Purification of phospholipids recovered from thin-layer chromatograms for infrared spectral analysis

A. H. Duthie* and Stuart Patton

Department of Dairy Science, Pennsylvania State University, University Park, Pennsylvania

SUMMARY An application of the Folch washing procedure for the removal of contaminants from phospholipid classes recovered from thin-layer chromatograms is described. The infrared spectra from the purified phospholipids are satisfactory for identification. The pattern of the major milk phospholipids separated on a thin-layer plate is presented.

Authorized for publication (8/13/64) as paper no. 2924 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This work was supported by PHS Research Grant H-3632 from the National Institutes of Health, U.S. Public Heath Service.

* Present address: Department of Animal and Dairy Science, The University of Vermont, Burlington, Vermont.

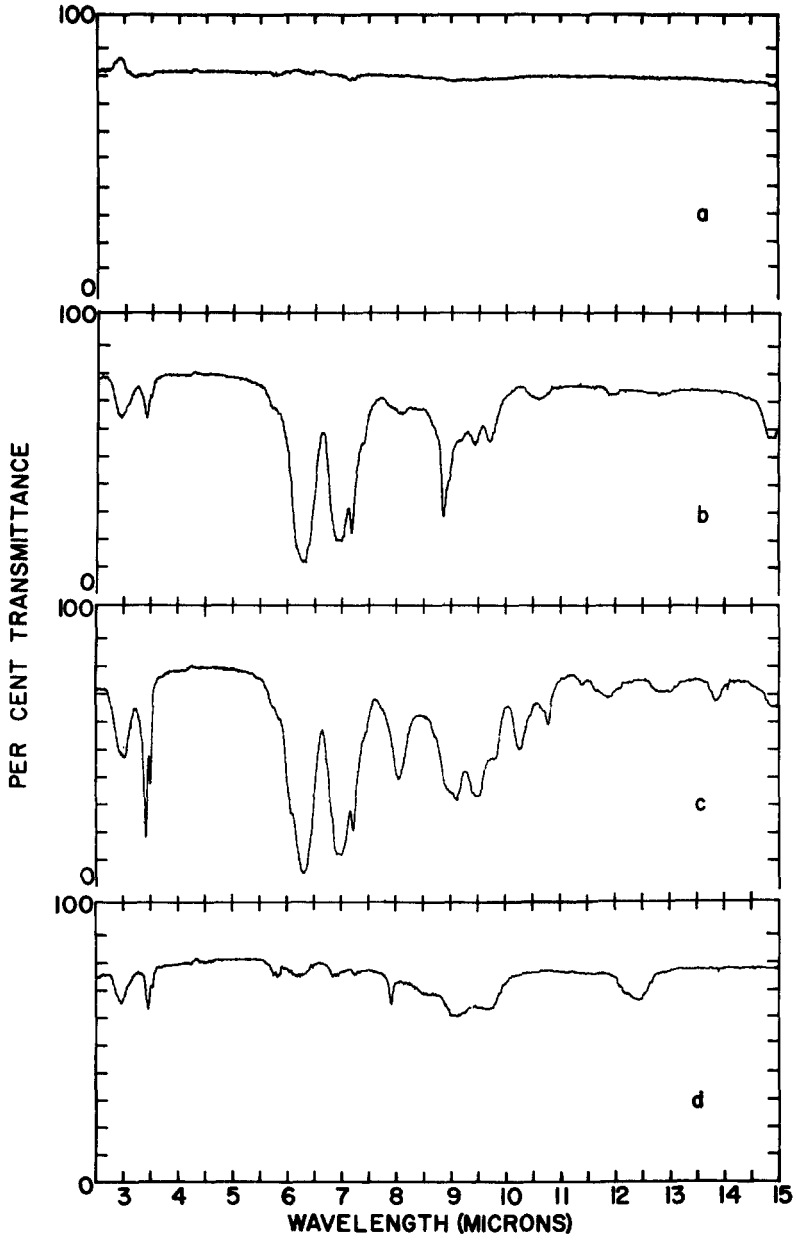

Fig. 1. Infrared spectra of KBr disks. Eluates from basic TLC zones added to approximately 300 mg of KBr. (*a*) Control disk. (*b*) Eluate from a TLC zone containing no sample. (*c*) Eluate from TLC zone containing reference sphingomyelin. (*d*) Eluate from TLC zone containing no sample, after having been washed by the method of Folch et al.

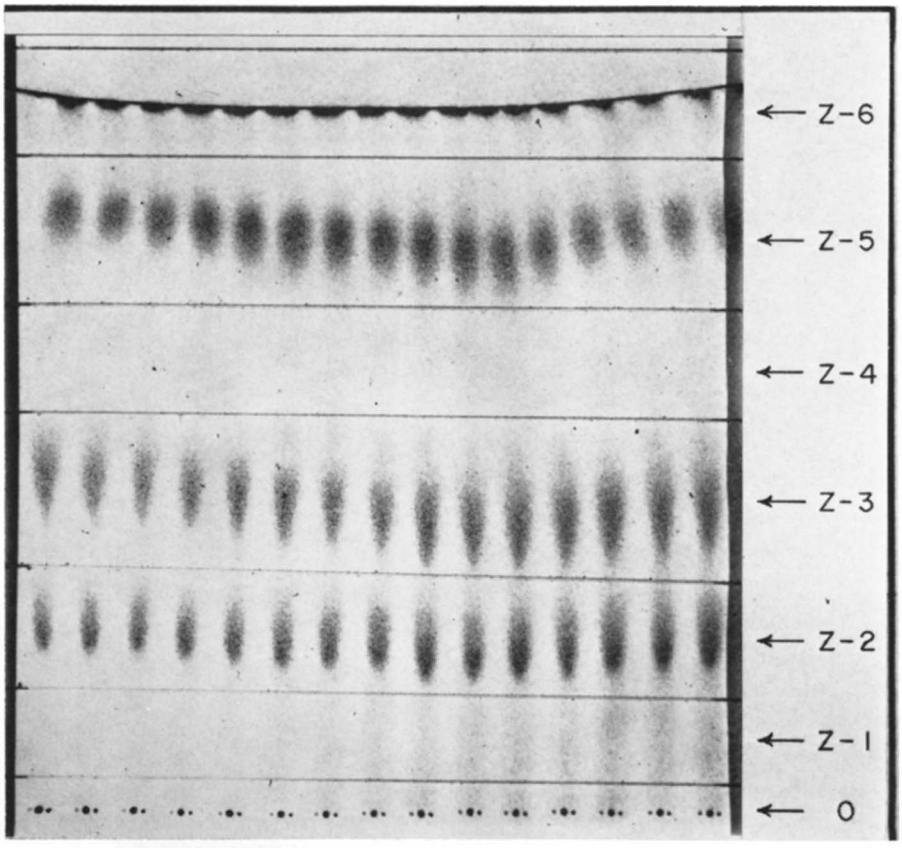

FIG. 2. A TLC chromatogram of milk phospholipids. Developing solvent: chloroform–methanol–acetic acid–water 100:50:16:8 (v/v). Detection: sprayed with iodine in methanol solution and after iodine had vaporized sprayed with ammonium molybdate–perchloric acid for permanent record. A phospholipid sample containing 131.9 μg of P was applied. Zone identification: O, origin; Z-1, lysophosphatidyl choline; Z-2, sphingomyelin; Z-3, phosphatidyl choline; Z-4, not identified; Z-5, phosphatidyl ethanolamine; Z-6, material at solvent front.

KEY WORDS thin-layer chromatogram · recovered phospholipids · purification · Folch wash · infrared spectra · milk

IN AN INVESTIGATION of methods for recovering phospholipids from milk (1, 2), thin-layer chromatography (TLC) and infrared spectral analysis were used to study the recovered components. Conventional KBr disks were prepared containing extracts of areas from thin-layer plates, and the disks were examined in a Perkin Elmer Model 137 Infrared Spectrophotometer. The spectra were compared with those of highly purified phospholipids reported by Smith and Freeman (3) among others.

Although all components extracted from plates rechromatographed as homogeneous units, the quality of spectra initially obtained was poor because of the presence of a contaminant (see Fig. 1) which was not removed by several recovery methods (4, 5). It is not known whether this contaminant originated from the Silica Gel G (not prewashed), the solvent system (chloroform–methanol–acetic acid–water), their interaction, or some other source. However, a simple expedient enabling satisfactory spectra to be obtained was found. This consisted in washing, by the method of Folch et al. (6), the extracts of plate areas which had been obtained by using a short column of silicic acid (7). In essence, the sample, dissolved in 12 ml of chloroform–methanol 2:1 (v/v), was washed with 3 ml of 0.04% $CaCl_2$ solu-

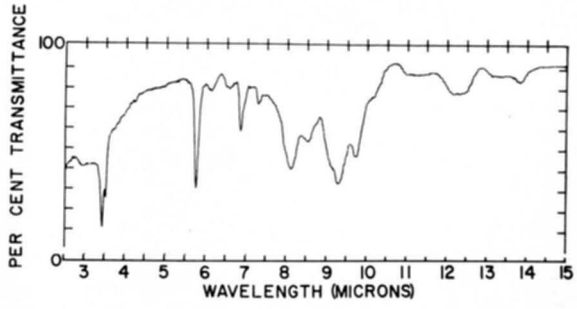

FIG. 3. Infrared spectrum of material eluted from zone 5, Fig. 2, showing it to be phosphatidyl ethanolamine. Eluate was washed by the method of Folch et al. (6) and then added to approximately 300 mg of KBr.

tion. The lower layer was evaporated in the presence of KBr and the residue was compressed into a disk.

The effect of the Folch wash on the infrared spectra is shown in Fig. 1. A TLC pattern on a basic plate (8) of phospholipids derived from milk is shown in Fig. 2. The infrared spectrum from Zone Z-5, identifying its component as phosphatidyl ethanolamine, is given in Fig. 3. Components of other zones were similarly characterized (spectra not shown) as indicated in the legend of Fig. 2.

Manuscript received September 2, 1964; accepted December 14, 1964.

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