High-Performance Liquid Chromatography Analysis of Peanut Phospholipids. I. Injection System for Simultaneous Concentration and Separation of Phospholipids

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ABSTRACT: This paper discusses the details of a high-performance liquid chromatography method for the simultaneous concentration and separation of phospholipids or other trace compounds by direct oil injection using two different solvent systems. The system equilibrates and concentrates phospholipids on a silica column using hexane. At the same time, an analytical column is equilibrating and separating phospholipids using two binary solvent mixtures. This system eliminates a preconcentration step previously accomplished by solid-phase extraction, open-column chromatography, and other previously used methodology. Other advantages include: a 40% reduction in analysis time, elimination of a second transfer of labile compounds, decreased solvent use, and a simpler array of solvents to separate phospholipids. The method described has broader applications, such as trace organic compounds in water supplies, and trace metals with appropriate modifications for the particular analysis.

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KEY WORDS: HPLC, injection system, peanuts, phospholipids, trace compounds.

Peanuts are widely marketed in the form of snack foods. Phospholipids, even though low in concentration, contribute to the smoothness, texture, and mouthfeel of many of these products as well as contributing to the stability of the product through their inherent antioxidant properties (1,2). Phospholipids in peanuts are the major constituents of cell membranes, and they have a high degree of unsaturation. When peanuts are damaged, cells become leaky and release enzymes, amino acids, carbohydrates, metals, and cations (3–6). These metabolic changes result in production of off-flavors in the raw product (4). In many cases the crude oil is unusable for edible purposes and/or otherwise difficult to refine.

Analysis of the phospholipid fractions can serve as a good indicator of peanut quality as affected by physical damage. Since the concentration of phospholipids in peanuts is only about 1%, concentration prior to analysis by high-performance liquid chromatography (HPLC) is necessary to give useful profiles and to obtain enough of the individual compounds for further analysis (7,8). This paper describes an HPLC method for simultaneous concentration and analysis of phospholipids. This method greatly reduces analysis time, solvent use, and the complexity of solvent arrays normally required. The method also can be applied to many other types of analyses involving other trace compounds by the adaptation of appropriate solvent systems.

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EXPERIMENTAL PROCEDURES

Materials. All the solvents used in the extraction of lipids from peanut seed and for HPLC analysis of phospholipids were obtained from Fisher Scientific (Fair Lawn, NJ). Phospholipid standards were obtained from Sigma Chemical Company (St. Louis, MO).

Lipid extraction. Total lipids were extracted from peanut seed with chloroform/methanol (2:1, vol/vol) in an Omni blender (Sorvall-Omni, Norwalk, CT) for 1 min. The blended material was filtered, and the resultant cake resuspended in chloroform and blended again. Again the solution was filtered and water saturated with NaCl was added to remove nonlipid components. The filtrate was washed three times prior to removing the solvent by flash evaporation from the lipid material. The crude lipid fraction was used for HPLC analysis.

Description of the analysis system. An HPLC injection system was designed for simultaneous concentration and analysis of phospholipids by direct oil injection using two different solvent systems (J.A. Singleton and L.F. Stikeleather, unpublished U.S. patent application filed in 1994 by USDA). This two-valve system (Fig. 1) allows one pump (pump #1) to equilibrate and concentrate phospholipids onto a silica column using hexane regulated by a second pump (#2). While the analytical column is equilibrating, phospholipids are separated using two binary solvent mixtures of 2-propanol/hexane (4:3) and 2-propanol/hexane/water (8:6:1.5) ratio. The oil is injected onto the concentrator column which adsorbs the phospholipids while removing triacylglycerol (98% of the oil). The valves are automatically switched by digital controls

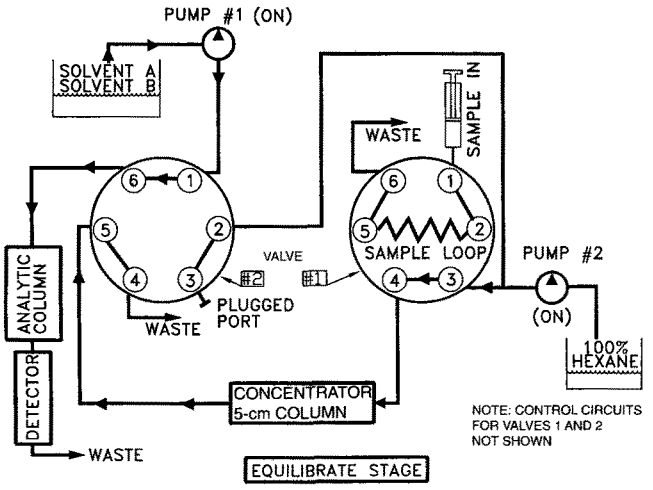

FIG. 1. Valving configuration and solvent flow diagram for equilibration stage.

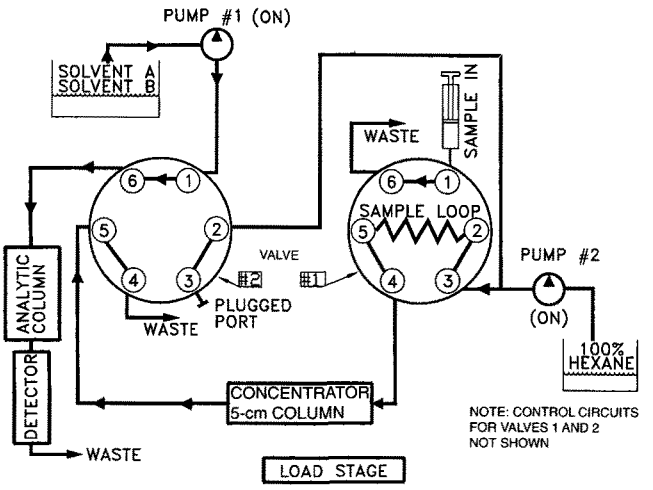

FIG. 2. Valving configuration and solvent flow diagram for the loading stage.

to inject the concentrated phospholipids onto the column using the binary solvent from pump #1.

HPLC injection system design. A prototype system was developed using two six-port valves (Model AH-60; VALCO Inst. Co., Houston, TX) mounted on a Varian 5000 (Palo Alto, CA) ternary HPLC interconnected with an injection loop, an external single piston pump, a Humphrey pneumatic switch (valve), concentrator column, and semiprep column. Figure 1 shows the valve positions and solvent flow path in the equilibration stage. Pump #2 was connected to port #3 on valve #1. The concentrator column (50 mm × 4 mm 40 μ silica) is connected to port #4 on valve #1 and to port #5 on valve #2 which vents through port #4 to a waste flask. Pump #1 was connected to port #1 on valve #2 and the analytic and/or semiprep column (100 mm × 8 mm 6 μ silica; Waters Chromatography Division, Millipore, Marlborough, MA) was connected to port #6 and to the detector. The solvent used to equilibrate the analysis column was 2-propanol/hexane (4:3, vol/vol). The solvent used to equilibrate the concentrator column was 100% hexane. In this configuration, both columns were equilibrated simultaneously using two different solvent systems. During the equilibration stage, 1 mL of peanut oil was injected into the sample loop (valve #1).

Figure 2 shows the valve configuration and the solvent flow path in the load stage. Valve #1 was rotated by control output from the microprocessor, and the oil in the sample loop was injected onto the concentrator column. The phospholipids were adsorbed on the concentrator column, and the bulk triglyceride fraction was eluted to waste.

In the analysis stage (Fig. 3) pump #2 is turned off temporarily, and an air switch (not shown) activates valve #2 which directs solvent (2-propanol/hexane, 4:3, vol/vol) from pump #1 through the concentrator column. The adsorbed phospholipids are eluted onto the analysis column and separated. When the phospholipids have been eluted from the concentrator column (2 min), both valves are switched again, placing the configuration back in the load position (Fig. 2).

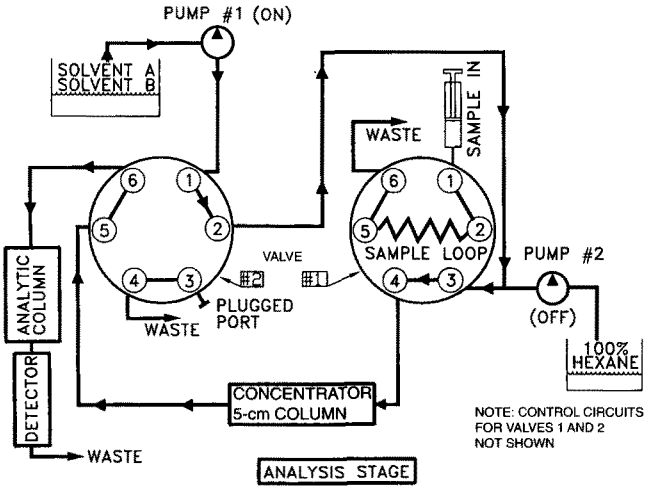

FIG. 3. Valving configuration and solvent flow diagram for the analysis stage.

The concentrator column can be reloaded for the next analysis while the phospholipids are being analyzed.

RESULTS AND DISCUSSION

HPLC of peanut phospholipids. The major phospholipids of peanut oil are phosphatidic acid, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylcholine. Figure 4 shows a typical HPLC chromatograph for undamaged peanuts after postharvest treatment. Samples were injected automatically onto the HPLC column using a 1-mL sample loop. Individual phospholipids were identified by retention time of known standards that had been previously characterized by mass spectrometry (J.A. Singleton, unpublished data).

Commercial potential and advantages. The hardware and appropriate software could be incorporated into a commercially available HPLC unit by adding another valve, appro-

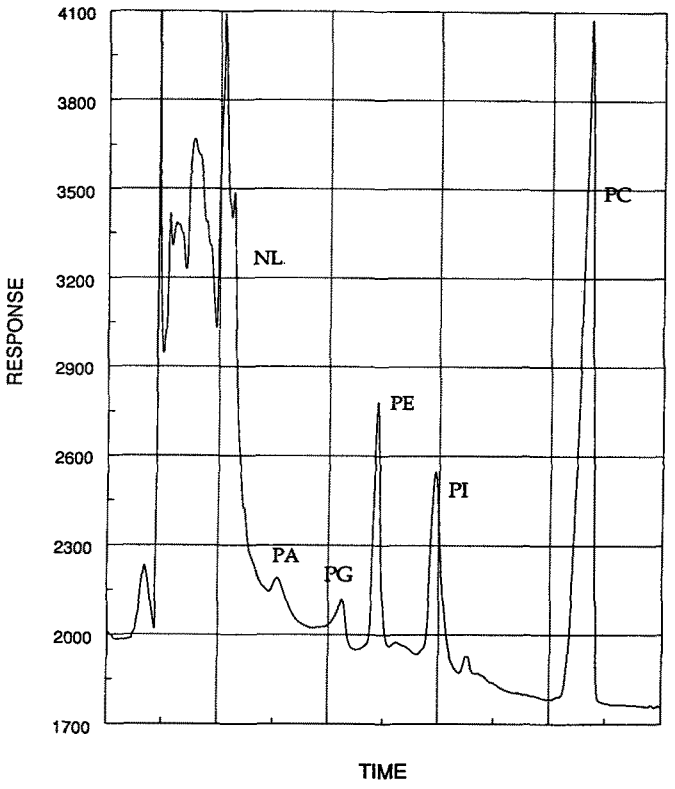

FIG. 4. Phospholipid profile of undamaged peanuts, NL, neutral lipids; PA, phosphatidic acid; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PC, phosphatidylcholine.

priate plumbing, the software necessary for controlling multiple external events for valve switching, and circuitry for controlling an external pump or additional small low-pressure pump in the same HPLC unit. Since the methodology and apparatus can be adapted to prep chromatography, it is especially suitable for the concentration of phospholipids from plant materials and other sources for commercial purposes.

This system eliminates a preconcentration step previously accomplished by solid-phase extraction, open-column chromatography, and other previously use methodology. Other advantages include analysis time reduction (approximately 40%), elimination of a second transfer of labile compounds, decreased solvent use, and the use of a less complex array of solvents to separate phospholipids from other lipid components. The described method has broader applications as it can be modified for analysis of trace organic compounds and trace metals in water by using appropriate columns, solvents, and parameters for the particular analysis.

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