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Short communication

Chromatographic tank designed to obtain highly reproducible high-performance thin-layer chromatograms of gangliosides and neutral glycosphingolipids ☆

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Abstract

Although high-performance thin-layer chromatography (HPTLC) is one of the most useful methods in glycosphingolipid studies, there are some difficulties with the reproducibility of the chromatographic patterns. When mixtures of solvents with different vapour pressures are used as the mobile phase, saturation of the tank is a critical point for obtaining reproducible chromatograms. A chromatographic tank designed to give optimal saturation conditions, resulting in highly reproducible chromatograms, is described.

Glycosphingolipids (GSLs) are amphiphatic molecules with a hydrophobic moiety formed by an N-acylated long-chain amino alcohol (ceramide) and a hydrophilic moiety of one to several sugars. They are membrane components with the oligosaccharide protruding into the extracellular space. Their oligosaccharide variability and topographic distribution make them potential key molecules in the interactions of the cells. They have been involved in several important cellular processes such as cell recognition, growth regulation, development and oncogenesis [1,2].

Ion-exchange and normal-phase liquid chromatography are the most common methods used for analytical studies of GSLs. Since high-performance silica gel became available, high-performance thin-layer chromatography (HPTLC) has become a powerful analytical tool in the GSL field [3]. However, the major difficulty with HPTLC is the low reproducibility of the chromatographic pattern obtained after different runs. In our experience, in order to obtain a reproducible pattern, the critical point is the saturation of the chromatographic tank. When mixtures of solvents having different vapour pressures are used, if the tank is not saturated the ascending solvent mixture becomes enriched in the less volatile solvent.

For the separation of GSLs, mixtures of chloroform, methanol and water are used and as the ascending solvent mixture will be enriched in

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water, it will become more polar, affecting the migration of the GSLs. Although saturation of the tank can be obtained within 3–4 h, when the tank is opened in order to put the plate in, saturation is lost to a variable extent. One way to solve this problem is to put the plate inside the tank, not contacting the solvent; only after saturation is obtained should the plate be permitted to touch the solvent mixture. Under these conditions we were able to obtain reproducible chromatograms. However, the long time required for saturation of the tank constitutes an obvious disadvantage.

Here, we describe a tank into which a small fan is introduced, thus reducing the saturation time to ca. 3 min instead of the usual 3–4 h. Fig. 1 shows a diagram of this chromatographic tank. The lid, which contains the small fan, must be kept tightly closed by using a silicone-rubber seal. The solvent mixture and the plate are put simultaneously into the tank.

There are several possible ways to keep the plate out of contact with the solvent. We found it practical and easy to hang it up using a small clamp and a string. The fan is turned on for 3 min and then the plate is lowered until it touches the solvent. Using this system, we were able to obtain perfect reproducibility of the chromatograms in a minimum of time. Several runs with GSLs (gangliosides and neutrals) were carried out using the same batch of solvent mixture. The slowest and fastest migrating chromatograms of the series are shown in Fig. 2; it can be seen that the general aspects of the patterns are similar and the variability of the R_F values is very small: in every case, the relative standard deviation of the R_F value found for different GSLs was of the order of 3–4% of the media.

Although we have not described the tank previously, we have already used it to study very complex GSL patterns such as insect GSLs [4] and antibodies to GSL [5,6]. In addition, the tank has been successfully applied to the HPTLC analysis of other compounds such as amino acids (urine and plasma samples), nucleotides and phospholipids.

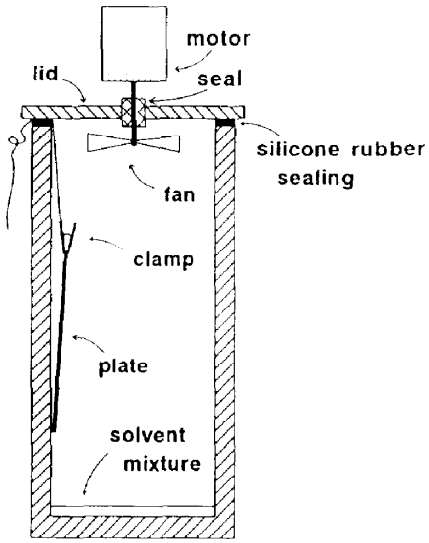

Fig. 1. Cross-section of the chromatographic tank.

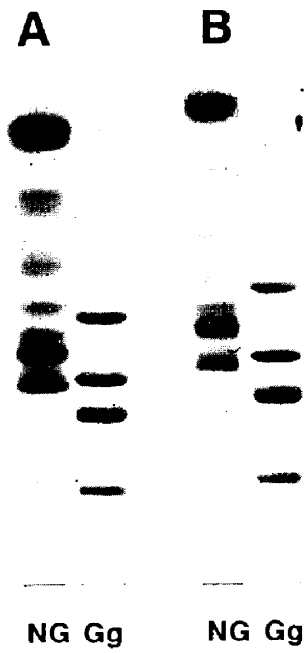

Fig. 2. Chromatograms of gangliosides (Gg) and neutral glycosphingolipids (NG). Human meconium neutral glycosphingolipids and an artificial mixture of gangliosides (total human brain gangliosides plus GM2 and GM_3) were separated on HPTLC plates (Merck, Darmstadt, Germany) using chloroform–methanol–0.2% aqueous $CaCl_2$ (60:37:8) and stained with orcinol reagent. The chromatograms are (A) the slowest and (B) the fastest migrating ones from a series of runs made in the course of 1 week.

Acknowledgements

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