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An improved one-dimensional thin-layer chromatographic separation of neutral lipid classes

A number of thin-layer chromatographic procedures were examined in an attempt to implement a rapid one-dimensional procedure suitable for densitometric quantitation[1-3]. None of the systems attempted gave satisfactory separations of neutral lipids from serum. One method reported required plates which were 34 cm long and required 2 to 3 h for development[4]. A system for separation of micro quantities of neutral lipids has been described by BIEZENSKI *et al.*[5]. The system described here was devised for routine separation of neutral lipid samples and quantitation by densitometry.

Materials and methods

Glass plates and spreader were obtained from Quickfit Reeve Angel, Clifton,

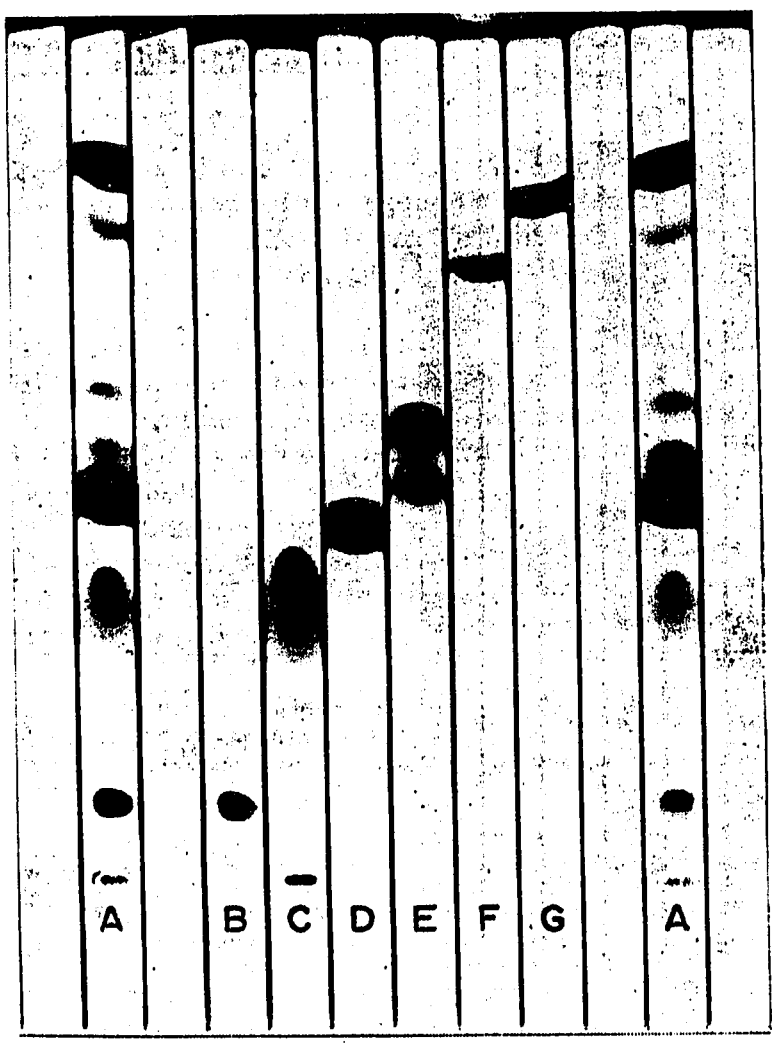

Fig. 1. Thin-layer chromatogram of standard neutral lipid classes. A = Mixtures of standard neutral lipids; B = monoglyceride; C = free fatty acid; D = sterols; E = 1,2- and 1,3-diglycerides; F = triglyceride; G = sterol esters.

N.J., U.S.A. Adsorbosil-5 silica gel was obtained from Applied Science Laboratories, State College, Pa., U.S.A. Spectroquality *n*-hexane and petroleum ether (boiling range 38.8–54.6) were obtained from Matheson, Coleman, and Bell, East Rutherford, N.J., U.S.A. Reagent grade methanol and anhydrous diethyl ether were obtained from Mallinckrodt, St. Louis, Mo., U.S.A.

Individual and mix standard neutral lipids were obtained from Supelco, Inc., Bellefonte, Pa., U.S.A. Spotting syringes and repeating dispenser were obtained from Hamilton Company, Whittier, Calif., U.S.A.

Glass plates, 20 × 20 cm, were coated with a 250 μ thick slurry of silica gel and air dried at ambient temperature for about 2 h. The air dried plates were scribed into 1.0 cm wide lanes which permitted multiple samples per plate. Samples containing 200 μg of lipid in 10 μl of chloroform–methanol (95:5) were spotted under nitrogen as a narrow band in the center third of a lane. The spotting solvent was allowed to evaporate and the plate transferred into a TLC tank lined with filter paper. The plate was developed to a height of 15 cm in a solvent of *n*-hexane–diethyl ether–methanol

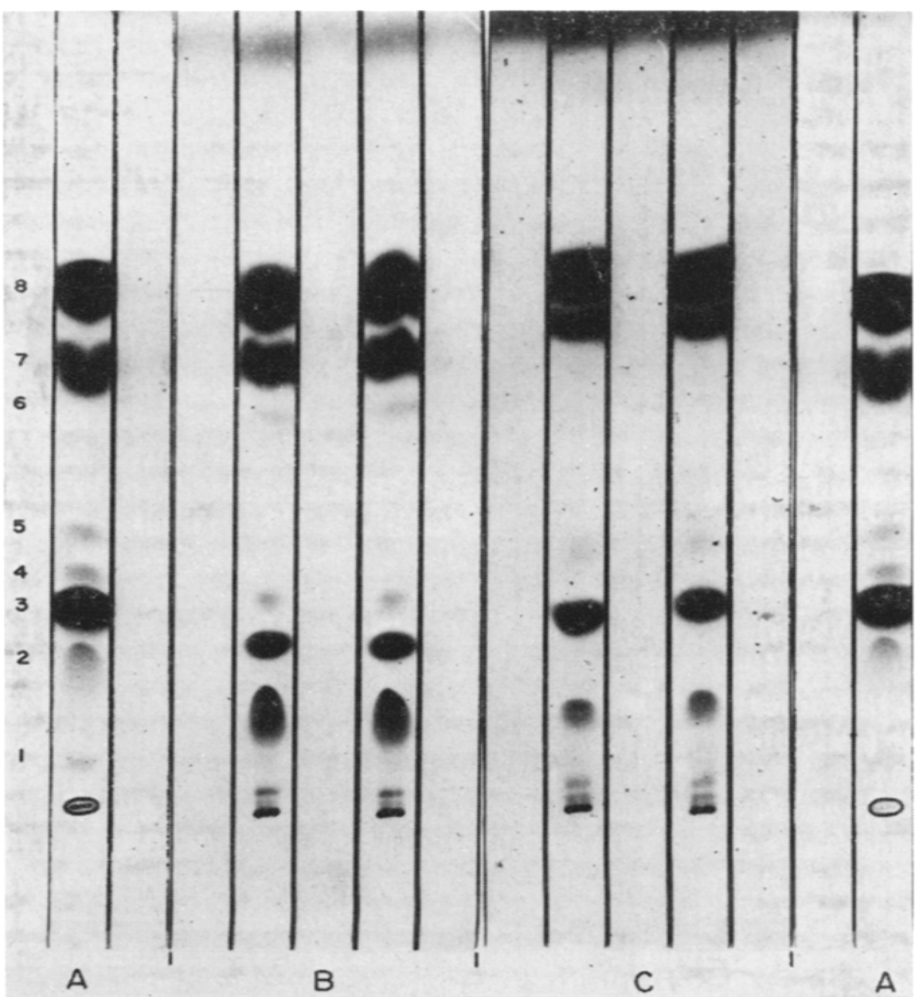

Fig. 2. Thin-layer chromatogram of serum neutral lipids and a standard neutral lipid mix. 1 = Monoglyceride; 2 = free fatty acid; 3 = sterols; 4 = 1,2-diglyceride; 5 = 1,3-diglyceride; 6 = unknown; 7 = triglyceride; 8 = sterol esters; A = mixture of standard neutral lipids; B = rat serum; C = baboon serum.

(90:30:5). Subsequent to development, the plate was placed in a dry nitrogen atmosphere for 5 min and then transferred into a second TLC tank lined with filter paper. The plate was developed in petroleum ether to the top and then left in the tank for an additional 25 min. Following development the plate was removed from the TLC tank, air dried at ambient temperature, sprayed with a 20% aqueous solution of ammonium bisulfate[6], and charred at 170° for 90 min for lipid class detection. Total development including preparation of the plate for charring takes 1½–2 h.

Discussion

Fig. 1. illustrates a typical chromatogram obtained by the above method with neutral lipid standards. Fig. 2 illustrates a typical chromatogram, obtained by this method, with serum neutral lipids. Environmental and mechanical changes such as temperature, relative humidity, and lot variations in silica gel can affect the demonstrated separations. These changes can be alleviated by varying the height of development in the first solvent, the time the plate is left in the second solvent after full height development is attained, or by altering the amount of methanol in the first solvent.

Although methanol is incorporated as a solvent component in phospholipid chromatography it has been used very little in neutral lipid class separations. Solvents such as methanol have such a high polarity that triglycerides and sterol esters are not resolved and move on or very near the solvent front. The use of methanol, in this system, increased the polarity sufficiently to move the monoglyceride spot above the origin. This cannot be accomplished with most neutral lipid solvents. Subsequent development of the plate in a solvent such as petroleum ether, with a very low polarity, then moves the sterol esters above the triglycerides with little or no effect on the other neutral lipid classes. This second solvent also leaves the sterol esters below the solvent front which enhances quantitation. The use of these two solvent systems in one dimension accomplishes the desired separations with multiple samples per plate.

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