A Simple and Cost-Effective Gram-Scale Chromatographic Method for the Purification of Soybean Phospholipids

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ABSTRACT: A method was developed for the preparative fractionation of soybean lecithin to enable the study of the functional properties of pure soybean phospholipids. Hereby, a coarse and irregularly shaped silica gel was used as the stationary phase, whereas the mobile phase consisted of three mixtures of hexane, 2-propanol, and water with increasing polarity. These solvents were included in a step gradient, which was formed by an isocratic pump connected to a solvent switcher. With this system, two grams of soybean lecithin were fractionated. The purity was evaluated by analytical high-performance liquid chromatography, and the recovery was estimated from concentration determinations by flow injection analysis. From these results, it was concluded that 60 to 75% of the three major soybean phospholipids could be recovered with a purity of at least 93%. Only 1.5 L of solvents were needed for both the column equilibration and the elution of all soybean phospholipids. *JAOCS 72,* 1073–1075 (1995).

KEY WORDS: Mass detector, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, soybean lecithin.

Soybean lecithin is a commercial by-product of vegetable oil production. Due to its high phospholipid content, this material is widely used as a natural emulsifier, stabilizer, baking improver, and wetting agent (1,2).

Because the different phospholipid classes present have strongly deviating functional properties (3–5), the availability of a simple and cost-effective preparative soybean lecithin separation method is highly desirable. However, most methods published up to now are characterized by a low loading capacity and/or high solvent consumption (6–10). In the present study, a simple and cost-effective method has been elaborated that allows handling two grams of lecithin in a single run. In essence, it is based on a previously described semi-preparative method (11).

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MATERIALS AND METHODS

The solvents, equipment, and analytical high-performance liquid chromatography (HPLC) method used have been described previously (11). For preparative-scale purposes, 15–40-μm silica gel 60 for column chromatography (Merck, Darmstadt, Germany) was selected; this coarse powder was packed into a 250 × 20.0 mm preparative column (S.F.C.C., Neuilly-Plaisance, France). To avoid particulate contamination of the columns, a 0.2-μm Uptight prefilter (Upchurch Scientific Inc., Oak Harbor, WA) was inserted.

The flow rate was kept constant at 10 mL/min throughout the whole experiment. During the first two minutes, the sample, containing one gram of lecithin per 10 mL of solvent (hexane, 2-propanol, and water in a 55:44:2 volumetric ratio), was introduced *via* the pump. Subsequently, a 55:44:4.0 mobile-phase mixture was used from 2 to 45 min. After 15 min of elution with a 55:44:5.7 mixture, a 55:44:7.0 mixture was selected.

The elution was followed on-line with a ultraviolet absorbance detector at 214 nm and off-line with a flow injection analysis (FIA) system. Throughout the whole experiment, an FIA of 10–15 mL were collected.

RESULTS AND DISCUSSION

In our previously described semi-preparative method, 100 mg of soybean lecithin was fractionated on a 250 × 4.6 mm column with 150 mL of solvent. Hence, direct scaling-up of the method to a 250 × 20 mm column would necessitate at least 3 L of solvent. Therefore, a third, more polar solvent mixture, containing hexane, 2-propanol, and water in a 55:44:7.0 volumetric ratio, was introduced. Figure 1 shows that the fractionation was completed within 140 min, so that only 1.4 L of solvents were used.

From the elution profiles of the major soybean phospholipids, as represented in Figure 2, it becomes obvious that collected fractions are highly purified and cross contamination is negligible. In case of phosphatidylinositol (PI), the initial fractions are slightly contaminated. This is caused by the fact that the PI front coincides with the solvent front of the second solvent mixture of the solvent program, so that all more-

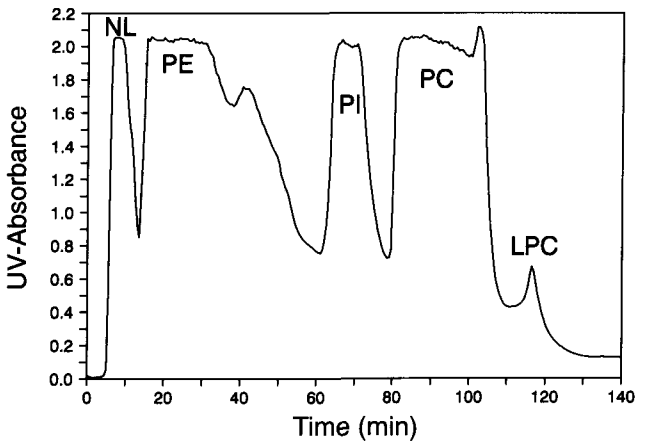

FIG. 1. Fractionation of 2 g soybean lecithin on the preparative column. The mobile phase, whose flow rate was kept constant at 10 mL/min, was a ternary mixture of hexane, 2-propanol, and water. After 45 min, the composition was changed from 55:44:4.0 to 55:44:5.7, and after 60 min, a 55:44:7.0 mixture was used. UV, ultraviolet; PE, phosphatidylethanolamine; NL, neutral lipids; PI, phosphatidylinositol; PC, phosphatidylcholine; LPC, lysophosphatidylcholine.

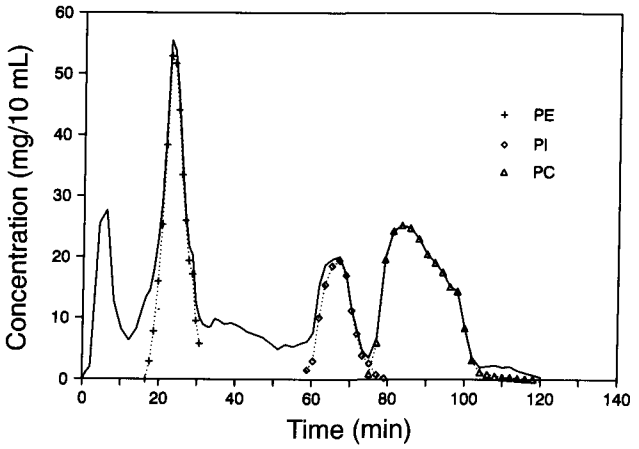

FIG. 2. Off-line chromatogram obtained by flow injection analysis of the fractions collected during the separation of 2 g soybean lecithin on a 250 × 20.0 mm column. Besides the overall mass concentration, the concentrations of the major soybean phospholipids have been included. See Figure 1 for abbreviations.

polar components residing on the stationary phase are then eluted. If a higher purity is required, the polarity of the second solvent mixture should be slightly reduced, so that the solvent front and the PI front become resolved.

By keeping track of the volume of the fractions, it was calculated that 1541 mg of the lecithin was recovered. Because 20 mL of a solution containing 100 mg/mL was applied, about 25% was lost. This may be explained in part by the fact that about 15% of the soybean lecithin did not dissolve in the solvent mixture used. Besides, a precipitate was observed in some fractions; FIA only measures the amount dissolved.

Under our experimental conditions, 86% of phosphatidylethanolamine, 69% of PI, and 78% of phosphatidylcholine were recovered. This means that about 30% of the PI present in the soybean lecithin got lost. This was mainly due to its steady elution together with phosphatidic acid and phosphatidylserine. Figure 3 shows that recovery of the major soybean phospholipids is determined to a large extent by the purity requirements. Thus, only 43% of PI was recovered with an overall purity of 96.4%, whereas 66% was recovered with an overall purity of 89.1%. For the sake of completeness, it should be mentioned that the purity of the PI fractions can be improved by delaying the 55:44:4.0 to 55:44:5.7 switch. However, due to the steady elution of PI with the former solvent mixture, the recovery is badly affected, so that the overall efficiency can hardly be changed. Several subfractions can be collected throughout the elution of the PI peak. After analytical HPLC to detect their compositions, these multiple fractions can be combined to optimize the recovery or the purity.

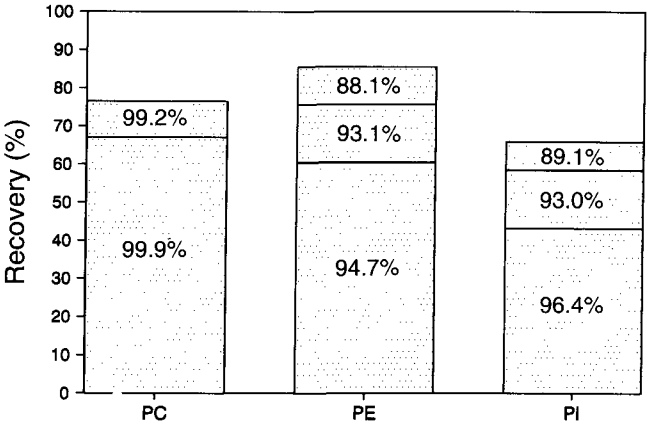

FIG. 3. Graphical representation of the relationship between purity and recovery of the main phospholipids originating from the separation of 2 g soybean lecithin on a 250 × 20.0 mm column. See Figure 1 for abbreviations.

From these results, it follows that the proposed method enables the purification of two grams of soybean lecithin. Hereby, only 1.5 L of solvent is used for column equilibration and separation of the phospholipids, which compares favorably to the method proposed by Hanras and Perrin (9); they used 7.2 L of solvents to fractionate 2.1 g of phospholipids. Besides, the latter method necessitates gradient elution, so that expensive equipment is needed. On the other hand, in the method proposed, the separation is accomplished by a step gradient, so that a simple isocratic pump, in connection with a solvent switcher, may be used. Besides, a low-cost coarse and irregularly-shaped silica gel has been used as the stationary phase.

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