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Note

Separation of neutral lipids on chromarods

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The use of chromarods[1], quartz rods to which silica gel is fused at high temperature, for the separation of neutral lipids for quantification by means of a flame ionisation detector in the commercially available Iatroscan TH-10 analyser has been described by several workers[2–5]. All utilised solvent systems for the initial chromatographic separation that were modified from those used in conventional thin-layer chromatography and consisted of hexane–diethyl ether mixtures. In this communication, it is shown that improved separations can be achieved if chlorinated solvents are used.

EXPERIMENTAL

The Iatroscan TH-10 (Iatron Lab.) was supplied by Life Science Labotatories Ltd. (Luton, Great Britain). Lipid standards were the purest grade available and were supplied by Sigma (London) (Poole, Great Britain) while solvents (AnalaR grade) were from BDH (Poole, Great Britain).

The chromarods were stored overnight in the racks provided with the instrument in groups of ten in a developing tank containing distilled water to a depth such that it was in contact with the adsorbent layers. The rods were activated by passing through the flame ionisation detector immediately before use. Standard mixtures of lipids in chloroform (10–80 mg/ml) were applied by means of disposable pipettes (2 μl) to the rods. The developing solvent of greatest utility consisted of 1,2-dichloroethane–chloroform–acetic acid (92:8:0.1). After an initial development in this solvent, the rods were air-dried at room temperature for 15 min then were subjected to a second development in the same direction.

RESULTS AND DISCUSSION

The chromatographic properties of the silica gel bound to chromarods were somewhat different from the silica gel with binders commonly used in conventional thin layer chromatography; solvents of slightly lower polarity must be used with chromarods to obtain comparable mobilities of lipid classes. With hexane–diethyl–ether–acetic acid mixtures as developing solvent, cholesterol esters, triacylglycerols, cholesterol and phospholipids were found to be readily separable but diacylglycerols were not separable from cholesterol and free fatty acids tended to overlap the triacylglycerols. Replacement of the diethyl ether component of the mixture with other

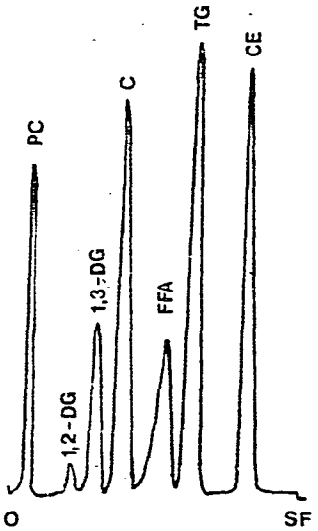

Fig. 1. Separation of neutral lipid classes on chromarods. PC = phosphatidylcholine; 1,2- and 1,3-DG = 1,2- and 1,3-diacylglycerols respectively; C = cholesterol; FFA = free fatty acids; TG = triacylglycerols; CE = cholesterol esters; O = origin; SF = solvent front.

solvents such as acetone, ethyl acetate, ethanol or chloroform did not bring about significant improvements nor did replacement of the hexane component with benzene or toluene. However, with solvent systems containing entirely chlorinated solvents *i.e.* 1,2-dichloroethane and chloroform (with 0.1% acetic acid to effect sufficient mobility of the free fatty acids) satisfactory separation of most of the common lipid classes was achieved as illustrated in Fig. 1. Free fatty acids migrated between triacylglycerols and cholesterol and 1,2- and 1,3-diacylglycerols were separable from each other and from cholesterol. As the rods age (approx. 50 analyses), the quality of the separation starts to deteriorate although this can in part be restored by varying the chloroform content of the mixture. Better separations were achieved with a double development with the solvent system listed than with a single development with a slightly more polar solvent system.

Some problems remain in obtaining satisfactory quantification of results. Within each set of rods, a given rod will distort the quantification of a particular standard mixture in a distinctive way. Quantification was dependent on the nature of the sample, the response of the detector to sterols was found to be greater than anticipated, and on the amount of sample applied to the rod. It was also affected by slight deviations in the correct geometry of the rod with respect to the detector. These problems are currently being studied.

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