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Note

Separation of bile acids from neutral lipids on thin-layer chromatograms

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The quantitative analysis of bile acids in biological materials cannot be achieved unless they are separated from other substances, particularly the neutral lipids. The different physicochemical properties of bile acids, free and conjugated, make it difficult to separate them together from neutral lipids. The separation technique which include liquid–liquid chromatography, ion-exchange chromatography and adsorption chromatography have been summarized in a recent review[1]. These techniques are all time consuming and some bile acids separate with the neutral lipid. Various solvent systems have been proposed for the separation of bile acids and its conjugates on thin-layer chromatograms[2–5]: the present system described here is a useful addition. This technique separates the bile acids in one narrow band in which both free and conjugated bile acids are close together to permit recovery from the chromatograms for quantitative analysis of total bile acids.

MATERIALS AND METHODS

The following bile acids were used: taurocholic acid, glycodeoxycholic acid, cholic acid, hyodeoxycholic acid (Calbiochem, San Diego, Calif., U.S.A.), lithocholic acid, chenodeoxycholic acid (Applied Science Labs., State College, Pa., U.S.A.), glycocholic acid, glycochenodeoxycholic acid, taurodeoxycholic acid, taurochenodeoxycholic acid, deoxycholic acid (Sigma, St. Louis, Mo., U.S.A.). These bile acids were dissolved in methanol. Neutral lipid was butter dissolved in chloroform–methanol (2:1). The solutions to be tested were applied on 20 × 20 cm pre-coated thin-layer plate silica gel of 0.25 mm thickness (E. Merck, Darmstadt, G.F.R., distributed by Brinkmann, Westbury, N.Y., U.S.A.: Catalogue No. 5763). 10-μg samples of bile acids, free and conjugated, were applied as spots of 4-mm diameter and 1.5 cm above the bottom edge of the plate. Volumes of 0.5–1.0 μl of gallbladder bile, collected from surgical specimen, were applied directly to the plate using a Hamilton microliter syringe. Mixtures of bile acids and fat were also run for separation. The plate was then placed in a commercial chromatographic chamber (Gelman, Ann Arbor, Mich., U.S.A.) and was run with a 20:25:6:4 mixture of isopropyl alchol (Baker analysed

reagent, J. T. Baker, Phillipsburg, N.J., U.S.A.), ethyl acetate (Mallinckrodt, St. Louis, Mo., U.S.A.), water and ammonia (DuPont, Wilmington, Del., U.S.A.), for 2 h at room temperature (23–25°). The plate was removed from the chromatographic chamber, dried in air and sprayed with the copper–molybdenum spray reagent[4] or developed in iodine vapor in order to make the components visible.

RESULTS AND DISCUSSION

The R_F values of different bile acids are given in Table I. All bile acids, free and conjugated, lie between glycocholic acid (R_F = 0.40) and taurochenodeoxycholic acid (R_F = 0.55), which, being close together, form a relatively narrow band. The lipid fraction runs ahead of the bile acids along with the solvent. For quantitative analysis of total bile acids, the narrow band between glycocholic acid and taurochenodeoxycholic acid can be scraped off from the chromatogram after visualization with iodine vapor and extraction with methanol and then analysed enzymatically. The recovery of bile acids from thin-layer plates by such procedure has been reported to be very good[5,6]. This method is very simple, time saving, and does not require hydrolysis of the conjugated bile acids. The method does permit quantitative analysis of total bile acids, free and conjugated, from biological materials.

TABLE I

R_F VALUES OF BILE ACIDS

Bile acid	*Free*	*Glyco-*	*Tauro-*
Chenodeoxycholic acid	0.48–0.49	0.48–0.49	0.53–0.55
Deoxycholic acid	0.48–0.49	0.46–0.47	0.51–0.53
Cholic acid	0.42–0.43	0.40–0.41	0.49–0.50
Lithocholic acid	0.50–0.52	—	—
Hyodeoxycholic acid	0.48–0.49	—	—

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REFERENCES

1 P. Eneroth and J. Sjövall, *Methods Enzymol.*, 15 (1969) 237.
2 A. F. Hofmann, in A. T. James and L. J. Morris (Editors), *New Biochemical Separation*, Van Nostrand, London, 1964, p. 261.
3 P. Eneroth, *J. Lipid Res.*, 4 (1963) 11.
4 S. K. Goswami and C. F. Frey, *J. Chromatogr.*, 89 (1974) 87.
5 F. W. Koss, D. Mayer and H. Haindl, in H. V. Bergmeyer (Editor), *Methoden der enzymatischen Analyse*, Band II, Verlag-Chemie, Weinheim, 1970, p. 1824.
6 Y. Ghoos, G. Vantrappen and D. Mayer, *J. Chromatogr.*, 76 (1973) 425.