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Note

Thin-layer chromatography of gangliosides

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Thin-layer chromatography (TLC) is the most powerful technique for the identification and determination of gangliosides in animal tissues[1–10]. Recent methods have used pre-coated silica gel plates[9,11] with detection by charring or spraying with specific reagents, and with direct densitometry of the plates[12–14]. However, most of the solvent mixtures[1–10] give incomplete separations of brain gangliosides and are time consuming and very sensitive to temperature. We describe here the use of a solvent that gives extremely reproducible results and can achieve the separation of all brain gangliosides in 90 min. With recently available high-performance thin-layer chromatographic (HPTLC) plates the same separation can be obtained within 30 min for amounts as low as 0.5–1 μg of total ganglioside sialic acid.

EXPERIMENTAL

Materials

Pre-coated silica gel plates (DC-Fertigplatten, 20 × 20 cm), HPTLC plates (für nano-DC, 10 × 5 cm cut from 10 × 10 cm plates) and orcinol were obtained from Merck (Darmstadt, G.F.R.).

Gangliosides were purified from pig and human brain in the Centre de Neurochimie (Strasbourg, France). Brain gangliosides from patients with Sanfilippo disease were kindly supplied by Dr. A. Federico. Standard individual gangliosides were a generous gift from Dr. W. Ziegler and Dr. L. L. Sarlieve. All other chemicals were of analytical grade and were used without further purification.

The scanning was carried out with a Vernon densitometer.

Chromatography

Ganglioside mixtures were dissolved in chloroform–methanol (2:1) at a total concentration of 1 mg/ml of sialic acid. Individual ganglioside concentrations were 0.1 mg/ml of sialic acid. For both chromatographic systems the solvent used was methyl acetate–isopropanol–2.5 mg/ml aqueous potassium chloride (45:30:20, v/v).

At the macro-scale level, 10 μl of the ganglioside solutions were applied in 2-cm streaks 1.8 cm from the bottom edge of the plate. Ascending chromatography was performed for 90 min in a conventional TLC tank coated with filter-paper.

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At the micro-scale level, the equivalent of 1 μl of the ganglioside solutions was applied, with a glass capillary, in a 5-mm streak 8 mm from the bottom edge of the plate. Chromatography was performed for 30 min in a small closed glass container.

The spots on the plates were revealed by spraying with orcinol–hydrochloric reagent[15].

RESULTS AND DISCUSSION

With appropriate amounts of ganglioside (see below), the solvent used gives very reproducible separations of all common brain gangliosides (G_7, G_{M3}, G_{M2}, G_{M1}, G_{D3}, G_{D1a}, G_{D1b}, G_{T1} and G_{Q1}) (Figs. 1 and 2). Chromatography for 90 min gives a better separation than chromatography for 6 h with the commonly used solvent system chloroform–methanol–2.5 mg/ml aqueous potassium chloride (60:35:8, v/v)[9]. In particular, the high resolution between G_{D1a} and G_{D1b} allows the detection of

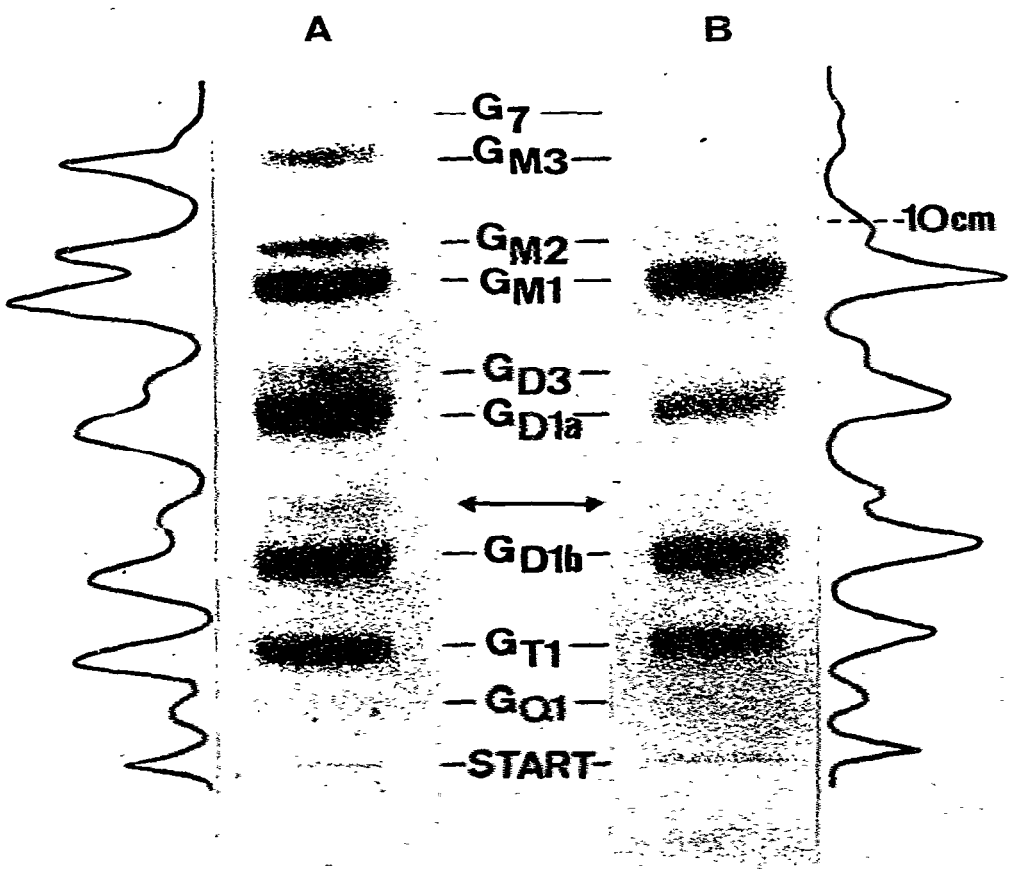

Fig. 1. TLC and densitometry of gangliosides on conventional thin-layer plates (20 × 20 cm) [90 min in the solvent methyl acetate–isopropanol–2.5 mg/ml aqueous potassium chloride (45:30:20, v/v)]. A, Sanfilippo desease; B, normal human brain. Total 6 μg of sialic acid.

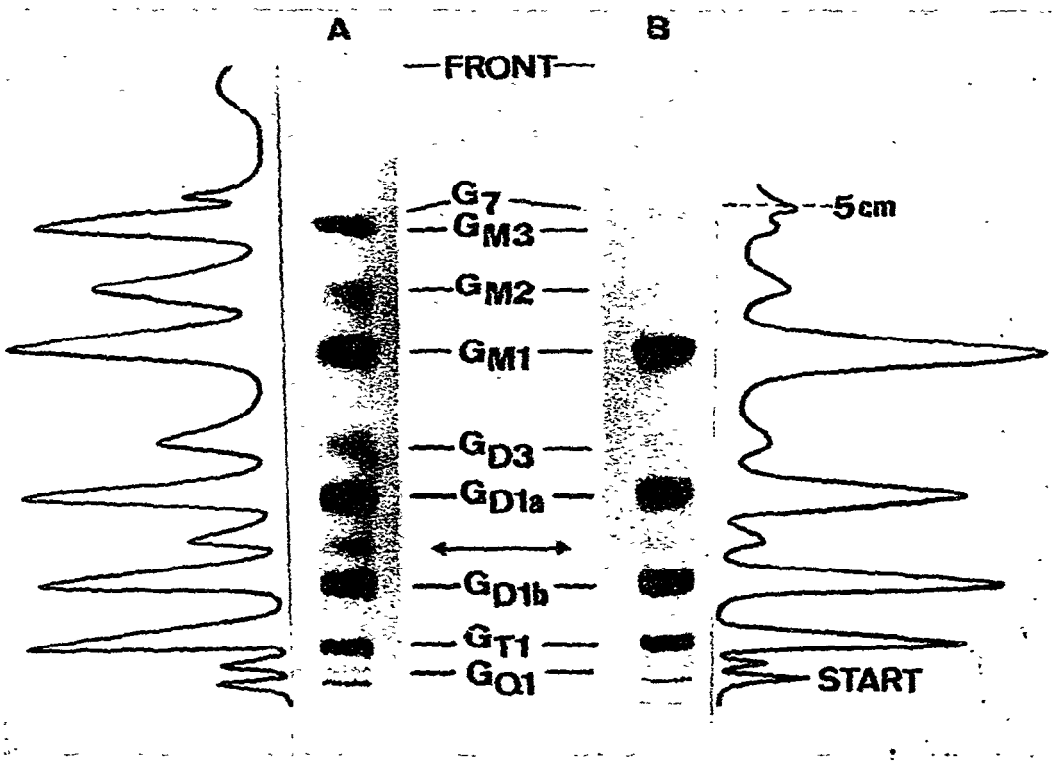

Fig. 2. TLC and densitometry of gangliosides on HPTLC plates (10 × 5 cm); 30 min, 0.5 μg of total sialic acid. A and B as in Fig. 1).

another spot with a migration comparable to that of component "y" found by Tettamanti[16]. It should be emphasized that this component is particularly abundant in Sanfilippo brain ganglioside extract (and should correspond to G_{D2}) but is also present in smaller amounts in normal human brain and rat cerebellum.

Variation of the temperature between 18° and 25° and errors of up to 5% in the proportions of the components of the solvent did not affect the separation significantly.

Tailing of the spots was observed only when too large amounts of gangliosides were applied to the plate, *viz.*, at the macro-scale level more than 2 μg of sialic acid of each ganglioside and at the micro-scale level more than 0.2 μg. Overloading of the plate also modified the R_F values of major gangliosides and thus affected the separation of neighbouring compounds. This is of particular importance for the densitometric determination of individual gangliosides.

At the macro-scale level, determination by densitometry using the present solvent is possible with the same degree of confidence as obtained with other solvents (Fig. 1). Further, for G_7, G_{M3} and G_{M2} the spots are more concentrated and the detection is more sensitive than with other solvents. Densitometry of the HPTLC plate is also possible; in fact it is easier, as the separation is better, there is less tailing and the spots are more concentrated. In this micro-system, the sensitivity is more than 10 times higher. However, the colour background is not negligible, owing to

impurities eluted from the plate, and the peak areas must be determined by planimetry.

The high resolving power of this system, its high sensitivity and its rapidity should be particularly useful in control and screening studies. Further, if gangliosid more complex than G_{Q1} are suspected, an increase in the proportion of potassium chloride solution from 45:30:20 (v/v/v) to 45:30:25 (v/v/v) would allow the detection or separation of such slowly migrating compounds.

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