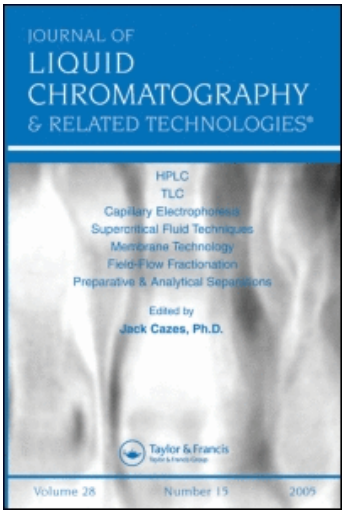

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A NEW BONDED-PHASE FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS

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ABSTRACT

A silica bonded-phase with carboxylic acid functionality and a side chain of three carbon atoms has been synthesized for HPLC analysis. Mixtures of nucleic acid bases, PTH-amino acids, phospholipids and ethanolic extract of dry ginger powder were analyzed to demonstrate its performance. Nucleic acid bases and PTH-amino acids were separated by cation-exchange mechanism whereas phospholipids experienced combination of cation-exchange and normal-phase resolution. The resolution of gingerols in the dry ginger powder was achieved by reversed-phase mechanism.

INTRODUCTION

Syntheses of strong cation-exchange materials have been reported by Wheals (11) and Jandera et al. (10).

These phases utilize silica which have ion-exchanging groups (aromatic or alkyl sulfonic acids) chemically bonded to their surface. However, literature does not record any synthesis of similar type of weak cation-exchanger. The present investigation, therefore, is directed to synthesize and demonstrate the performance of a cation-exchange material having carboxylic acid functionalities and side chains of three carbon atoms.

EXPERIMENTAL

Materials

Synthetic phosphatidylglycerol (PC), soybean phosphatidylethanolamine (PE), phosphatidic acid (PA), bovine brain sphingomyelin (SPH) were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). HPLC grade solvents, ammonium formate and ammonium dihydrogen phosphate were purchased from E. Merck Chemicals (Cherryhill, NJ). Uracil, thymine, cytosine and adenine were purchased from Sigma Chemical Company (St. Louis, MO). PTH-cysteic acid, PTH-aspartic acid, PTH-serine, PTH-asparagine and PTH-methionine sulfone were purchased from Pierce Chemical Company (Rockford, IL). Hexahydro-curcumin was isolated from ginger root extract on silica gel according to the condition described by Tadakazu et al. (8). Ginger root was purchased from a local market. Dry ginger powder was obtained from Spice Island Inc. (San Francisco, CA). Gingerol-6, gingerol-8 and gingerol-10 were isolated from ginger root extract on Whatman PartiSphere-5, C-18 column (3). o-Nitroaniline and methyl benzoate were purchased from Fluka Chemical Company (Hauppayge, NY).

Columns

PartiSphere-5 silica and carboxypropyldimethyl-chlorosilane from Whatman Inc. (Clifton, NJ) were condensed. The weak cation-exchange material thus

obtained was packed by slurrying in acetone at a pressure of 7,000 psi. The cation-exchange capacity of the end product was determined by titrating with 0.01 N NaOH solution. PartiSphere-5, C-18 cartridge columns were obtained from Whatman Inc. (Clifton, NJ).

Sample Preparation

Solutions of specific sample for phospholipid mixture were prepared as follows: 20 mg SPH in 2 ml of chloroform, 20 mg of PG in 0.5 ml of chloroform, 13 mg of PE in 0.5 ml of chloroform and 10 mg of PA in 1 ml of chloroform.

The extract of ginger powder was prepared according to conditions described by Tonnesen et al. (9). One or two grams of the dry powder or crushed ginger root was dispersed in 3 to 5 ml of ethanol, stirred for 15 minutes and then centrifuged.

Solutions of PTH-amino acids were prepared by dissolving 5 mg of individual amino acid (PTH-cysteic acid, PTH-aspartic acid PTH-serine, PTH-asparagine and PTH-methionine) in 5 ml of water.

Solutions of nucleic acid bases were prepared as follows: 15 mg of uracil in 5 ml of water, 20 mg of thymine in 5 ml of water, 50 mg of cytosine in 50 mg of water and 50 mg of adenine in 50 mg of water.

HPLC Analysis

HPLC was performed using a variable wavelength UV detector, Spectroflow monitor SF-770 (Kratos Analytical, Ramsey, NJ), RI detector, Differential Refractometer R 401 (Waters Associates, Inc., Milford, MA), a programmable solvent delivery system, Series 3B (Perkin-Elmer Corp., Norwalk, CT), a manual injection valve with 50 ul loop (Valco Instruments Co., Houston, TX) and a chart recorder (Laboratory Data Control, Riviera Beach, FL).

Water containing 0.01 M $NH_4H_2PO_4$ (pH 4.5) was used as a mobile phase to analyze nucleic acid bases. PTH-amino acids were analyzed by using water containing 0.001 M $NH_4H_2PO_4$. A mixture of acetonitrile, methanol, water and ethanol (400:100:34:2.5, V/V) containing 0.01 M ammonium formate was used as a mobile phase to analyze phospholipids.

A linear gradient starting from water (100%) to methanol (100%) was adopted for reversed-phase separations of gingerols from ginger powder of ginger root extract and methyl benzoate was used as an internal standard.

RESULTS AND DISCUSSION

A silica bonded phase with carboxylic acid functionality and a side chain of three atoms has been synthesized for HPLC analysis. This bonded phase has the cation-exchange capacity of 0.09 m eq/g. Mixtures containing various kinds of organic components were resolved to demonstrate its performance. Cytosine and uracil were used as the test components to test the packed columns (Fig. 1). The performance parameters of the column are shown in Table 1. The chromatographic properties of the cation exchanger were good. Average column efficiency of 85,455 plates/meter was obtained. The asymmetric ratios of uracil and cytosine were 1.34 and 1.54 respectively. A mobile phase containing 0.01 M $NH_4H_2PO_4$ resolved a mixture of nucleic acid bases by cation-exchange mechanism (Fig. 2). Similarly a mixture of PTH-amino acids such as PTH-cysteic acid, PTH-aspartic acid, PTH-serine, PTH-asparagine and PTH-methionine sulfone was resolved using water containing 0.001M $NH_4H_2PO_4$ as a mobile phase (Fig. 3). A mixture of phospholipids such as phosphatidylglycerool, phosphatidylethanolamine, phosphatidic acid and

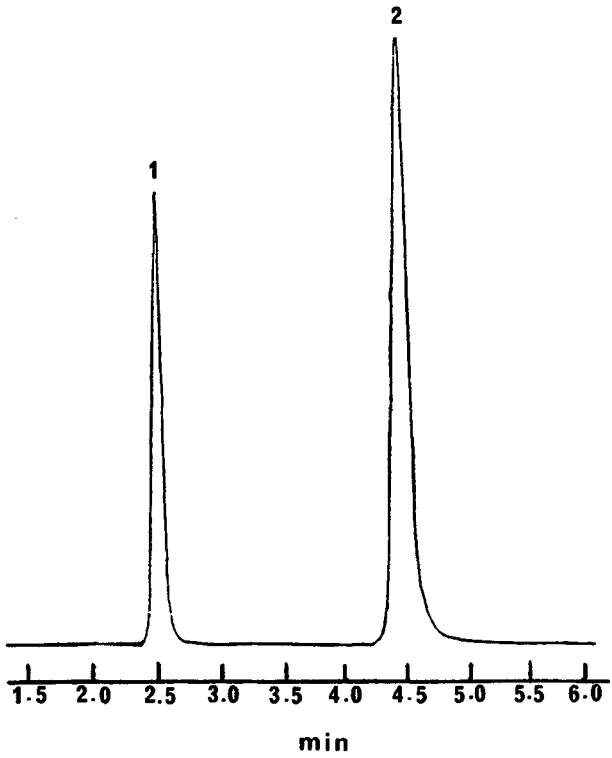

Figure 1. 1. Urasil, 2. cytosine.
Mobile phase: 0.01M $NH_4H_2PO_4$ (pH 4.5); Flow rate: 0.7 ml/min; λ_{max}: 254 nm; Sample volume injected: 2 µl; Sample description: uracil (2 ml) and cytosine (5 ml); Column: WCX, 11 cm x 4.6 mm (I.D.)

TABLE 1. Performance Parameters of the WCX Column

Solute	Efficiency plate/meter	Asymmetric Ratio
urasil	91,455	1.34
cytosine	79,455	1.54
Average Plates / Meter : 85,455		

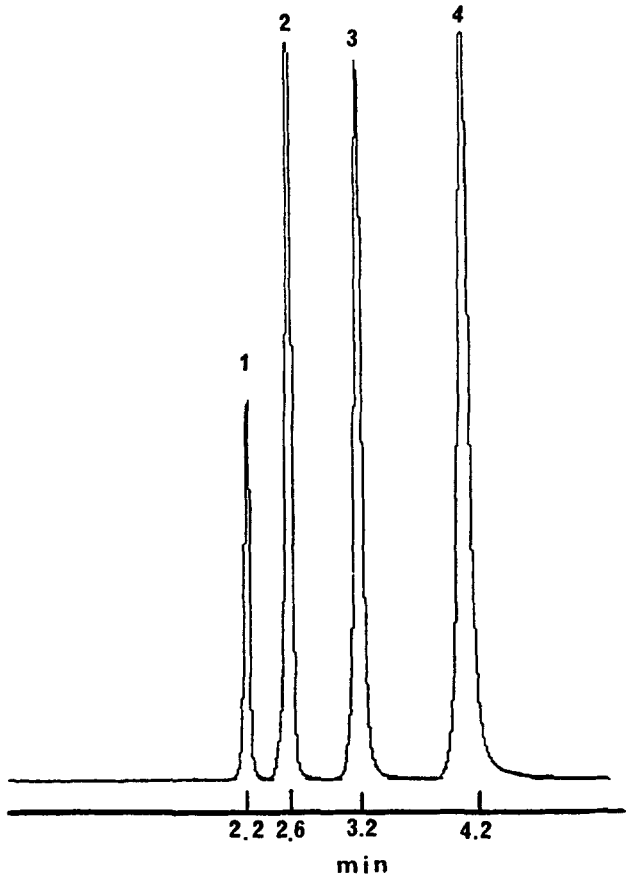

Figure 2. 1. Uracil, 2. thymine, 3. cytosine, 4. adenine.
Mobile phase: 0.01M $NH_4H_2PO_4$ (pH 4.5); Flow rate: 0.7 ml/min; λ_{max}: 254 nm; Sample volume injected: 2 µl; Sample description: uracil (2 ml), thymine (10 ml), cytosine (5 ml) and adenine (5 ml); Column: WCX, 11 cm x 4.6 mm (I.D.).

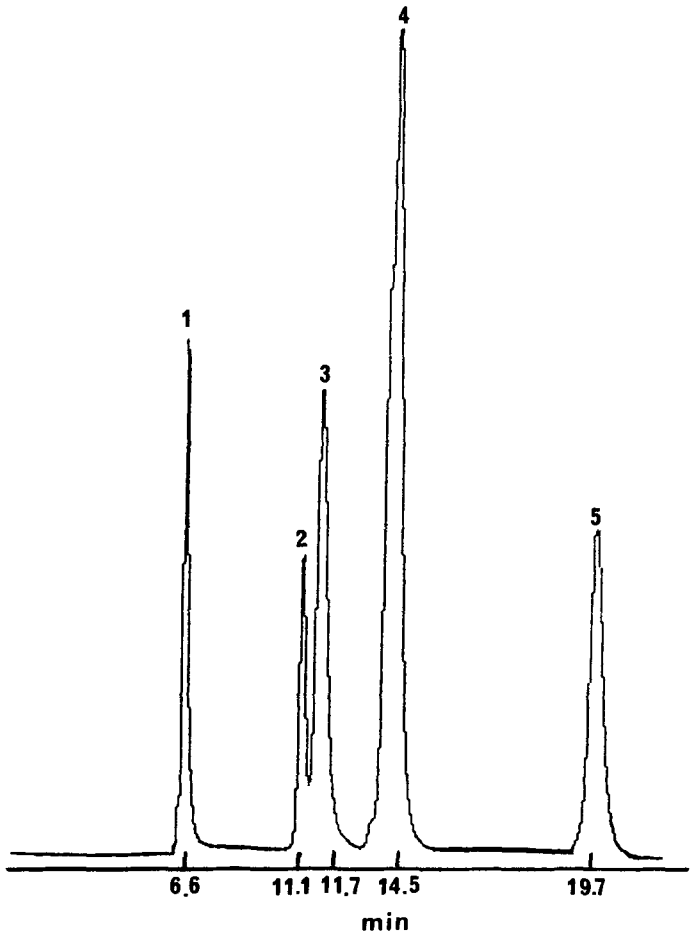

Figure 3. 1. PTH-cysteic acid, 2. PTH-aspartic acid, 3. PTH-serine, 4. PTH-asparagine, 5. PTH-methionine sulfone.
Mobile phase: 0.001M $NH_4H_2PO_4$ (pH 3.5); Flow rate: 0.2 ml/min; Detector: RI; Sample volume injected: 3.9 µl; Sample description: PTH-cysteic acid (1.5 ml), PTH-aspartic acid (0.3 ml), PTH-serine (0.5 ml), PTH-asparagine (1.3 ml), PTH-methionine sulfone (0.3 ml); Column: WCX, 11 cm x 4.6 mm (I.D.) (three columns in series).

sphingomyelin was resolved through mixed mechanism (Fig. 4). The amino phospholipids like phosphatidylethanolamine and sphingomyelin separated through cation-exchange mechanism while phosphatic acid and phosphatidylglycerol experienced normal phase resolution. A mixture of acetonitrile, methanol, water and ethanol containing 0.01M ammonium formate was used as a mobile phase. Use of buffer concentration more than 0.09M in the mobile phase resulted in loss of resolution. Same was true for the resolution of nucleic acid bases. A loss of resolution was observed in the case of PTH-amino acids when a buffer concentration more than 0.001M $NH_4H_2PO_4$ was used. The three carbon atom chain of the weak cation exchanger (WCX) phase provided a negligible hydrophobic interaction and has been found to be useful in preventing multiple band or zone formation of a single phospholipid species (2). This type of problem has been encountered when reversed-phase columns such as C-18 of C-8 were used to resolve phospholipids (6).

Reversed-phase analysis of ethanolic extract of dry ginger powder is exhibited in the Fig. 5. The pungency of ginger products is due to the presence of gingerol compounds (6-, 8- and 10-gingerols) (4). Thin layer chromatography and gas chromatography has been frequently used to analyze gingerols (5). However, due to the poor resolution of the former and incomplete retro-aldol reactions during the analysis of the latter, high performance liquid chromatography has been preferred (7). Baranowski et al. (1) has found reversed-phase C-18 columns to be suitable for resolving gingerols with phenolic groups in their skeletons. The order of resolution in the present separation (Fig. 5) has been observed to be the same as reported earlier by Chen el al. (3) on C-18 reversed-phase column.

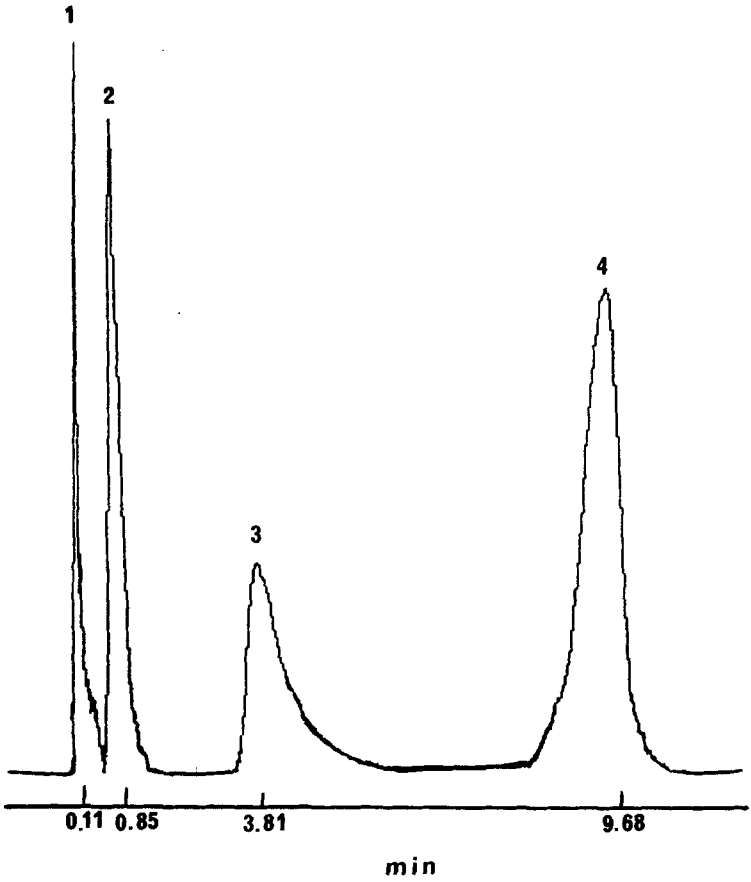

Figure 4. 1. PG, 2. PE, 3. PA, 4. SPH. Mobile phase: acetonitrile, methanol, water and ethanol (400:100:34:2.5, V/V) containing 0.01 M ammonium formate; Flow rate: 3 ml/min; λ_{max}: 203 nm; Sample volume injected: 20 ul; Sample description: PG (10 µl), PE (25 ul), PA (300 ul) and SPH (500 µl); Column: WCX, 11 cm x 4.6 mm (I.D.).

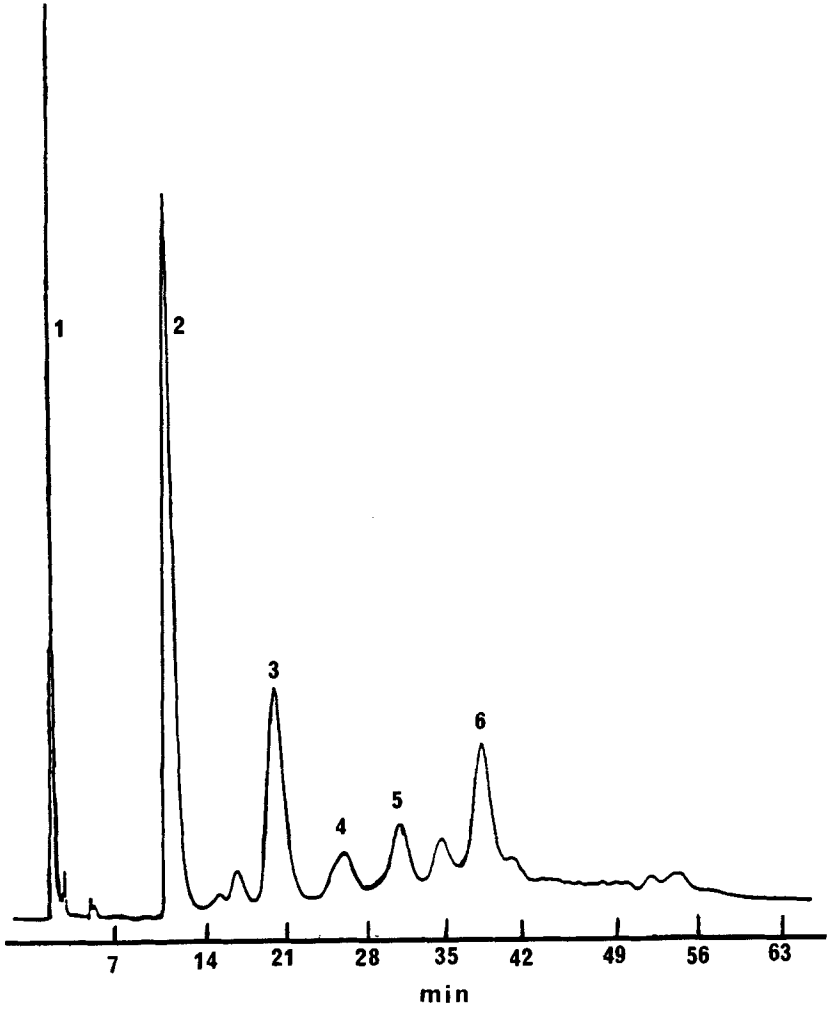

Figure 5. 1. Unknown (23%), 2. methyl benzoate (internal standard), 3. gingerol-6 (6.24%), 4. gingerol-8 (8.8%), 5. gingerol-10 (10.5%), 6. hexahydrocurcumin (17%). Mobile phase: a linear gradient from water (100%) to methanol (100%). The analysis time was 50 Minutes, the first 40 minutes in gradient elution. Flow rate: 1 ml/min; λ_{max}: 282 nm; Sample volume injected: 3 µl; Sample description: 2 g of the dry ginger powder was extracted with 5 ml of ethanol. Column: WCX, 11 cm x 4.6 mm (I.D.) (three columns in series).

ACKNOWLEDGEMENT

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