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Note

Separation of phospholipids on chromarods

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Separation and quantitation of phospholipid classes is commonly achieved by thin-layer chromatography (TLC) and chemical methods for determination of inorganic phosphorus after elution of separated phospholipids from the TLC plate. Though such methods are widely established and used, they are time consuming, requiring tedious sample handling and transfer through a number of stages where potential loss of sample may occur.

In view of the expanding interest in simple and rapid methods using flame-ionization detection following lipid separation on silica-coated quartz rods, we wish to report a system which accomplishes separation and quantitation of neutral lipids and individual phospholipids in about 3 h. Although our application has been mainly on the separation and quantitation of mitochondrial phospholipids, this method is generally applicable to a wide variety of complex lipid extracts.

EXPERIMENTAL

Mitochondria were isolated from rat heart by standardized methods[1] and total lipid extracted with 20 volumes of chloroform–methanol (2:1) containing 50 μg 2-ethoxyquin as antioxidant followedby 20 volumes of chloroform–methanol–28% aqueous ammonia (35:5:2) with 50 μg 2-ethoxyquin[2]. Extracts were evaporated to dryness at 40°C under nitrogen and dissolved in 0.2 ml chloroform, containing 2.8 mg/ml nonadecane as an internal standard, to give a final phospholipid concentration of *ca.* 20 mg/ml.

Chromarods (type S obtained from Technical Marketing Assoc., Mississauga, Canada) were routinely stored in 10 *N* sulphuric acid. Prior to use each chromarod was washed 4 times in distilled water, dried 5 min at 110°C and then activated by passage through the flame-ionization detector (FID; Iatroscan Model TH-10, obtained from Technical Marketing Assoc.). Lipid extracts (2 μl) were applied to each chromarod using a syringe (Precision Sampling Corp., Baton Rouge, LA, U.S.A.). The rods were developed 30 min in light petroleum (b.p. 72.4–106.7°C)–diethyl ether (85:15) to separate neutral lipids. This system separates the internal standard, triglyceride, free fatty acids and free cholesterol with phospholipids remaining at the origin. After air drying 20 min, the rods were transferred to the FID scanning frame.

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Half of the rods were scanned in full for quantitation of neutral lipids and total phospholipid. The remaining rods were scanned to the end of the cholesterol band only, thus leaving the phospholipids intact at the origin on the chromarod. Rods were reactivated by scanning the upper portion of the rod a second time. Separation of phospholipids was accomplished in chloroform–methanol–water (80:35:3). The chromatography tank was viewed with a desk light positioned behind the rods and the chromatography stopped with the solvent front 0.5 cm from the end of the silica coat. The rods were air dried 20 min and scanned in full.

Chromarods were scanned through the FID at 1 min per rod. The hydrogen flow-rate was 80 ml/min and the air flow-rate was 2 l/min. Both differential and integral outputs from the FID were recorded using 100 mV full scale deflection.

RESULTS AND DISCUSSION

In our laboratory this system is used for separation of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, sphingomyelin and lysolecithin (Fig. 1). Identification of neutral lipids, individual phospholipids, and calculation of response factors was accomplished with known standards. The amount of water present in the second solvent system was found to be very critical to the clear resolution of these seven phospholipids. A previous report[3] utilizing chloroform–methanol–water (80:35:5) was found to separate lysophosphatidylcholine, sphingomyelin, phosphatidylcholine and phosphatidylethanolamine, but was unable to separate cardiolipin, phosphatidylserine and phosphatidylinositol when present in the same phospholipid mixture. Variation in quantity applied to the rods was controlled for by use of the internal standard.

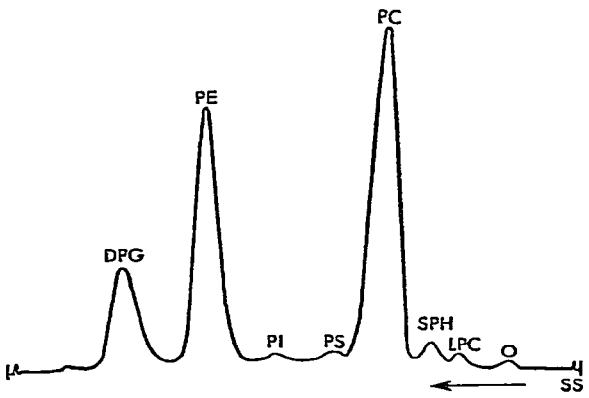

Fig. 1. Mitchondrial phospholipids separated on chromarods S. Phospholipids were separated in chloroform–methanol–water (80:35:3). The recorder chart speed was 10 in./min. SS = Start of scan; O = application point; LPC = lysophosphatidylcholine; SPH = sphingomyelin; PC = phosphatidylcholine; PS = phosphatidylserine; PI = phosphatidylinositol; PE = phosphatidylethanolamine; DPG = diphosphatidylglycerol.

With this method highly reproducible Iatroscan analyses for phospholipids were obtained within 3 hours after lipid extraction had been completed. This represents a considerable time-saving compared to more conventional methods of TLC.

Additionally, the high sensitivity of the method requiring the application of only very small quantities of lipid to each chromarod, and the need for sample handling at only the rod application stage make the use of combination TLC–FID a valuable tool for analyses of complex lipid extracts from a wide variety of sources.

Minor technical problems must be coped with before satisfactory quantitation of samples can be made. For example, as a result of the slightly different responses elicited by individual rods within original sets, sorting and matching of several sets of rods according to the response observed with a standard mixture must first be undertaken. Repeated analysis of standard mixtures will enable calibration of response factors. However, as the response obtained is relative to the individual laboratory's standardized operating conditions these factors must be determined individually for each new set of chromarods by any laboratory undertaking these analyses.

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