Thus, by appropriate choice of conditions, one can use this technique to isolate multi-gram quantities of α-, γ-, and δ-tocopherols. It should be noted, however, that the small quantity of β-tocopherol which may be present in naturally occurring mixtures can not be separated from γ-tocopherol since these isomeric tocopheryl esters have similar rates of deacylation.

A brief study of secondary amines other than pyrrolidine revealed that pyrrolidine is uniquely suited for reaction under mild conditions. Diethylamine, neat or in refluxing CH_2Cl_2 gave no deacetylation. Morpholine did not react in CH_2Cl_2 at reflux, but deacetylation of 4 in the presence of 1 was accomplished in neat morpholine at room temperature in 8 hr.

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The Analysis of Phospholipids in Soy Lecithin by HPLC

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ABSTRACT

A high performance liquid chromatography (HPLC) method is described for the HPLC analysis of major phospholipids in soy lecithin. The method entails dissolving soy lecithin in chloroform prior to analysis. The HPLC determination uses a normal phase column and a mobile phase of acetonitrilemethanol-H_3PO_4 with detection at 205 nm. The data presented illustrates that the method is rapid, accurate and precise for the determination of phospholipid in soy lecithin.

INTRODUCTION

Soy lecithin is a commonly used emulsifier in the manufacture of confectionery and other food stuffs (1). The surfactant behavior is influenced by the properties of the phospholipids, since each phospholipid has a different acyl chain and polar head group. Current methodology for the analysis of phospholipids is restricted to thin layer chromatography (TLC) (2) or time consuming methods based on acetone insolubles (3).

In many instances, TLC solvents could be transferred directly to HPLC. In this case, the systems used for TLC contained $CHCl_3$, which eliminated the use of direct HPLC detection since the ultraviolet (UV) cutoff for LC grade $CHCl_3$ is much greater than 205 nm. Several authors (4,5) have reported the use of various derivatives for phospholipid determination. The direct detection of these compounds is also possible using the concept of end absorption (6). The mobile phase of McCluer and Jungawala (6) consisted of 65/21/14 $CH_3CN/CH_3OH/H_2O$ on a Porasil column. It permits direct detection of phosphatidyl choline but not other phospholipids.

This mobile phase has been previously used in our laboratory (8). The other mobile phase, suggested by Hax and VanKessel (9), permitted direct detection and achieved separation but required the use of a gradient. The work of Chen and Kou (10) illustrated the separation of various phospholipids with direct detection and an isocratic mobile phase. We chose to utilize this approach for the determination of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol and phosphatidyl serine in soy lecithin.

MATERIAL AND METHODS

The HPLC used consisted of an M6000A Solvent Delivery System (Waters Associates), a Model 710B WISP (Waters Associates), a Model 720 System Controller (Waters Associates), a Model 730 Data Module (Water Associates), and a Model 165 UV Detector at 205 nm (Beckman Instruments). The HPLC Column used was a 3.9 mm × 30 cm μPorasil (Waters Associates). The HPLC mobile phase was 780/10/9 (v/v/v) $CH_3CN/CH_3OH/85\%H_3PO_4$ flowing at 2.0 mL/min.

Standards of phosphatidyl choline. phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidyl inositol from soybeans were obtained from Sigma Chemical Company and dissolved in $CHCl_3$ to a final concentration of 0.1 μg/μl. Standards were kept refrigerated when not in use and prepared fresh weekly.

Samples were obtained from various manufacturers and dissolved in $CHCl_3$ prior to analysis to final concentration of 0.1 μg/μl.

RESULTS

Samples of soy lecithin containing varying amounts of the various phosphatides were analyzed, and typical compositions are shown in Table I.

Precision studies were conducted using both standard solutions and extracts of a soy lecithin. Data are summarized in Table II.

Lower limits of the various phosphatides varied from 100 ng/injection for phosphatidyl serine to 350 ng/injec-

TABLE I

Typical Phospholipid Composition of Various Soy Lecithins (% Composition)

Description	Phosphatidyl choline	Phosphatidyl ethanolamine	Phosphatidyl inositol	Phosphatidyl serine
Soy lecithin I	11.3	12.2	11.6	N.D.
Soy lecithin II	11.2	13.3	4.4	N.D.
Soy lecithin III	16.4	6.4	21.3	N.D.

N.D. = not detectable; n=2.

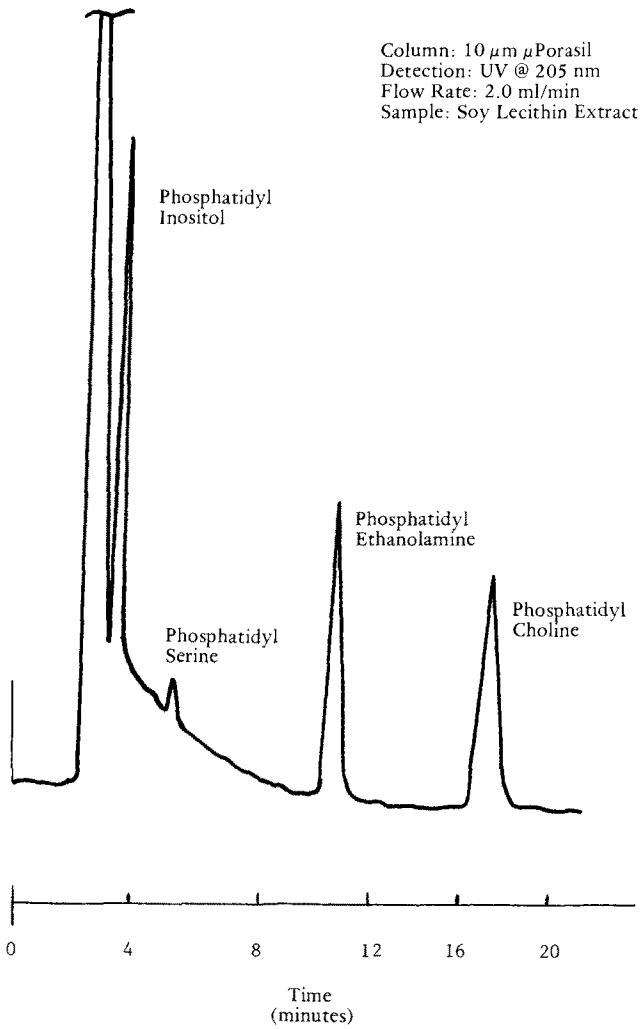

FIG. 1. Chromatogram of soy lecithin extract.

tion for phosphatidyl choline. All of the phosphatides exhibited excellent linearity with regression coefficients of 0.98-0.99 over a 100-fold range.

The separation of soy lecithin phospholipid can be seen in Fig. 1. To confirm peak identity in the sample, all peaks were scanned using the accessory on the UV Detector and compared with authentic standards.

TABLE II

Sample Precision Study

Phospholipid	Conc (μg)	% Cv
Phosphatidyl inositol	2	4.3
Phosphatidyl serine	2	3.9
Phosphatidyl ethanolamine	2	2.4
Phosphatidyl choline	2	3.8

The work presented in this study provides a rapid, precise HPLC method for monitoring the major phospholipids in soy lecithin. Work is continuing in our laboratory to apply a modification of this method to the major phospholipids in chocolate.

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