Quantitative recovery of lecithins after argentation chromatography on thin layers

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SUMMARY The lecithins of rat liver are resolved into three fractions by chromatography on a thin layer of silica gel impregnated with silver nitrate. Each of these fractions is placed in a small column containing a cation-exchange resin, and the lecithins are eluted free from silver nitrate. Recoveries average 96%.

KEY WORDS silver nitrate · silica gel · thin-layer chromatography · lecithins · cation-exchange resin · recovery · quantification

PUBLICATIONS BY Haverkate and van Deenen (1) and Arvidson (2) have described separations of lecithins according to their degree of unsaturation by argentation TLC. Neither of these reports has given a procedure for eliminating the silver nitrate from the recovered lecithin. Without this, determination of phosphorus by the Fiske-Subbarow procedure (3) is difficult, because of the formation of an insoluble black precipitate which interferes with color development. Arvidson (4) described in detail a method which combines argentation TLC and reversed-phase partition TLC on hydrophobic kieselguhr to yield eight phosphatidyl cholines. This method, while obviously suited for highly refined separations, is too elaborate and time-consuming for routine work. More recently, Nakayama and Kawamura (5) described a simple method for separating biliary lecithin into subgroups by argentation TLC, but they had difficulty in determining the lecithin after extraction from the plates.

The purpose of this communication is to describe a modified method for separating linoleoyl, arachidonoyl, and docosahexaenoyl lecithins on silver nitrate TLC, and for eliminating the silver nitrate during extraction of the compounds from the silica gel.

Methods. Plates 20 × 20 cm are coated with a slurry of 55 g of silica gel without binder (Kieselgel DS-O, Camag D.O., Muttenz, Switzerland, obtained through Arthur H. Thomas Co., Philadelphia, Pa.) and 3 g of calcium sulfate in 100 ml of distilled water containing 11 g of silver nitrate. The slurry is spread 0.5 mm thick with an anodized aluminum applicator that is impervious to silver nitrate (Quickfit Reeve Angel Co., Clifton, N.J.). The plates are allowed to dry in the air for 1–2 hr and can be stored in the absence of light without desiccation for up to 3 wk. They are activated before use by heating at 110°C for 60–90 min.

Abbreviation: TLC, thin-layer chromatography.

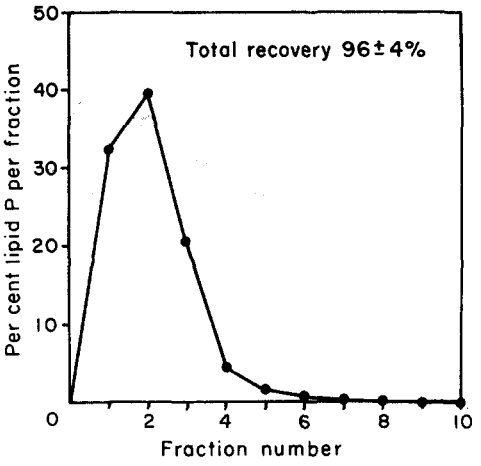

FIG. 1. Elution of lecithins from silver nitrate-impregnated silica gel on columns containing cation-exchange resin (1–2 g of AG50W-X8). Column loads varied from 37.6 to 102.7 μg of lipid P, and recoveries ranged from 92.8 to 100.2% (mean 96%) in five experiments. 5-ml fractions were collected.

The plate is placed under a plastic cover 22 × 22 × 2 cm, which has a hole at one end for applying nitrogen and a slot at the other end to allow the sample solution to be applied to the plate (6). Solutions of lecithin in chloroform are applied to the plate under a stream of nitrogen with 2–10 μg of lipid P on each spot and as much as 150 μg on each plate. The plates are developed in chloroform–methanol–water 125:50:6 for 60–90 min. On completion the plates are placed under the plastic cover and dried under a stream of nitrogen, and the spots are made visible by spraying with 0.002% 2,7-dichlorofluorescein (Eastman Kodak Co., Rochester, N.Y.) in methanol. The spots are outlined with a needle under UV light and scraped into small columns, 15 cm × 1 cm I.D., containing 1–2 g of cation-exchange resin (AG50W-X8, 200–400 mesh, H^+ form, Bio-Rad Laboratories, Richmond, Calif.) which has been washed twice with the eluting solvent. The lipid is eluted with 50 ml of methanol–acetic acid–water 94:1:5; as much as 80% of the lecithin is eluted in the first 10–20 ml of eluate (Fig. 1). The eluate is taken to dryness on a rotary evaporator at 35–40°C under reduced pressure, and dissolved in chloroform–methanol 2:1 for analysis. Chromatography on the cation-exchange resin completely eliminates the silver nitrate from the eluate, and 92–100% of the lipid is recovered as indicated by phosphorus determinations (Fig. 1). At the same time, the resin absorbs almost all the 2,7-dichlorofluorescein from the silica gel, and the clear eluate can be used for liquid scintillation counting without interference by color quenching.

Results. Fresh rat liver lecithin purified by TLC, when chromatographed on silver nitrate impregnated silica gel plates as described, separated into three fractions. All fractions contained palmitic and stearic as well as small amounts of oleic acid. In the first fraction (fastest

TABLE 1 RAT LIVER LECITHINS: FATTY ACID COMPOSITION OF FRACTIONS SEPARATED BY TLC

Sample	Fatty Acid						
	16:0	16:1	18:0	18:1	18:2	20:4	22:6
	% *by weight*						
Intact	30.7	0.5	20.9	6.6	12.1	21.8	7.4
Fraction 1	30.5	0.5	16.9	10.5	*41.6*	0.0	0.0
Fraction 2	16.5	0.4	24.5	3.5	0.7	*53.6*	0.0
Fraction 3	14.9	1.6	16.2	4.7	0.6	19.0	*43.0*

Values represent means of eight separate analyses on an F & M Model 400 gas chromatograph. Fatty acids designated by chain length: number of double bonds.

running; R_f about 0.49) linoleic acid was the predominant constituent fatty acid, in the second fraction (R_f about 0.34) arachidonic acid; and in the third fraction (R_f about 0.24) docosahexaenoic acid. The results of the fatty acid analysis of whole rat liver lecithin and the three subgroups are shown in Table 1. Docosahexaenoic acid may be partially or completely lost unless the rat liver is extracted fresh, the lecithin is kept in a freezer under nitrogen, the material is applied to the TLC plates under nitrogen, and the plates are dried under nitrogen.

This method is simple, and useful for the routine separation and quantitative analysis of linoleoyl and arachidonoyl lecithins which are the major fractions of intact lecithin.

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REFERENCES

1. Haverkate, F., and L. L. M. van Deenen. 1965. *J. Lipid Res.* **6:** 78.
2. Arvidson, G. A. E. 1965. *J. Lipid Res.* **6:** 514.
3. Bartlett, G. R. 1959. *J. Biol. Chem.* **234:** 466.
4. Arvidson, G. A. E. 1967. *J. Lipid Res.* **8:** 155.
5. Nakayama, F., and S. Kawamura. 1967. *Clin. Chim. Acta.* **17:** 53.
6. Cruess, R. L., and F. W. Seguin. 1965. *J. Lipid Res.* **6:** 441.