Silicic acid thin-layer chromatography of conjugated and free bile acids

Margarito N. Chavez and Charles L. Krone

Gastroenterology Section, University of Arizona College of Medicine, and the Veterans Administration Hospital, Tucson, Arizona 85712

Summary The separation of free bile acids, taurine conjugates, and glycine conjugates from one another was achieved by thin-layer chromatography, using a solvent system of isopropanol–glacial acetic acid 93:7 In a solvent system of hexane–methylethylketone–glacial acetic acid 56:36:8 (v/v), it was possible to separate cholic, chenodeoxycholic, deoxycholic, and lithocholic acids. Application to a biological bile sample was demonstrated.

Supplementary key words thin-layer chromatography · bile acids separation · biological applications

Several thin-layer chromatography (TLC) procedures have been reported that separate conjugated and free bile acids (1–5). However, investigators have found it difficult, or have been unable, to separate free or conjugated chenodeoxycholic acid (CD) from deoxycholic acid (DC), although minimal or suboptimal separation has been achieved by chromatographing the methyl esters of these two bile acids (6). Bruusgaard (7) and O'Moore and Percy-Robb (2) have reported good separation of free CD and DC but the separations could not be reproduced using EM5763 precoated TLC plates. This report details a new TLC solvent system that allows separation of free bile acids, glycine conjugates, and taurine conjugates from one another, and a second solvent system for the separation of the four free bile acids found in human bile in a more satisfactory manner than previously reported.

Methods

TLC glass plates (20 × 20 cm), precoated with silica gel (0.25 mm thick) (E.M. Laboratories Inc.,

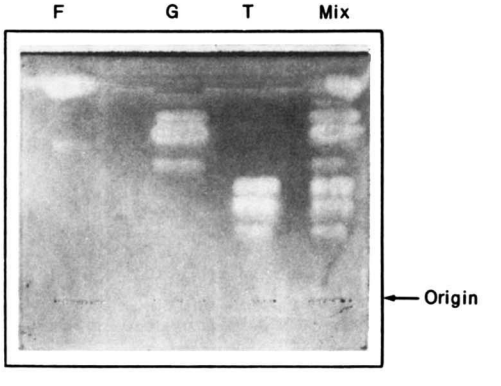

Fig. 1. Separation of free bile acids, glycine conjugates, and taurine conjugates with isopropanol–glacial acetic acid 93:7. Each spot represents 20 μg of bile acid. The plate was developed for 3.5 hr. From left to right: F, a mixture of cholic, chenodeoxycholic, deoxycholic, and lithocholic acids; G, glycine conjugates of the bile acids; T, taurine conjugates of the bile acids; and Mix, a mixture of the free bile acids, and glycine and taurine conjugates.

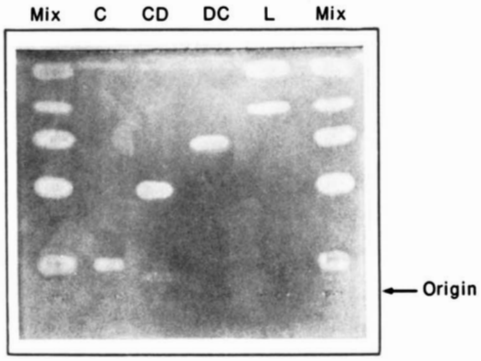

Fig. 2. Separation of free bile acids by Petcoff's solution. Each spot represents 20 μg of bile acid. The plate was developed for 2 hr. From left to right: mixture of free cholic, chenodeoxycholic, deoxycholic, and lithocholic acid (the uppermost band is a contaminant of lithocholic acid, probably 3-ketolithocholic acid); cholic acid (C), chenodeoxycholic acid (CD), deoxycholic acid (DC), and lithocholic acid (L); and the mixture.

500 Elmsford, N.Y.), and either the free acid or the sodium salts of bile acid standards (Supelco, Inc., Supelco Park, Bellefonte, Pa.) were used. Bile and standards were dissolved in methanol and applied to the plate with a Hamilton syringe. When quantitative analysis of bile was desired, the plates were first washed in the developing solvent; otherwise they were used as they came.

Figs. 1 and **2** show typical separations of the classes of bile acids and the free bile acids alone and in mixtures, respectively. The spots were revealed by spraying the plate with water (3). Isopropanol–glacial acetic acid 93:7 (v/v) was used for class separation. With this system (Fig. 1) the bottom three bands are taurocholic acid, taurochenodeoxycholic acid with taurodeoxycholic acid as one spot, and taurolithocholic acid as the third band from the bottom. The fourth, fifth, and sixth bands (from the bottom) are glycocholic acid, glycochenodeoxycholic acid with glycodeoxycholic acid, and glycolithocholic acid, respectively. The top single band represents the free bile acids. The mixture clearly shows

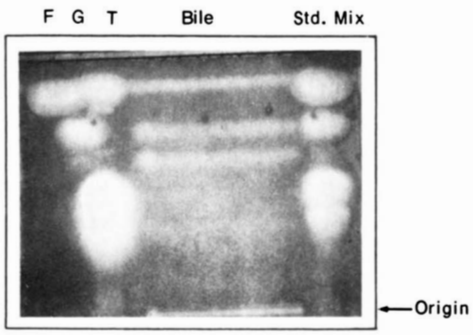

Fig. 3. TLC class separation of human bile and standards. See Fig. 1 for abbreviations.

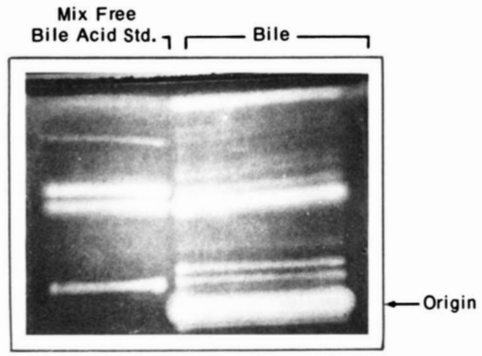

Fig. 4. TLC of free bile acid standards mixture and of hydrolyzed human bile. The four standard bands are, from bottom to top, cholic acid, chenodeoxycholic acid, deoxycholic acid, and lithocholic acid.

that this system can separate the classes of bile acids that can be eluted with appropriate solvents.

The second solvent system[1] (**Fig. 2**) consists of hexane–methylethylketone–glacial acetic acid 56:36:8 (v/v), and clearly separates all four free bile acids. With this system the separation between CD and DC widens as the solvent gets older (months).

Fig. 3 shows a typical class separation of extracted human bile and **Fig. 4** shows the separation of the free bile acids of the same bile after hydrolysis. Preliminary evidence suggests that the unmatched bands are 3-keto derivatives of free bile acids.

Table 1 shows the approximate R_f values of free and conjugated bile acids. Since these values vary

[1] Petcoff's solution, named in honor of Darryl Petcoff (deceased), formerly of Ivan Sorval, Inc., Helena, Mont. Mr. Petcoff cooperated with the authors on this project.

TABLE 1. R_f values for free and conjugated bile acids

	Solvent 1[a] (R_f 1.0 = 14 cm)	Solvent 2[b] (Petcoff's Solution)
Bile acid class		
Free bile acids	0.86	
Taurolithocholic	0.47	
Taurochenodeoxy with deoxycholic	0.38	
Taurocholic	0.29	
Glycolithocholic	0.71	
Glycochenodeoxy with deoxycholic	0.67	
Glycocholic	0.51	
Free bile acids		
Ketolithocholic		0.93
Lithocholic		0.78
Deoxycholic		0.67
Chenodeoxycholic		0.43
Cholic		0.21

[a] Isopropanol–glacial acetic acid 93:7 (v/v).
[b] Hexane–methylethylketone–glacial acetate acid 56:36:8 (v/v).

somewhat with each run and age of solution, it is advisable to use standards. These two systems have been applied and tested with satisfactory results in quantitative analysis of human bile. After elution, conjugated bile acids are hydrolyzed with sodium hydroxide or the Nair enzyme (8–10) and the free acids are extracted and rechromatographed in the free bile acid system. After the acids are eluted from the TLC plate their concentration is determined by the method of Iwata and Yamasaki (11). Complete details on human studies will be published later[2].

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[2] Chavez, Margarito N., and Charles L. Krone. Unpublished results.