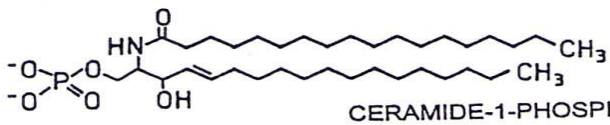
oligosaccharide



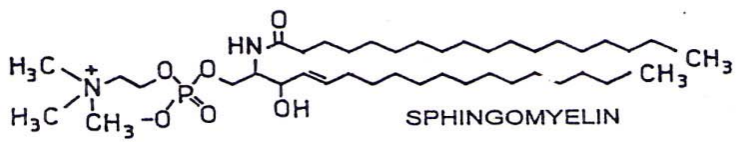
ceramide



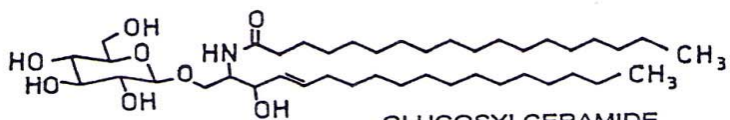
CERAMIDE



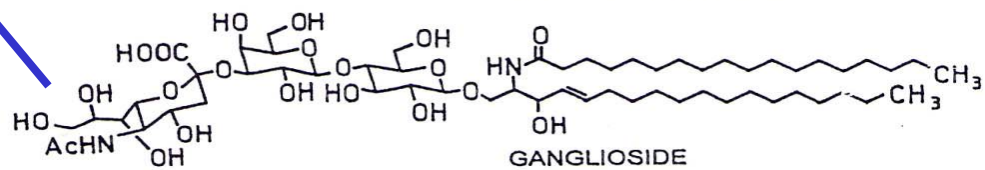
CERAMIDE-1-PHOSPHATE



SPHINGOMYELIN



GLUCOSYLCERAMIDE



GANGLIOSIDE

Gangliosides isolated on copolymer styrene-divinylbenzene columns

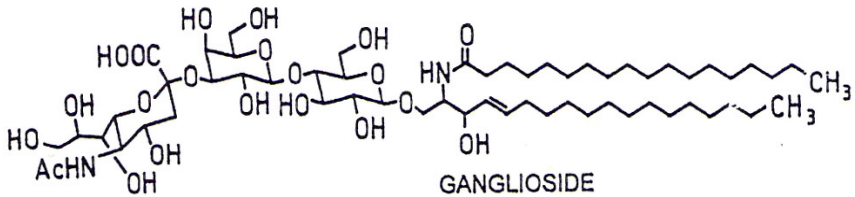
Popa et al., J. Lipid Res. 2002, 43:1335-1340

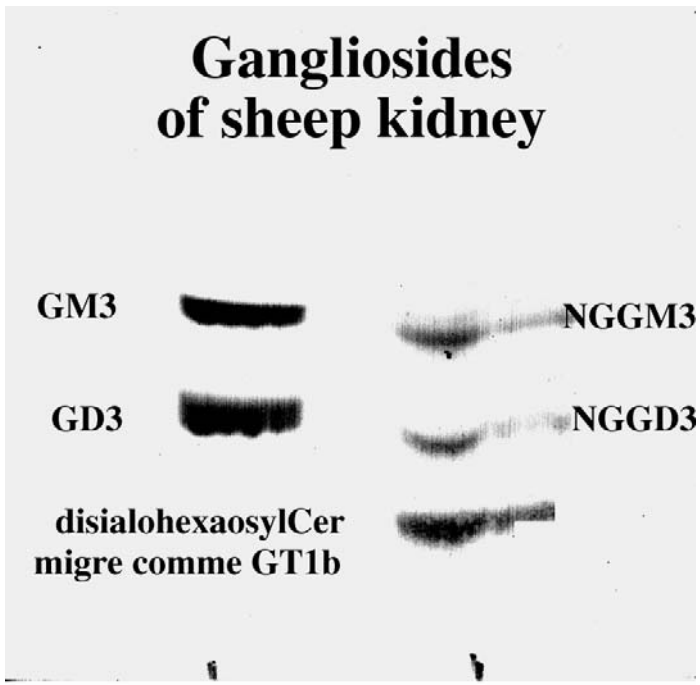
Apply on the TLC plate 1 to 5 µg of ganglioside

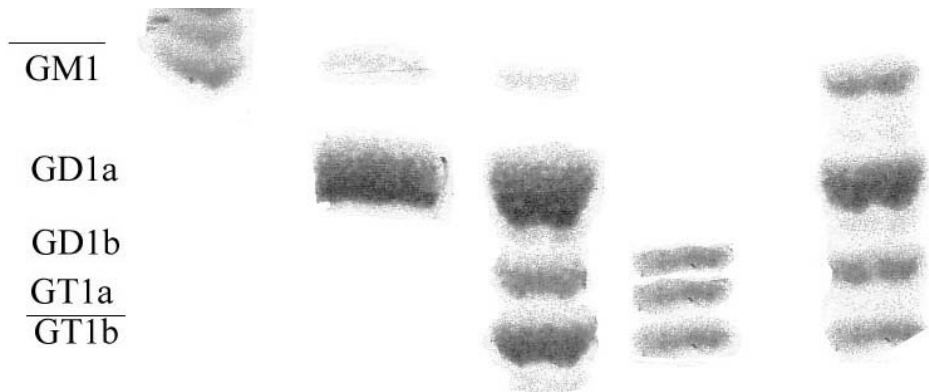
Migration in Ch/M/CaCl 0.2% 60/35/8 (by volume)

Visualization in resorcinol-HCl reagent, 120° C,

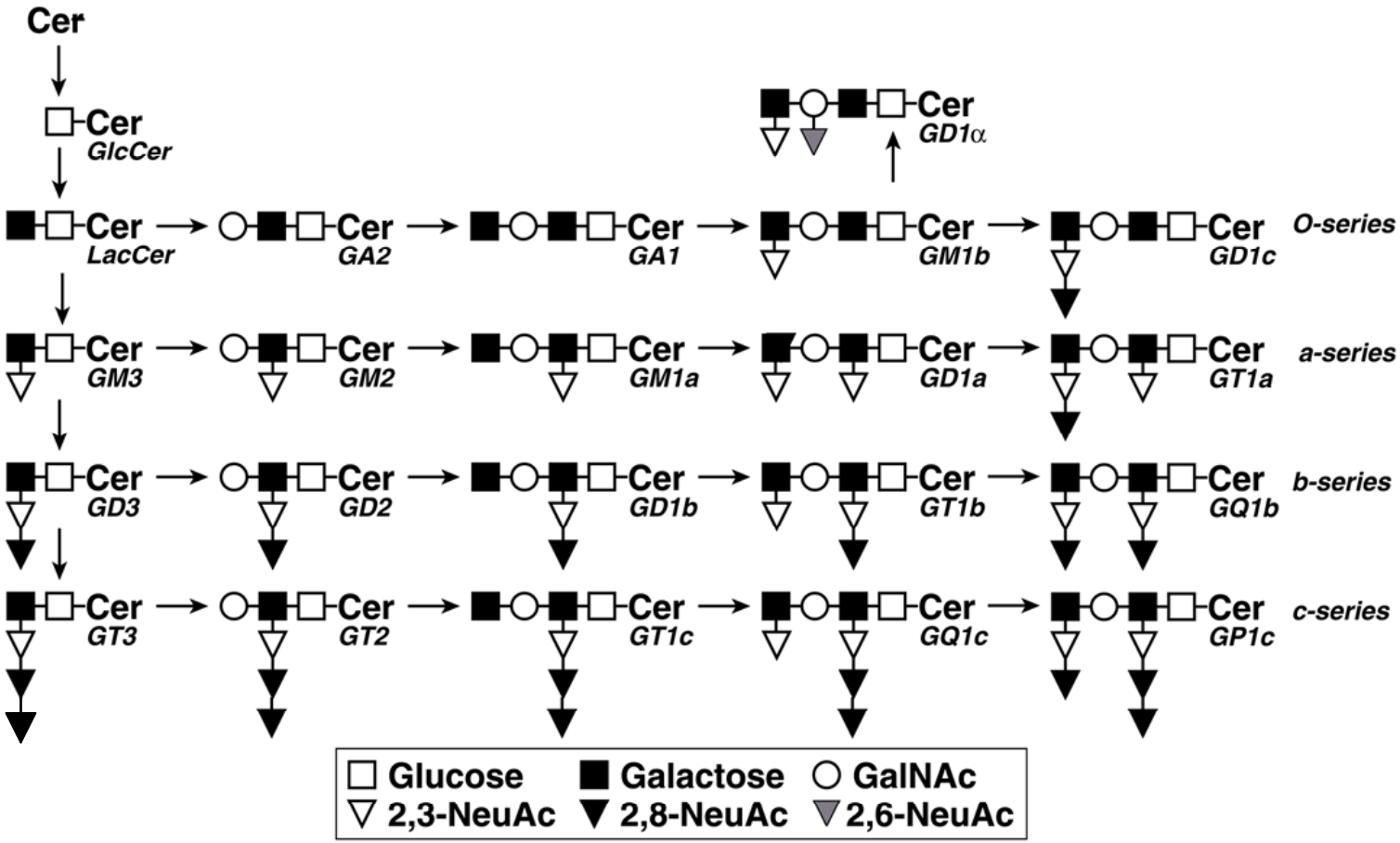
10 min







Ganglioside Biosynthetic Pathway



Analyses of different classes of lipids

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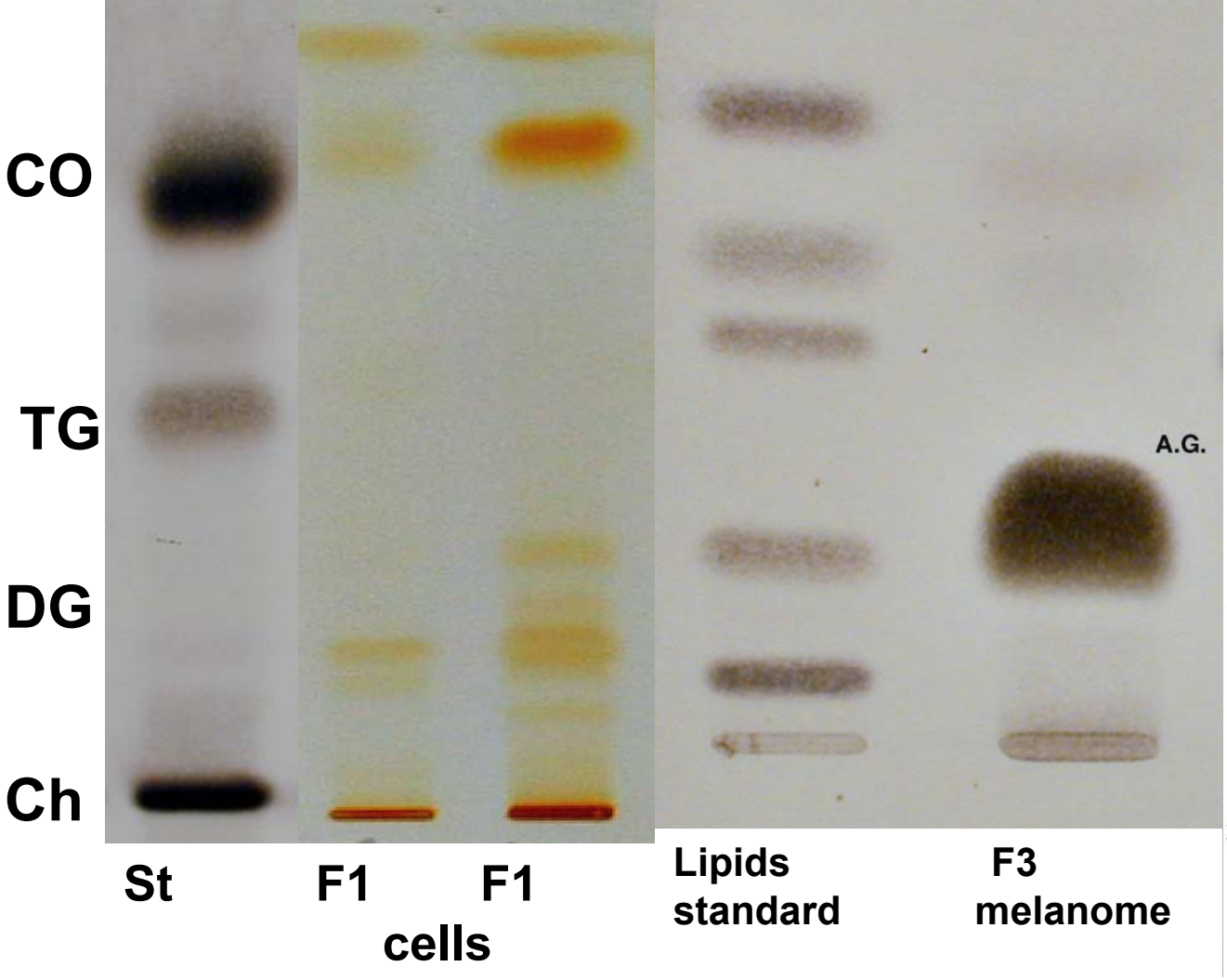
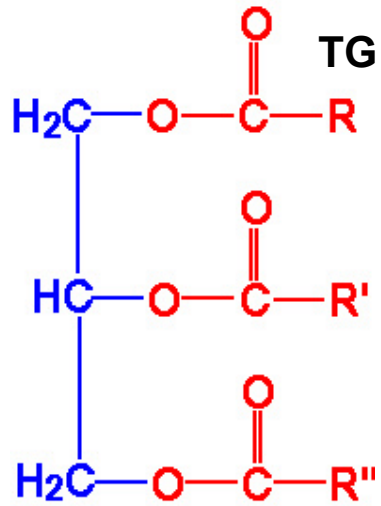
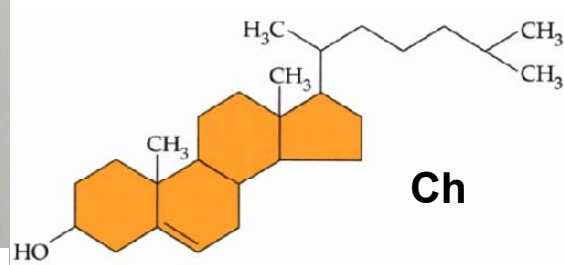
Fractions eluted from LC-NH2 columns	Standard for TLC	Solvent system	Visualization of the TLC
F1 Cholesterol (Ch), diglycerides, triglycerides, cholesterol-ester	Ch	Hexane/diethylether/acetic acid 70:30:1 (v/v/v)	Cu acetate 3 % in H3PO4 8% reagent
F2 Free ceramides and monoglycerides	Cer III, IV	chloroform/methanol 50:4 (v/v)	Cu acetate 3 % in H3PO4 8% reagent
F3 Free Fatty Acids (FFA) Free Hydroxy Fatty Acids	FFA	Hexane/ diethyl ether/acetic acid 70:30:1 (v/v/v)	Cu acetate 3 % in H3PO4 8% reagent
F4 Neutral Glycolipids Free Sphingoid Bases (SO, SA and PhytoSO)	CMH, CDH SO, SA	chloroform/methanol/water 65:25:4 (v/v/v)	Orcinol –H2SO4 reagent Ninhydrin reagent
F5 Neutral Phospholipids PC, PE, LPC and SM	SM, PE	chloroform/methanol/water 65:25:4 (v/v/v)	Dittmer and Lester reagent
F6 acidic phospholipids PI, PS and cardiolipin (CL) Gangliosides, sulphatides	PS CL	chloroform/methanol/water 65:25:4 (v/v/v)	Dittmer and Lester reagent Ninhydrin reagent







F1-cholesterol (Ch),diglycerides (DG), triglycerides(TG), cholesteryl-esters (CO)

F3- cholesterol,fatty acids, hydroxy fatty acids, cholesteryl-ester

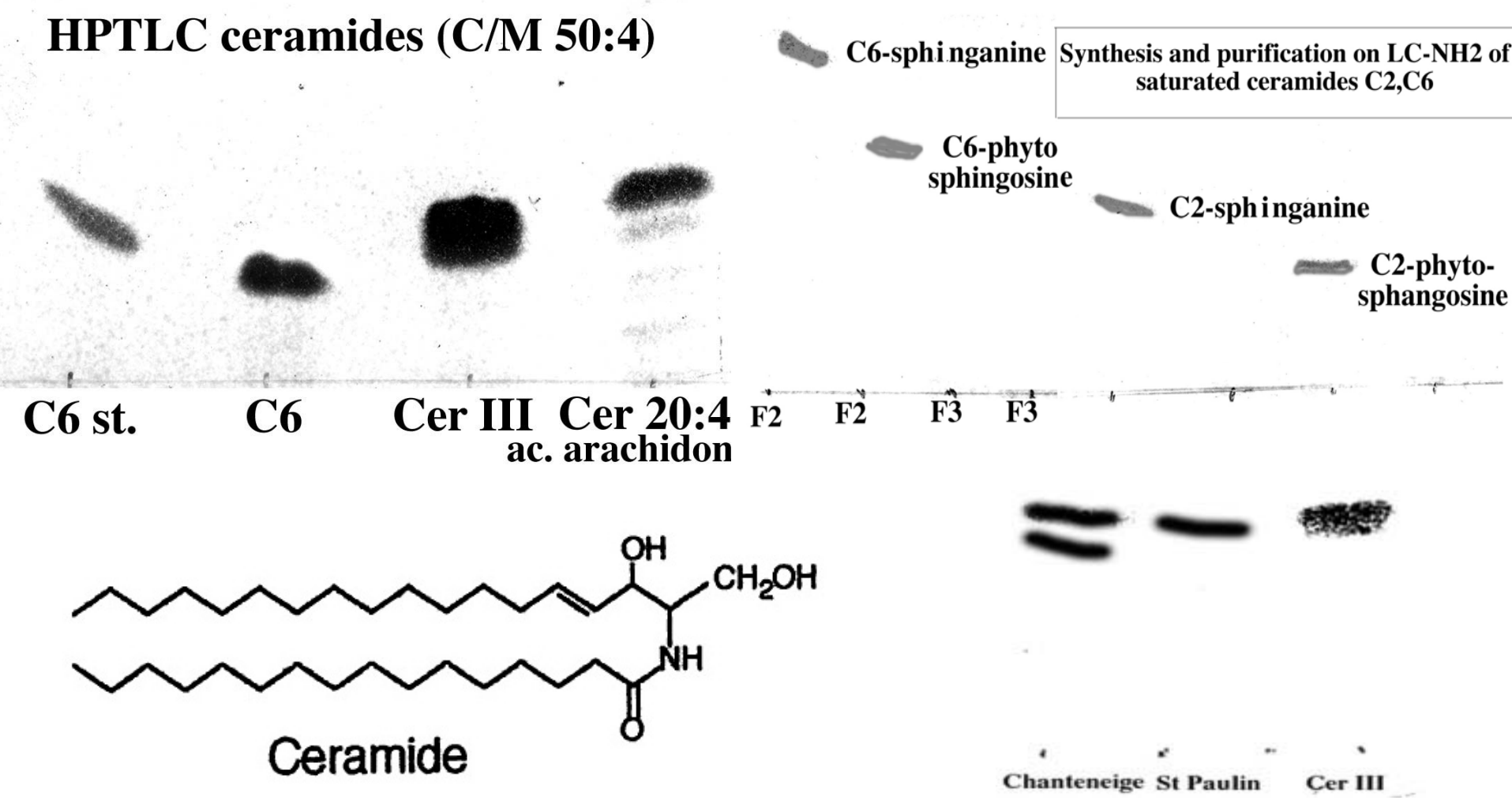
Migration in Hexane/ diethyl ether/ acetic acid 70:30:1 (by volume)

Visualization with reagent Cu acetate 3 % in H3PO4 8%

F2 ceramides fraction:

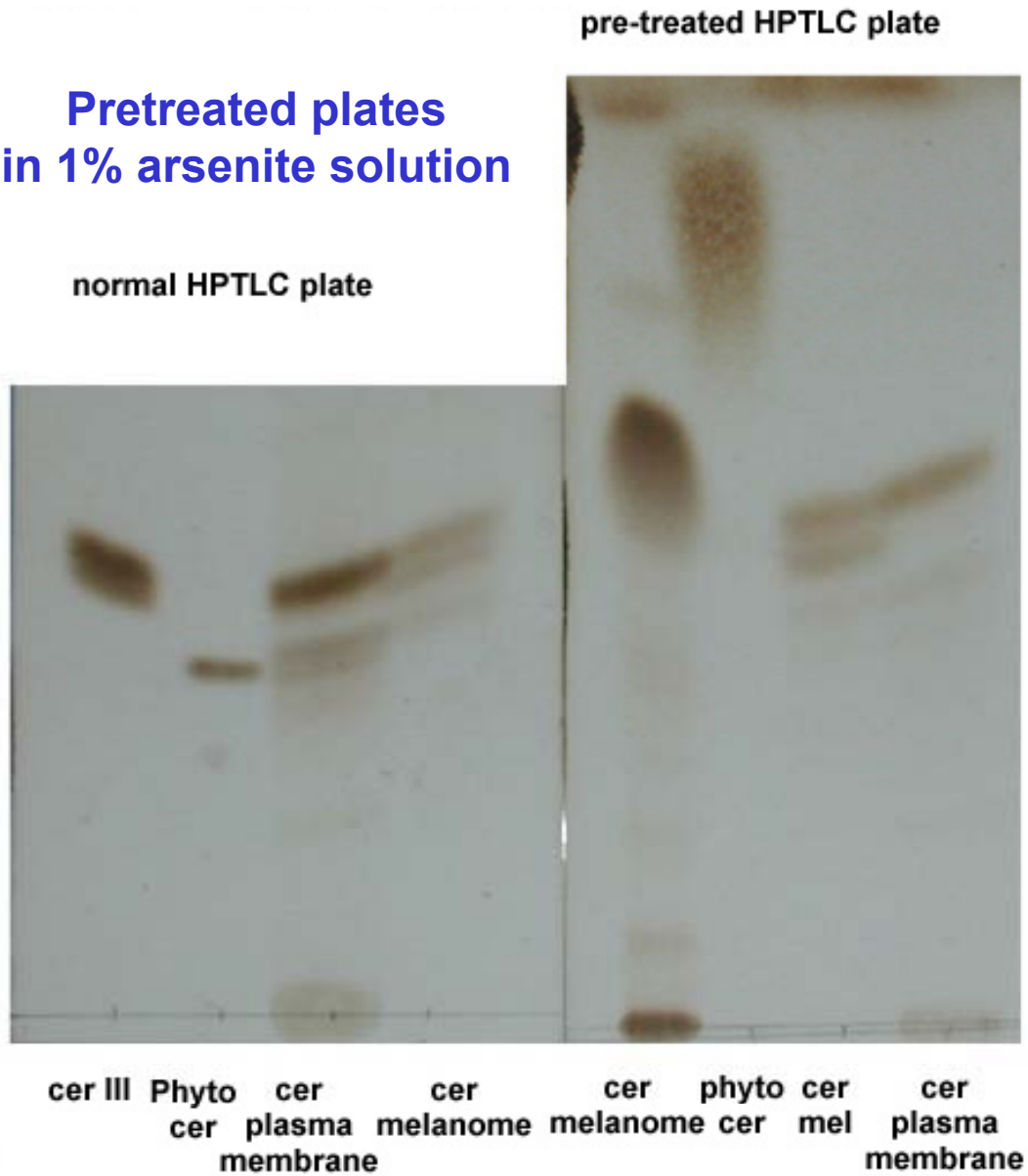
Migration in chloroform/methanol 50:4(by volume)

Visualisation with Cu acetate 3 % in H3PO4 8%, at 120° C



Analysis of ceramides extracted from melanoma tissues and from plasma membranes of cells

Pretreated plates in 1% arsenite solution



Analysis of ceramides extracted from cells and spent medium of cells

Cer III cerIV ceramide

cells medium

migration of the plates in chloroform-methanol 50/4 (by volume)
visualised in Cu acetate reagent at 150° C, 10 min.

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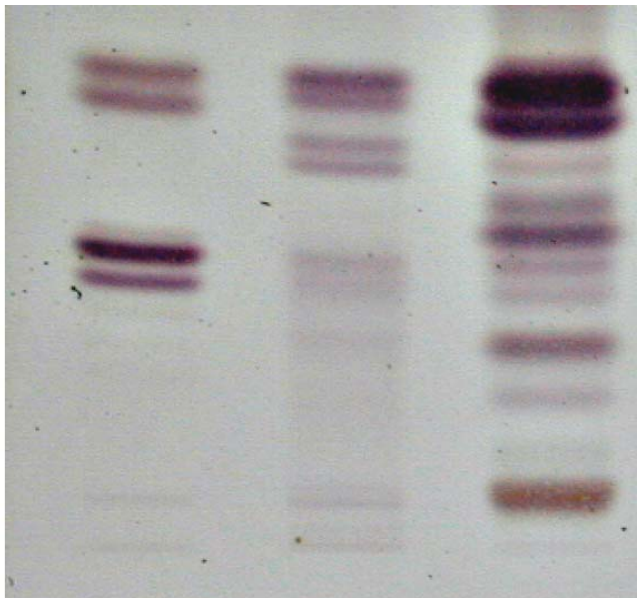
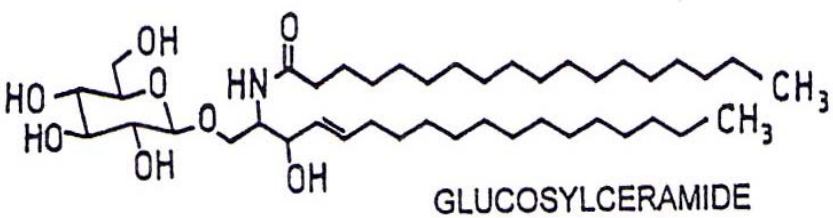
Analysis of neutral glycolipids and free long-chain bases

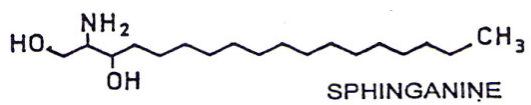


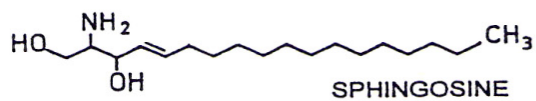
Melanoma standards | brain | melanoma cells

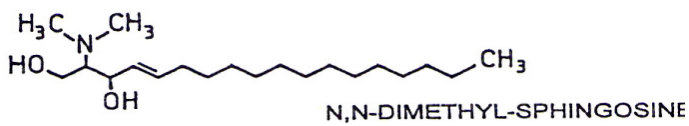
Separation of free long chain bases (sphingosine, sphinganine…) from neutral glycolipids (CMH, CDH, CTH...) on LC-WCX columns

Purification of neutral glycolipids and free-long chain bases on LC-NH2 columns
Migrated in chloroform/methanol/water 65/25/4 v/v
Visualised in orcinol-H2SO4 reagent

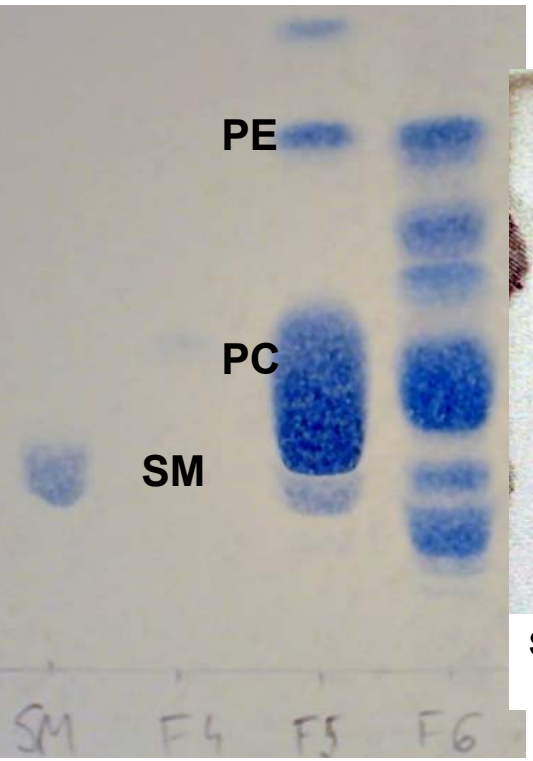


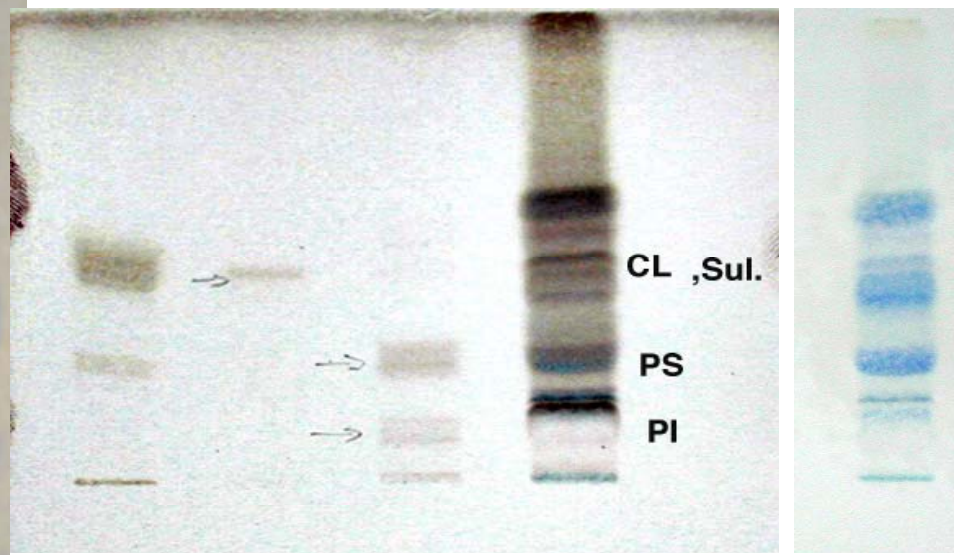




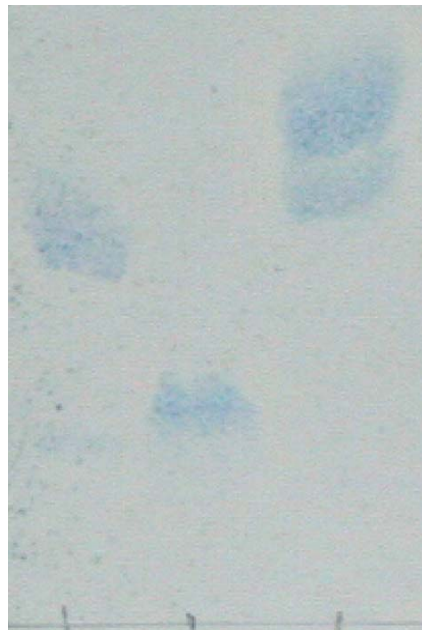


Analysis of neutral and acidic phospholipids



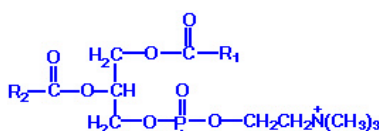


Sulphatides cardiolipin PS/PI human melanoma
visualised in orcinol/ in Ditmer reagent

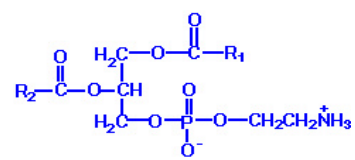


PS S-1P Cer-1P
PI

F5 Neutral Phospholipids PC, PE, LPC and SM
F6 acidic phospholipids PI, PS and cardiolipin (CL), Gangliosides, sulphatides
Migrated in chloroform/methanol/water 65:25:4 (v/v/v)
Visualised with Dittmer and Lester reagent or Ninhydrin reagent



Phosphatidylcholine (PC)



Phosphatidylethanolamine (PE)

TLC Immunostaining

Separate samples on an HPTLC plate×2.

↓

Dry the plate with a hair drier. → ① Detection with Orcinol / H_2SO_4 reagent.

↓

② Dip the plate in 0.4% PIM(polyisobutyl methacrylate) in hexane for 30 sec.

↓

Blocking with 1% BSA/PBS for 60 min at RT.

↓

Incubate the plate with 1st Ab diluted in PBS or with the sera at RT for 60 min.

↓

Wash the plate with 0.1%PBS 3min×5times.

↓

Incubate the plate with biotinylated antibody diluted in PBS at RT for 60 min

↓

Wash the plate with PBS several times.

↓

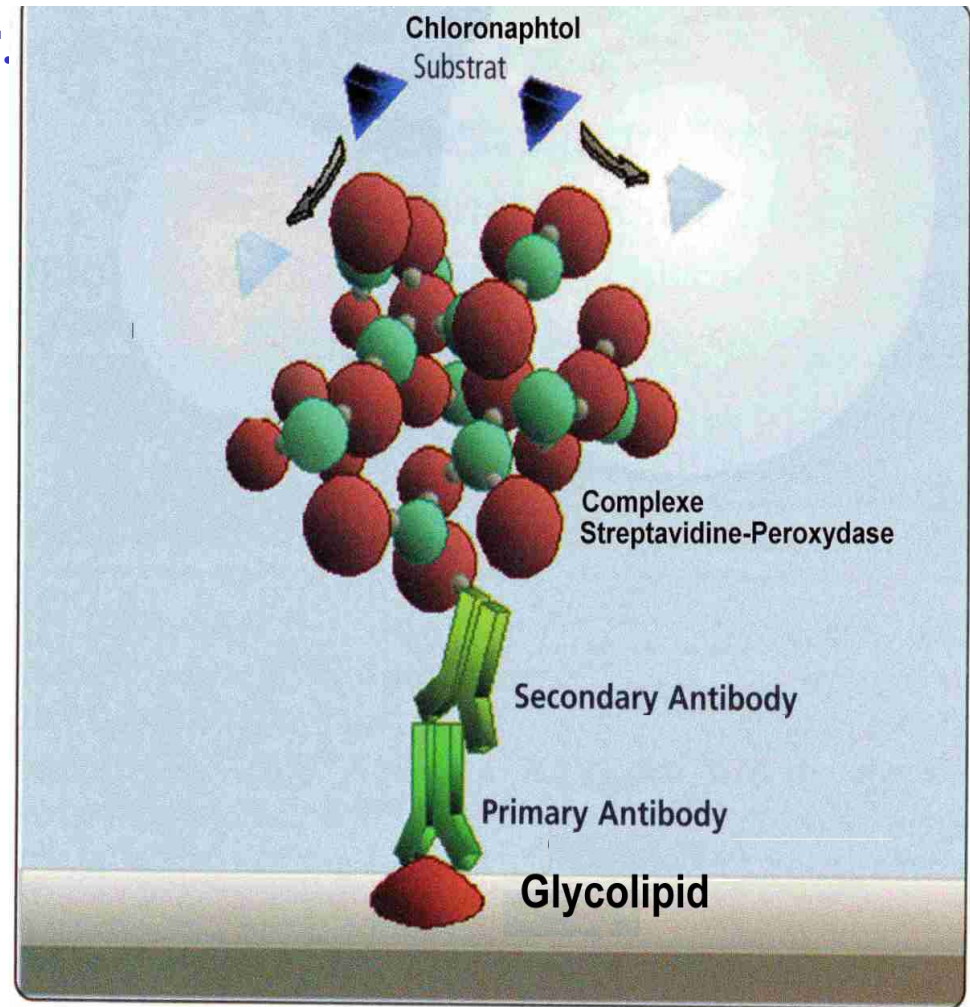
Incubate the plate with the complexe streptavidine-peroxidase at RT for 60 min

↓

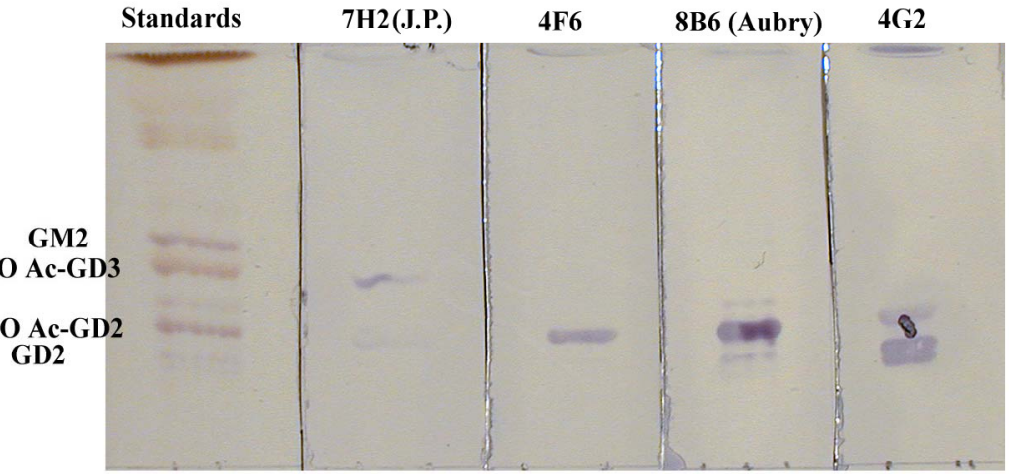
Wash the plate with PBS several times.

↓

Incubate with 4-Chloronaphtol solution to visualise

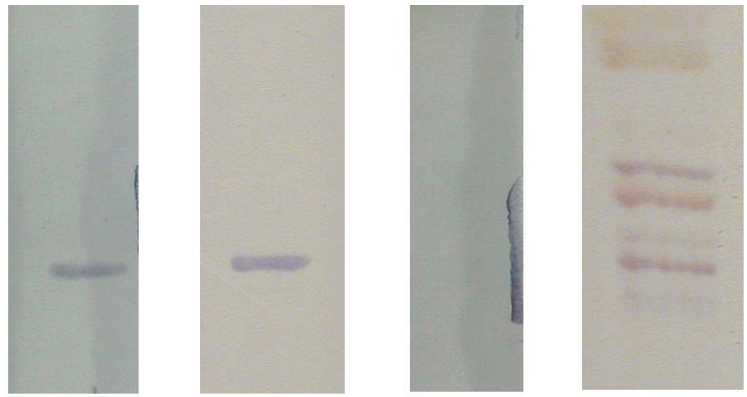


Examples of I-TLC versus TLC



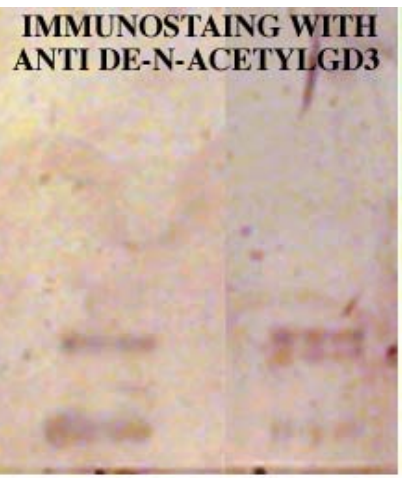
chemical identification with resorcinol

Immunodetection of different gangliosides with specific antibodies



Mab 3D2 | Control 4E6 | Mab 2C6 | melanoma (resorcinol)

TLC-Immunostaining on melanoma gangliosides with antiGD3 Mabs produced after immunization with peptide R4

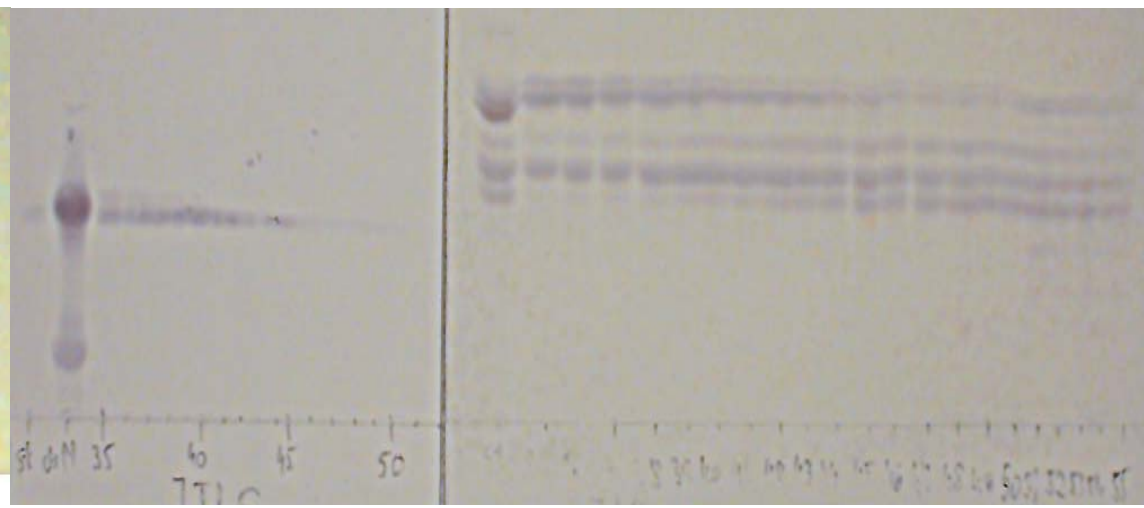


de-N-acetylated standard GD3

melanoma de-N-acetylGD3

melanoma gangliosides

fraction GD2



I-TLC with specific antibody to de-N-AcetylGD3 compare to TLC of HPLC samples from a melanoma ganglioside sample

TLC immunostaining (14G2a 5μg/ml)(KM641 10μg/ml)

1st Ab: KM641 (10μg/ml)
2nd Ab: α-mouse IgG-HRP 1/250

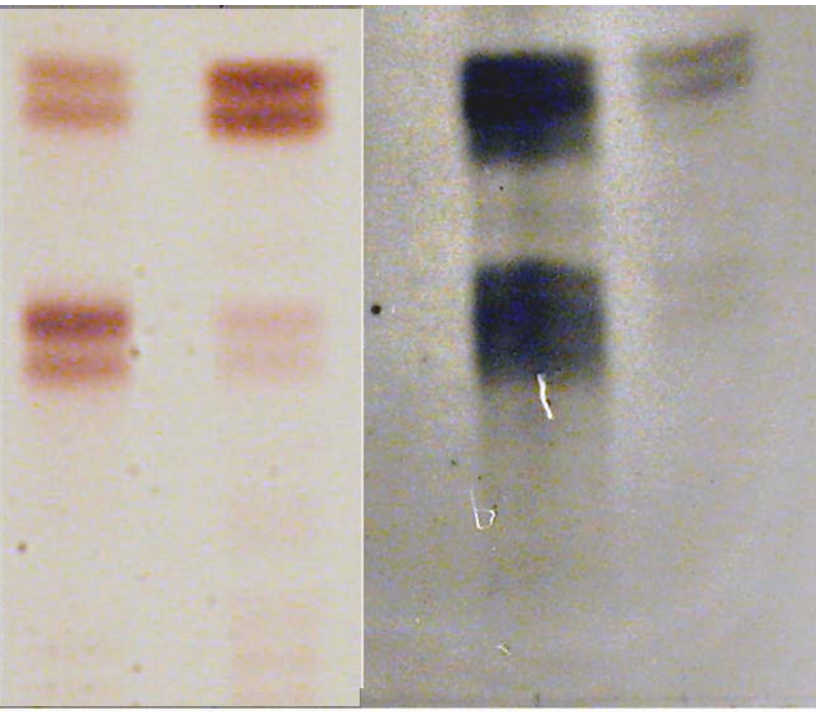
GD2 2μg | GD3 4μg | total ganglioside 4μl

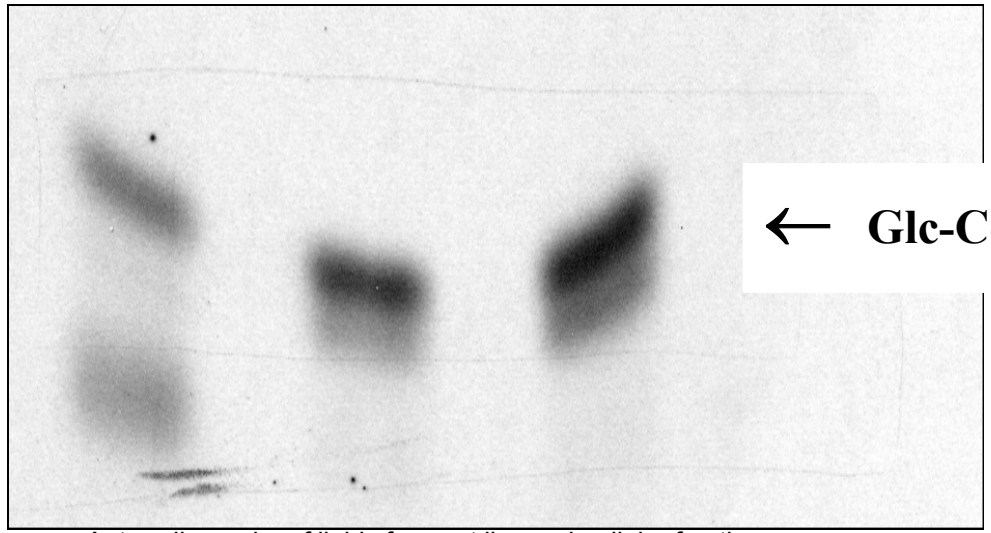
1st Ab: 14G2a (5μg/ml)
2nd Ab:α-mouse IgG-HRP 1/250

GD2 2μg | GD3 4μg | total ganglioside 4μl

GD2 0.08μg | GD3 4μg | total ganglioside 2μl

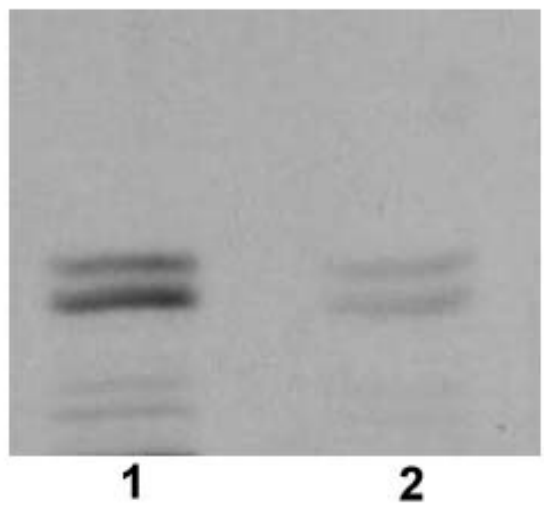
Autoradiography





Autoradiography of lipids from rat liver subcellular fractions incubated with UDP-[^{14}C]-glucose and exogenous ceramides. Extracted lipids from subcellular fractions (150 µg protein / incubation) were resolved by TLC using solvent system II on silica gel plates pre-run in 1 % borate. Lipids were isolated from : (1) whole microsomes, (2) MAM, (3) Golgi. Identification of labeled compounds was performed by co-migration with unlabeled commercial Glc-Cer

Informations obtained from the upper left TLC visualised by orcinol (left panel) comparatively with autoradiography of glycolipids (right panel), after exposure to a film for 2 months.
Method applied for monitoring of pathways of biosynthesis of lipids.



2D and 3D HPTLC of total lipids
Bodennec et al.,
J. Lipid Res.
2000, 41:1524-1531

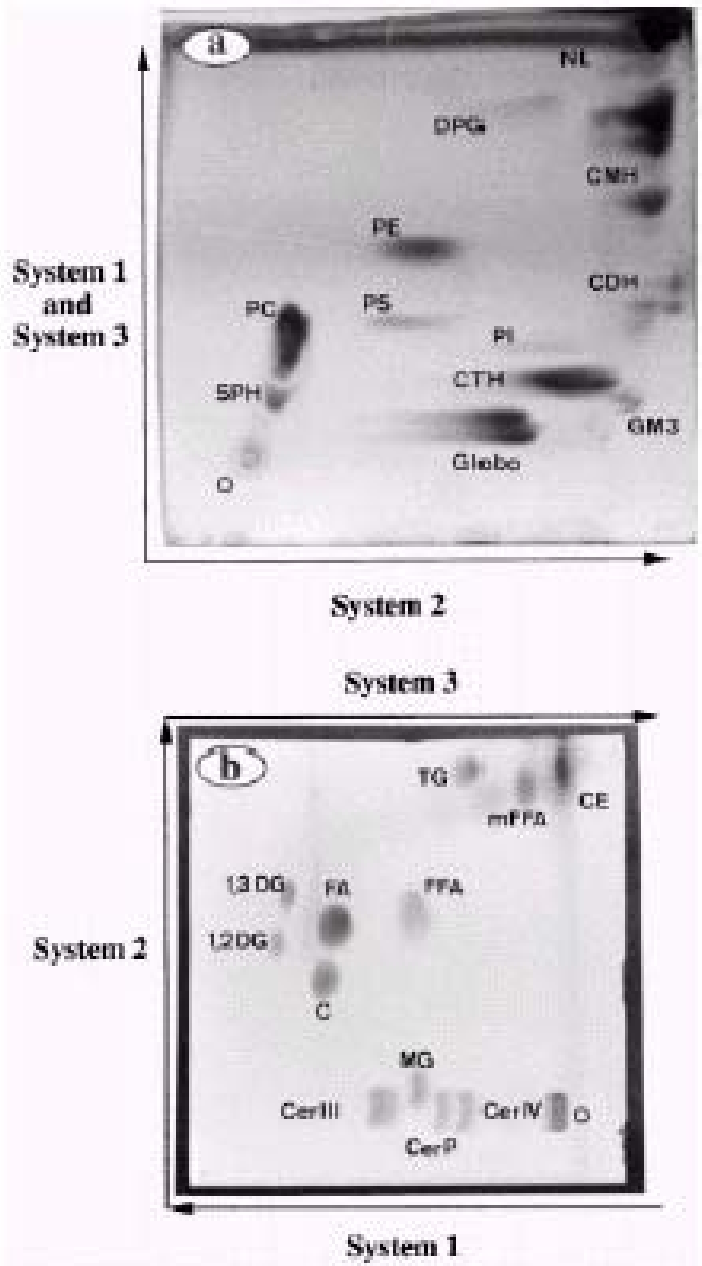
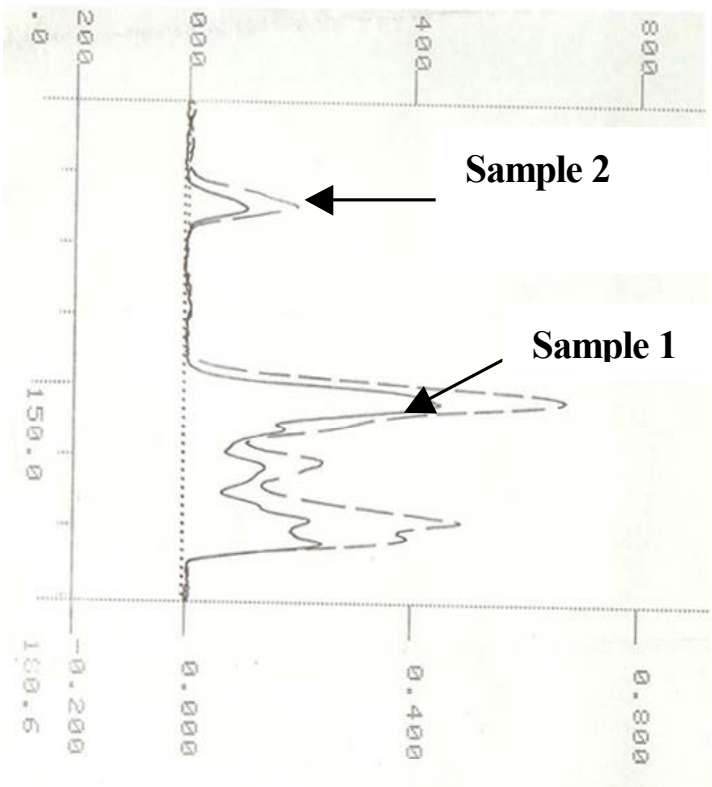
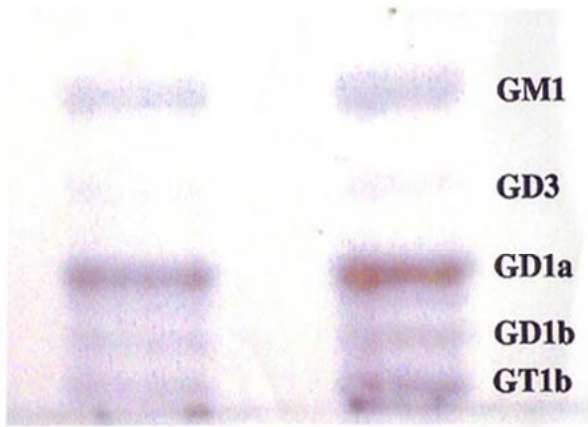


Fig. 2. Typical profile of a lipid mixture on separation by 2D- or 3D-HPTLC. These systems were used to check the recovery and cross-contamination between lipids in each fraction. (a) Profile of polar lipids on a 2D-HPTLC plate according to our published procedure (28). The plate was run consecutively in two directions as indicated, with different successive solvent systems: system 1, diisopropyl ether; system 2, tetrahydrofuran–acetone–methanol–deionized water 50:20:40:6 (v/v/v/v); system 3, chloroform–acetone–methanol–acetic acid–deionized water 50:20:10:15:5 (v/v/v/v/v). O, Origin. (b) Profile of neutral lipids on a 3D-HPTLC plate (22). The following solvent systems were used successively: system 1, chloroform–methanol 50:3.5 (v/v); system 2, petroleum ether–ethyl ether–acetic acid 40:60:0.1 (v/v/v); system 3, hexane–ethyl ether–acetic acid 80:20:1 (v/v/v). O, Origin. Abbreviations of individual lipids are given in text. Phospholipids, neutral glycosphingolipids, and free sphingoid bases remain at the origin during this 3D-HPTLC separation.

Densitometric analysis of the HPTLC plate following migration of gangliosides and visualisation with resorcinol-HCl





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Gg bovine brain

Wavelength set at 630 nm
zig-zag scan (10 mm) with square slit 1/1mm
***length of scanning is equal to that of the plate**

ChromatoScan CS-930, Shimadzu, Kyoto, Japan

Application:

Use of HPTLC plates in qualitative and quantitative manner

for physical detection with iodine

for chemical detection with specific reagents ,

for immunochemical detection with antibodies,

for radiochemical detection of labeled spots (autoradiograms)

Conclusion:

This method gives useful informations about the nature of the lipids before going to more sophisticated analytical methods such as HPLC, mass spectrometry, gas chromatography.