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Short Communication

Determination of gangliosides in parenteral dosage form by high-performance liquid chromatography*

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Introduction

Gangliosides are glycosphingolipids containing one or more residues of sialic acid, and are thought to be important in some physiological functions such as regulation of cell growth. It has been suggested that they play a key rôle in the development and in the repair of the nervous system [2, 3]. Up to now HPLC methods have not been employed for the analysis of gangliosides in pharmaceutical dosage forms [4–6]. Several preparative and semi-preparative methods by HPLC have been developed both in normal [7] and reversed-phase [8] modes but do not provide the necessary speed with suitable accuracy and specificity required for routine analysis.

In this paper we report a qualitative and quantitative analysis of a mixture of gangliosides in parenteral dosage form (Cronassial) taking account that for this special purpose a short, sensitive, precise and accurate method is highly desirable.

Experimental

Materials

Standard gangliosides (GM1, GD1a, GD1b, GT1b) from Fidia (Abano Terme, Italy); acetonitrile HPLC grade and $Na_2HPO_4 \cdot 2H_2O$ (Merck, Darmstadt, FRG); tetrabutylammonium hydroxide (Rudi Pont, Parsippany NJ, USA); Cronassial 100 injectable dosage form (Fidia, Abano Terme, Italy).

Equipment

Separation and quantitative determination were performed on a liquid chromatograph HP 1090M (Hewlett Packard, Palo Alto, CA, USA) equipped with a sample valve (Model 7410 Rheodyne) with a 5-μl loop. The outlet was connected to an UV–vis diode array detector HP 1040M controlled by a workstation HP 9000 model 310.

Analytical conditions

Normal-phase. Separations were performed using a 125 × 4.5 mm i.d. Hibar Lichrocart-NH_2 column (7 μm spherical particles). Elution was carried out by a gradient with mobile phase A = acetonitrile–5 mM phosphate buffer, pH 5.6 (83:17%, v/v) and B = acetonitrile–20 mM phosphate buffer, pH 5.6 (1:1%, v/v) with the following elution profile: 0–5 min A–B (85:15%, v/v); 5–15 min, A–B from (85:15%, v/v) to (80:20%, v/v); 15–17 min A–B (80:20%, v/v); 17–25 min A–B from (80:20%, v/v) to (70:30%, v/v); finally a gradient back to A–B (85:15%, v/v) in 10 min. The flow rate was 1 ml min^{-1} and the analytes

Abbreviations used: the nomenclature for gangliosides is in accordance with Svennerholm [1].

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were monitored by flow-through detection of UV absorbance at 215 nm, (bandwidth 4 nm).

Reversed-phase. Separations were performed using a 120 × 4.6 mm i.d. Hypersil C_8 column (5 μm spherical particles). Elution was carried out by a gradient with mobile phase A = 5 mM tetrabutylammonium hydroxide in water (pH 7.2) and B = 5 mM tetrabutylammonium hydroxide in acetonitrile. The gradient was as follows: 0–7 min A–B from (20:80%, v/v) to (25:75%, v/v); 7–10 min A–B (25:75%, v/v); finally a gradient back to A–B (20:80%, v/v) in 5 min. The flow rate was 1 ml min^{-1} and the analytes were monitored by flow-through detection of UV absorbance at 215 nm, (bandwidth 4 nm).

Standard solution

Normal-phase. Standard solution was prepared by mixing four standard gangliosides in 0.02 M phosphate buffer (pH 7.5) in order to have the following concentrations: GM1 5.250 mg ml^{-1}; GD1a 10.000 mg ml^{-1}; GD1b 4.000 mg ml^{-1}; GT1b 4.750 mg ml^{-1}. Quantitative assays were performed by means of an external standard procedure, using a calibration curve obtained by dilution of the standard solutions in the following ratios: 1:3; 1:5; 1:7; 1:10; the detector response was linear in the range considered. The regression equations are reported in Table 1. The precision of the chromatographic procedure was measured by 12 replicate injections of standard solutions (dilution 1:5). The standard deviations for GM1, GD1a, GD1b and GT1b were ±0.0709, ±0.1203, ±0.0761 and ±0.1004, respectively. The minimum detectability of each ganglioside was: GM1 = 0.43 mg ml^{-1}; GD1a = 0.83 mg ml^{-1}; GD1b = 0.33 mg ml^{-1}; GT1b = 0.39 mg ml^{-1}.

Reversed-phase. Pure standard GM1, GD1a, GD1b and GT1b from Fidia were directly chromatographed and diluted in order to obtain the calibration curves. Each standard ganglioside gave two peaks (C_{18} and C_{20} sphingosine) [8], which are the main two molecular species. For this reason we obtained eight regression equations for the four standards reported in Table 1.

Sample preparation

One millilitre of injectable dosage form was transferred to a tube and diluted to 5 ml with 0.002 M phosphate buffer (pH 7.5).

Triplicate 5-μl aliquots were directly chromatographed.

Results

Initial work was carried out using normal-phase conditions and by using the gradient system reported in the Experimental section, the retention time was considerably reduced in comparison to the method reported in the literature [7]. The resolution of standard gangliosides is shown in Fig. 1.

Twelve injectable dosage forms (Cronassial) were quantitatively analysed and the average values obtained were in good agreement with the stated amounts. A chromatogram of the injectable dosage form is shown in Fig. 2.

A further investigation was performed in reversed-phase in order to separate each ganglioside into its molecular species concerning the different sphingosine portion (C_{18} and C_{20}).

The results obtained show that the reversed-phase is suitable for the separation of the molecular species. Under these chromatographic conditions the pharmaceutical dosage form (Fig. 3) shows six peaks instead of eight.

Table 1
Linearity of the analytical procedures

	Normal-phase	Reversed-phase
GM1	$y = 4.63x - 0.0521$; $r = 0.996$	$y_1 = 4.20x + 0.033$; $r = 0.997$ $y_2 = 4.03x - 0.059$; $r = 0.999$
GD1a	$y = 4.16x - 0.0672$; $r = 0.993$	$y_1 = 136x + 0.36$; $r = 0.999$ $y_2 = 138x - 1.21$; $r = 1.000$
GD1b	$y = 2.37x - 0.0224$; $r = 0.997$	$y_1 = 165x - 4.72$; $r = 1.000$ $y_2 = 76.2x + 0.17$; $r = 0.997$
GT1b	$y = 2.71x - 0.0378$; $r = 0.999$	$y_1 = 150x + 2.18$; $r = 0.995$ $y_2 = 152x - 1.22$; $r = 1.000$

r = Correlation coefficient.

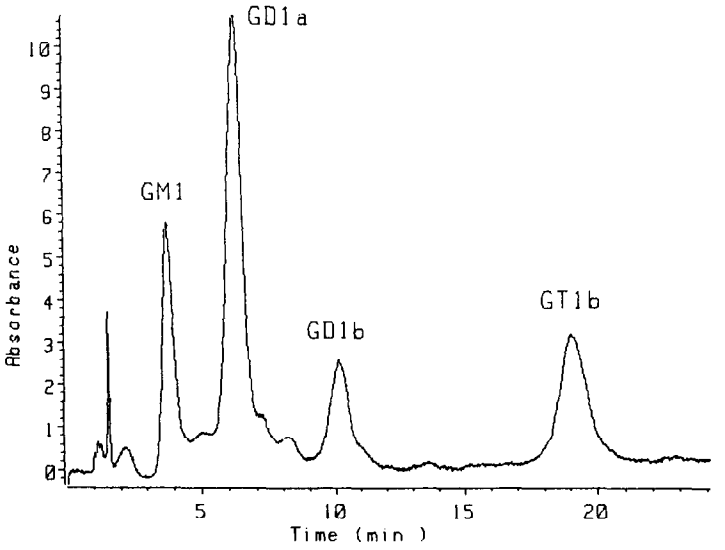

Figure 1
Chromatogram of mixture of standard gangliosides in normal-phase (conditions in text).

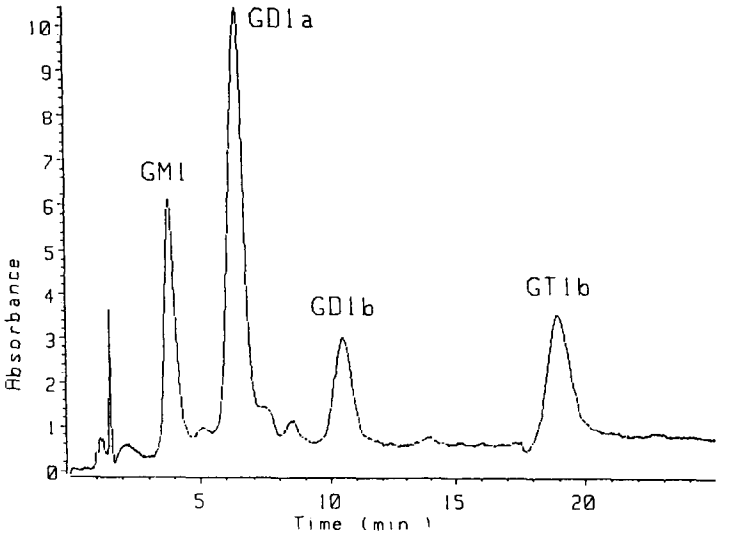

Figure 2
Chromatogram of the injectable dosage form (Cronassial) in normal-phase.

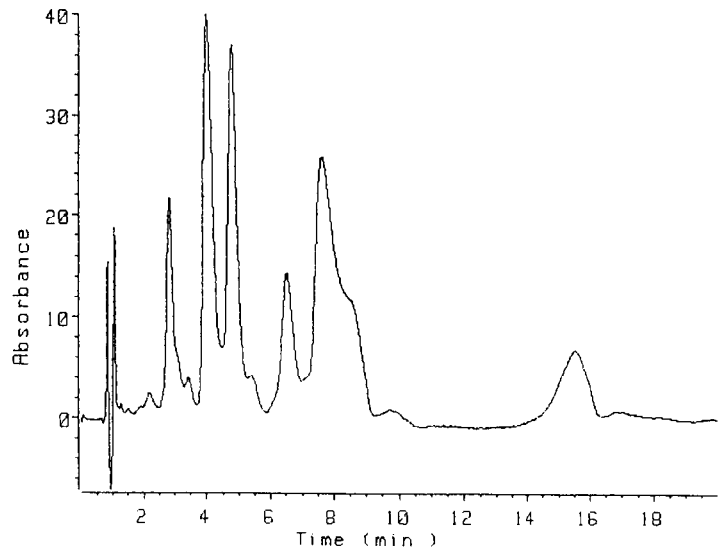

Figure 3
Chromatogram of the injectable dosage form (Cronassial) in reversed-phase (conditions in text).

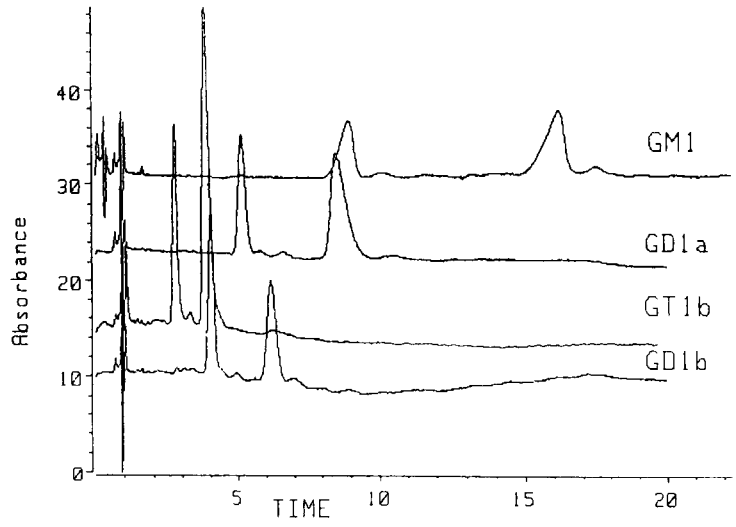

Figure 4
Superimposition of the chromatograms of each standard gangliosides in reversed-phase.

Figure 4, which is the superimposition of the chromatograms of each standard ganglioside, demonstrates that the six peaks are due to the overlapping of the first peak of GD1b with the second peak of GT1b and to the overlapping of the first peak of GM1 with the second peak of GD1a.

For this reason the method cannot be used quantitatively and needs further investigation.

References

[1] L. Svennerholm, *J. Neurochem.* **10**, 613–623 (1963).
[2] R.W. Ledeen, *J. Neurosci. Res.* **12**, 147–159 (1984).
[3] J.C. Samson, *Drugs Today* **22**, 73–107 (1986).
[4] S.K. Kundu and D. Dunn Scott, *J. Chromatogr.* **232**, 19–27 (1982).
[5] K. Miyazaki, N. Okamura, Y. Kishimoto and Y.C. Lee, *J. Biochem.* **235**, 755–761 (1986).
[6] J. Gottfries, P. Davidsson, J.E. Mansson and L. Svennerholm, *J. Chromatogr.* **490**, 263–274 (1989).
[7] G. Gazzotti, S. Sonnino and R. Ghidoni, *J. Chromatogr.* **348**, 371–378 (1985).
[8] S. Sonnino, R. Ghidoni, G. Gazzotti, G. Kirschner, G. Galli and G. Tettamanti, *J. Lip. Res.* **25**, 620–629 (1984).