Separation of Tri, 1,3, Di- and 1-Mono-oleoylglycerols and Oleic Acid by Flash Chromatography

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Separation of lipids is done mostly by various types of chromatography (1), although crystallization can be used for the purification of synthetic acylglycerols (2). When purifying synthetic triacylglycerols, we occasionally start with a reaction mixture which contains tri-, di- and monoacylglycerols and free fatty acids. It is difficult to obtain the desired compound from these mixtures by crystallization, and the quantities usually exceed the capacity of the silica gel column for resolution. In addition, we separate these lipids from lipolysates when investigating the specificity of lipases, but use thin layer chromatography (TLC) (3). Successful separations also have been achieved with high performance liquid chromatography (HPLC) (4). The quantities needed are small (4). Preparative separations of acylglycerols by column chromatography are tedious, and resolution is not always attained. Still et al. (5) have developed a rapid procedure for the routine purification of relatively large quantities of reaction mixtures which they named flash chromatography. The technique is simple and inexpensive; recoveries are good, and samples of 0.01–10.0 g can be separated in 10 to 15 min. Although the method is obviously applicable to lipids, it apparently has not been used to any extent. However, Prestwich et al. (6) purified fatty acids and Kodali and coworkers (7–10) purified 1,2-isopropylidene acylglycerols with the method.

The system for flash chromatography consists of a chromatographic column equipped with a Teflon stopcock and a 24/40 standard tapered or an appropriate ball and socket glass joint and a valve to control air flow. Details are given in (5), and columns can be purchased from J.T. Baker Chemical Co., Phillipsburg, New Jersey, or Aldrich Chemical Co., Milwaukee, Wisconsin, and others. Because optimum resolution on the column occurs when the solvent flow rate is about 5.0 ± 0.25 cm/min (hence the name, flash chromatography), particle size of the silica gel is critical. Still et al. (5) obtained their best results with 400–230 mesh (40±63 μm silica gel 60, E. Merck No. 9385). We obtained the silica gel (No. 7030-0, 40 μm) from J.T. Baker, but it is available elsewhere. J.T. Baker also provided a brochure containing useful instructions. Still et al. (5) give information on column size and load and the transfer of separations by TLC to flash chromatography.

We prepared and tested five separate mixtures of trioleoylglycerol, oleic acid 1,3-dioleoylglycerol and 1 (3)-monooleoylglycerol containing approximately equal weights of these compounds and ranging in total weight from 1.033 to 2.179 g. Fifteen cm of silica gel was added to a glass column (3.2 × 61 cm). The test mixture was dissolved in hexane:ethyl ether (70/30, v/v) to make a 20% solution. The solution was poured into the column and forced into the silica gel with air pressure (25 psi), although N_2 would be better for lipids sensitive to oxidation. The quantities of solvent mixtures in Table 1 were then forced through sequentially at the desired flow rate of 5 cm/min. It was helpful to mount a ruler by the column for these measurements. Samples of 20–50 ml were collected in beakers. These volumes also can be used to measure flow. Separations were monitored by TLC on coated microscope slides using a solvent system of hexane:ethyl ether:acetic acid (70/30/1, v/v/v) (3).

The eluted fractions contained only one spot with an appropriate Rf when analyzed by TLC. Recoveries ranged from 89 to 94% (Table 1). As noted by Still et al. (5), greater amounts of solvent were required as the sample size increased. Therefore, other investigators will need to develop their own solvent systems for the amounts and types of materials they plan to separate. We did not determine the occurrence or extent of acyl

TABLE 1

Separation of Acylglycerols and Free Fatty Acids by Flash Chromatography[a]

Compound	Initial wt (mg)	Wt recovered (mg)	Recovery (%)	Solvent ratio (hexane:ethyl ether)	Amount (ml)
Trioleoylglycerol	386±123[b]	358±131	92.7	90/10	102± 15
	(255-571)[c]	(299-564)			(90-120)
Oleic acid	301±77	268± 66	89.0	90/10	266± 21
	(225-397)	(204-349)			(210-260)
1,3-dioleoylglycerol	368±120	342±128	92.9	90/10	175± 25
	(275-590)	(238-574)		40/60	(150-250)
1-monooleoylglycerol	376±138	353±145	93.8	100	220± 25
	(271-627)	(223-612)			(200-250)
Total	1432±322	1322±462	92.3		832±102
	(1033-2179)	(915-2099)			(750-1020)

[a]Method from Ref. 5.
[b]Mean of 5 samples ± SD.
[c]Range.

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migration of 2-mono- and 1,2 (2,3) diacylglycerols to the 1 (3)-mono- and 1,3-diacyl species when the former are eluted through a column of silica gel. Acyl migration could probably be eliminated by inclusion of boric acid in the silica gel (2). We did not determine if it is possible to separate a large amount of, for example, triacylglycerol from a relatively small quantity of cholesterol or neutral from polar lipids, but we did separate triacylglycerols from methyl or ethyl esters with hexane as the eluant. This is almost impossible with crystallization. However, large quantities of lipid (up to 10 g) could probably be rapidly separated with flash chromatography. The method should have a multitude of applications for the rapid separation of neutral and possibly other lipids.

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