Radioassay of lipid components separated by thin-layer chromatography*

JERRY L. BROWN AND JOHN M. JOHNSTON

Department of Biochemistry,
Southwestern Medical School,
The University of Texas,
Dallas 35, Texas

[Received for publication March 21, 1962]

» Thin-layer chromatography (TLC) as described by Stahl *et al.* (1) has received widespread use in the separation of lipids. Malins and Mangold (2) have reported a system for the separation of a complex mixture of lipids. Recently, Privett *et al.* (3) have reported a procedure employing three solvent systems for the separation of mono-, di-, and triglycerides. We have been interested in the separation of glycerides and fatty acids and, in addition, the quantification of radioactivity in each fraction. The present report describes a method by which these classes of compounds may be separated by TLC and their radioactivity determined by liquid scintillation counting methods.

Palmitic acid, oleic acid, diolein, tripalmitin, cholesterol, phosphatidyl ethanolamine, and sphingomyelin were obtained from commerical sources. The 1,2- and 1,3-dipalmitin were gifts from Dr. Fred Mattson of Procter and Gamble Co. Phosphatidyl choline was isolated from egg by the method of Hanahan *et al.* (4). Cholesteryl stearate was a gift from Dr. S. G. Eliasson, University of Texas, Southwestern Medical School. The C^{14}-labeled tripalmitin and C^{14}-palmitic acid were obtained from New England Nuclear Corp. Labeled α-monopalmitin was prepared according to the procedure of Hartman (5). Purification of the C^{14}-labeled compounds was effected by recrystallization followed by column and thin-layer chromatography. The solvents employed were CP or reagent grade. The *n*-hexane and diethyl ether were redistilled prior to use.

Glass plates (20 x 20 cm) were coated with a suspension of 30 g of Silica Gel G[1] in 60 ml of an 0.02% aqueous 2′,7′-dichlorofluorescein (DCF) solution to give a silicic acid layer 250 μ thick. The DCF solution was adjusted to pH 7 with 10% sodium hydroxide prior to its use in the preparation of the silica gel suspension. Incorporation of the DCF into the plates proved to be superior to spraying the chromatogram. Inclusion of the DCF in the silica gel increased the sensitivity of detection and reproducibility of the counting results. Seventy-five micrograms of the individual compounds, dissolved in benzene, were applied to the plate in a volume of 0.05 ml with the aid of a microsyringe. The mixtures used contained 75 μg of each component. The samples, either single components or a mixture of components, were applied as one spot approximately 5 mm in diameter.

The plates were developed 40 min, during which time the solvent front migrated approximately 120 mm. The chromatogram was then removed from the chamber and air-dried for 15 min. The dried chromatogram was viewed under ultraviolet light with a predominant wavelength of 3660 Å, and the spots were outlined. Each spot corresponding to a component was scraped into a mound on the plate by the use of a small blade. A scintillation vial was placed over the mound of silica gel and the plate and the vial simultaneously inverted to allow the silica gel containing the radioactive component to be transferred into the vial. Ten milliliters of a naphthalene-dioxane liquid scintillator (6) was then added to the vial. The activity recovered from the chromatograms was compared with that of a similar aliquot placed directly in the scintillation vial. The samples were counted in a Tri-Carb scintillation counter (Model 314AX, Packard Instrument Co., LaGrange, Ill.) at a photomultiplier setting of approximately 810 v in a 16–100-volt "window." All samples were checked for self-quenching by the internal

* Supported by research grants from the Robert A. Welch Foundation, Houston, Texas, and from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service (Grant A-3108).

[1] Obtained from Brinkmann Instruments, Inc., Great Neck, New York.

TABLE 1. RECOVERY OF RADIOACTIVITY FROM CHROMATOGRAPHICALLY SEPARATED LIPIDS

Component	Applied to the Chromatogram	Chromatographed as Single Component*		Chromatographed as Mixture*	
		Recovered	Recovery	Recovered	Recovery
	cpm	*cpm*	%	*cpm*	%
α-Monopalmitin	2,768	2,599	93.9	2,641	95.4
Palmitic acid	1,972	1,826	92.6	1,824	92.5
Tripalmitin	902	835	92.6	868	96.2

* Seventy-five-microgram samples.
* Seventy-five micrograms of each component.

standard method. No quenching was observed when the naphthalene-dioxane scintillation system was employed. The use of a toluene-based scintillator, however, resulted in considerable quenching of the sample. The quenching observed in the toluene system is probably due to the solubility of DCF in toluene.

The developing solvent employed for the separation of the lipid components was *n*-hexane–diethyl ether–acetic acid–methanol 90:20:2:3 (v/v). Unlined chambers were used. This developing solvent system offers distinct advantages in that the separation of fatty acids and mono-, di-, and triglycerides is accomplished on one plate instead of requiring three systems as previously reported (3). In contrast to a similar but less polar solvent employed by Malins and Mangold (2), with the present solvent system the monoglycerides migrate from the origin and therefore are separated from the phospholipids. The following R_f values are typical for the reported solvent system: tripalmitin, 0.90-0.96; oleic and palmitic acid, 0.60, 0.73; 1,3-dipalmitin and 1,3-diolein, 0.40-0.50; 1,2-dipalmitin and 1,2-diolein, 0.30-0.40; α-monopalmitin, 0.10-0.15; cholesterol, 0.33-0.40; cholesteryl stearate, 0.95-0.99. Phosphatidyl choline, phosphatidyl ethanolamine, and sphingomyelin failed to migrate.

The R_f values obtained for the lipid classes vary, but the order of elution is always the same. When diglycerides were chromatographed, two components were observed. Separation of diglycerides into 1,2- and 1,3-isomers by TLC has been previously reported (2). Co-chromatography of the diglyceride with purified samples of 1,2- and 1,3-diglycerides confirmed the identity of the two spots as the 1,2- and 1,3-isomers.

In Table 1 are given the results obtained when C^{14}-labeled α-monopalmitin, tripalmitin, and palmitic acid were chromatographed separately and as a mixture. As can be seen in the table, the recovery of radioactivity in the individual components was in excess of 90%. A range of sample sizes from 100 to 10 μg was studied, and in all cases the recovery was 90% or greater. In numerous experiments employing purified radioactive standards, the recoveries ranged from 90 to 102%.

The reported method has been utilized in the separation and radioactive assay of lipid extracts obtained from the hamster intestinal mucosa with similar results. In addition, this procedure has been employed in the assay of lipid mixtures containing C^{14} and H^3 with similar recoveries of both isotopes. The combination of thin-layer chromatography and liquid scintillation counting provides a simple rapid method in which the radioactive lipid mixtures can be chromatographically fractionated, visually observed, and assayed for radioactivity.

The technical assistance of Frances M. Liggett is acknowledged.

REFERENCES

1. Stahl, E. *Pharmazie* **11**: 633, 1956.
2. Malins, D. C., and H. K. Mangold. *J. Am. Oil Chemists' Soc.* **37**: 576, 1960.
3. Privett, O. S., M. L. Blank, and W. O. Lundberg. *J. Am. Oil Chemists' Soc.* **38**: 312, 1961.
4. Hanahan, D. J., M. B. Turner, and M. E. Jayko. *J. Biol. Chem.* **192**: 623, 1951.
5. Hartman, L. *Chem. and Ind. (London)* 711, 1960.
6. Bray, G. A. *Anal. Biochem.* **1**: 279, 1960.