Journal of Chromatography, 305 (1984) 512—513
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1921

Letter to the Editor

Quantitation of gangliosides in the picomolar range

Sir,

We wish to report a methodology for the reproducible quantitation of picomolar amounts of individual ganglioside species. The procedure, utilizing scanning densitometry of high-performance thin-layer chromatography (HPTLC) plates, requires 75 pmol of ganglioside sialic acid for a triplicate determination and 300 pmol of the ganglioside for a densitometric standard curve. By comparison, spectrophotometric assays require approximately 20 nmol of ganglioside sialic acid for quantitation [1, 2].

HPTLC plates (10 × 10 cm) from E. Merck (Darmstadt, F.R.G., 5 μm particle size silica gel 60) are prewashed by migration in chloroform—methanol (2:1) and activated at 140°C for 10 min. Samples to be quantitated and ganglioside standards in chloroform—methanol (2:1) are applied 10 mm from the plate bottom with a microsyringe (Hamilton No. 7005) in 1—5 μl under a constant flow of warm air (30—40°C). Ganglioside solutions are applied in small overlapping spots which coalesce into 3 × 1 mm bands. The mobile phase is chloroform—methanol—water—1% calcium chloride (60:35:7:1). To insure a saturated atmosphere in the development tank, plates are suspended above the mobile phase for 30 min prior to development. The front is allowed to reach 80 mm above the origin (approximately 25 min). Plates are dried at room temperature and then heated at 140°C for 10 min to remove all traces of solvent prior to spraying with freshly prepared resorcinol—hydrochloric acid reagent [1]. After spraying, plates are covered with clean glass covers and heated at 140°C for 10 min to visualize the blue ganglioside bands.

Ganglioside bands are scanned the same day at 580 nm in the transmission mode at 6 mm/min with a Shimadzu (Kyoto, Japan) dual-wavelength TLC scanning densitometer, Model CS-910. The slit length is adjusted to be 10% greater than the longest band after development and the slit width is 0.2 mm. The peak area of each band is determined after subtraction of the background absorbance at 720 nm by a data processor (Shimadzu C-R1B). Neither zig-zag function nor linearizer program is used.

Densitometric standard curves for gangliosides G_{M4}, G_{M1}, G_{D1a}, G_{D1b} and G_{T1b} are highly linear to 50 pmol of ganglioside sialic acid (Fig. 1). As little as 1 pmol of G_{M1} can be reproducibly detected with a signal-to-noise ratio of

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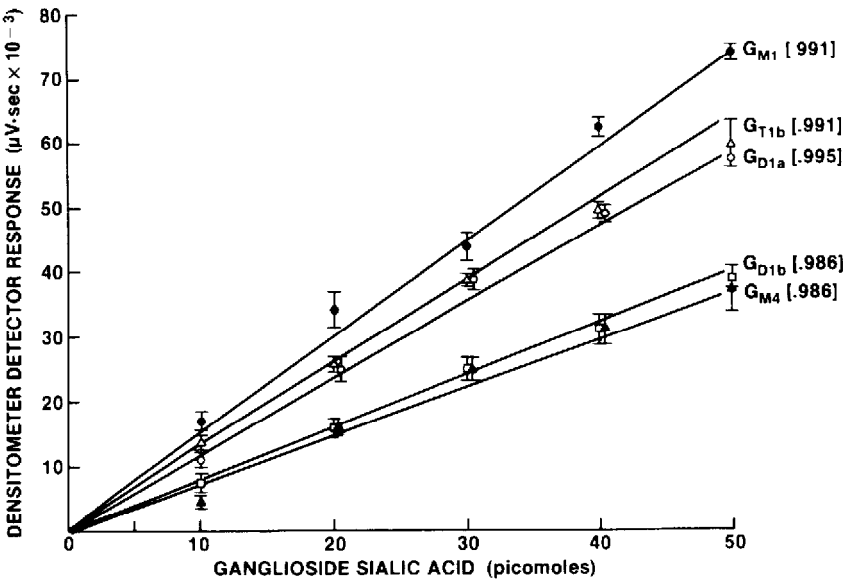

Fig. 1. Ganglioside densitometric standard curves. Densitometric values are the mean ± S.D. of triplicate samples. A separate HPTLC plate was used for each ganglioside species. Correlation coefficients for each standard curve are given in brackets.

5:1. This is 90 times more sensitive than the densitometric procedure of Ando et al. [3]. This enhanced sensitivity allows a more accurate assessment of ganglioside purity. Although sensitivity could be increased further by applying gangliosides in bands less than 3 mm, experimental errors increased significantly due to difficulty in alignment of the densitometer light slit.

To minimize errors due to differences in gel layers, spraying, and ganglioside band geometry [4], gangliosides to be quantitated should be developed on the same HPTLC plate with ganglioside standards used for densitometric standard curves. The variability of this method is 4%.

Picomolar amounts of individual ganglioside species are rapidly quantitated by this method, thus conserving purified gangliosides.

We acknowledge support from the National Multiple Sclerosis Society.

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(First received June 27th, 1983; revised manuscript received September 8th, 1983)