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Design and operation of a modified silica gel column chromatography

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Abstract

Liquid–solid chromatography (LSC) is the oldest of the various liquid chromatography methods. Despite the fact that high-performance liquid chromatography (HPLC) operation leads to better separation and analysis, classical column chromatography and thin-layer chromatography (TLC) are still widely practiced because of their convenience. In this study, a modified silica gel column chromatography was designed with the objective of reducing the amount of solvent required to achieve the same degree of separation as the classical silica gel column chromatography. The separation of squalene and fatty acid steryl esters (FASEs) from non-polar lipid fraction (NPLF) of soybean oil deodorizer distillate (SODD) was employed as a model system to test the effectiveness of this new design. Modified silica gel column chromatography process is feasible from economic point of view compare to classical silica gel column chromatography because it significantly reduces the amount of solvent and time required to achieve the same degree of separation. By employing modified silica gel column chromatography to obtain the squalene-rich fraction, the mobile phase volume and elution time required as fractions of those needed in classical silica gel column chromatography are 1/73 and 1/18, respectively. To obtain the FASEs-rich fraction, the corresponding mobile phase volume and elution time are 1/221 and 1/23, respectively of those needed in classical silica gel column chromatography.
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Keywords: Fatty acid steryl esters; Modified silica gel column chromatography; Soybean oil deodorizer distillate; Squalene

1. Introduction

Liquid–solid chromatography (LSC) or adsorption is the oldest of the various liquid chromatography methods. Despite the fact that high-performance liquid chromatography (HPLC) operation leads to better separation and analysis, classical column chromatography and thin-layer chromatography are still widely practiced because of their convenience. The successful use of liquid chromatography for a given problem requires the right combination of operating conditions: the type of column packing and mobile phase, the length and diameter of the column, mobile phase flow rate, elution temperature, and sample size (Snyder and Kirkland, 1979). Temperature is normally not used as separation variable in LSC. Most applications are carried out at ambient temperature.

A classical silica gel column chromatography was used to fractionate hydrocarbons from the unsaponifiable matter of both virgin and refined olive oil, using hexane as the eluent (Lanzón *et al.*, 1994). Also, a classical silica gel column chromatography was used to fractionate hydrocarbons and fatty acid steryl esters (FASEs) from the non-polar lipid fraction (NPLF) obtained from crude rice bran oil (Gunawan *et al.*, 2006) and soybean oil deodorizer distillate (SODD) (Gunawan *et al.*, 2008). When the NPLF of SODD was introduced into a classical silica gel column chromatography to isolate squalene, squalene (95.90% purity and 93.09% recovery) was obtained in the 2nd fraction after eluting the column with 10.96 L hexane. The continued elution of column with 33.13 L hexane yielded a 3rd fraction rich in FASEs (84.04% purity and 63.17% recovery). In another study, the separation of squalene from the unsaponifiable matter of olive oil deodorizer distillate was achieved by thin-layer chromatography (TLC), using mixture of solvent as mobile phase (Bondioli *et al.*, 1993).

Classical column chromatography and TLC are laborious, time consuming (Moreda *et al.*, 2001), and requiring the use of large amount of organic solvents (Lacaze *et al.*, 2007). A disadvantage of TLC compared to classical column chromatography is the limited possibility of understanding quantitative analysis since exhaustive recovery of the separate spot is not sufficiently precise (Moreda *et al.*, 2001). The advantages over classical column chromatography are that the procedure is more rapid, and the possibility of using specific reagents to immediately reveal the nature of the separated compound. Solid-phase extraction (SPE)

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Nomenclature

AOCS	American oil chemists' society
EI	electron impact
FASEs	fatty acid steryl esters
FFAs	free fatty acids
GCMS	gas chromatography mass spectrometry
h	hour
HPLC	high-performance liquid chromatography
HTGC	high-temperature gas chromatography
k'	equilibrium distribution factor
LSC	liquid–solid chromatography
n	total number of experiment
NIST	national institute of standards and technology
NPLF	non-polar lipid fraction
p	probability
S	standard deviation of the measures
SODD	soybean oil deodorizer distillate
SPE	solid-phase extraction
TLC	thin-layer chromatography
x	value of individual experiment
$\bar{x}$	mean value of three independent experiments

Greek symbol

α	significance level

has been developed to isolate squalene from virgin olive oil without any chemical treatment. Rapidity, reliability, use of minimal amounts of solvent and automation are the main advantages of SPE in comparison to TLC and classical column chromatography (Giacometti *et al.*, 2002). All the methods mentioned above are limited by small sample loading and using mixture of organic solvent in order to obtain good separations.

In this study, a new silica gel column chromatography was designed with the objective of reducing the amount of solvent required to achieve the same degree of separation as the classical silica gel column chromatography. The isolation of squalene and FASEs from the NPLF of SODD was employed as a model system to test the effectiveness of this new design. The effects of parameters, such as NPLF to silica gel mass ratio, flow rate and composition of mobile phase, and elution temperature, on the separation were systematically investigated.

2. Materials and methods

2.1. Chemicals and materials

Standard nonacosane, farnesene, cholesta-3,5-diene, squalene, fatty acids, α-, δ-, γ-tocopherol, monooleylglycerol, diolein, triolein, and tripalmitin were obtained from Sigma Chemicals Company (St. Louis, MO). Standard β-sitosterol (practical grade) was obtained from MP Biomedicals, LLC (Aurora, OH). All solvents and reagents were either of HPLC grade or analytical reagent grade and were obtained from commercial sources. SODD was donated by TTET Union Corporation (Tainan, Taiwan). TLC aluminum plates (20 cm × 20 cm × 250 μm) were purchased from Merck (Darmstadt, Germany). Silica Gel (70–230 mesh) was obtained from Silicycle (Quebec, Canada). Characteristics of the gel according to the manufacturer were: particle size: 60–200 μm; pore size: 60 Å; pH: 7; water content: 6%; and specific surface area: 500 m^2/g.

2.2. Extraction of NPLF from SODD

Solid suspension was removed from SODD at 40 °C by using a 7 μm Advantec filter paper (Toyo Roshi Kaisha Ltd., Tokyo, Japan). SODD was applied to modified soxhlet extraction, under the following operation conditions: solvent = *n*-hexane, SODD to silica gel mass ratio = 1:3 (w/w), extraction temperature = −6 °C, and extraction time = 11 h as described by Gunawan *et al.* (2008). The resulting hexane extractive, named NPLF, was used for this study.

2.3. Modified silica gel column chromatography

A modified version of the classical silica gel column chromatography (300 mm × 4 mm i.d. glass tube) equipped with a jacket, a valve to control the flow rate of eluent and a condenser system was employed in this study. Ethyl alcohol was used as the refrigerant. This was circulated in the jacket to control the column (packing region) temperature. A schematic drawing of the modified silica gel column chromatography is shown in Fig. 1. The siphon arm for operation in soxhlet extraction mode was closed by three-way valve. Silica gel (18–60 g) was kept in a furnace at 150 °C for 1 h to remove its water content. Next, a slurry of silica gel in hexane was poured into the column that was previously half-filled with hexane. The exit of the column was plugged with 1 g cotton to retain the silica gel and a thermo couple was inserted above the cotton. The hexane was allowed to drain slightly during packing. The top surface of the silica gel was covered by 1 g cotton, and three thermocouples were set inside the elution chamber at different locations. The hexane level was lowered until at controlled level (50 and 100 mL above the cotton at the top). NPLF (3 g) was put into the column at room temperature (23 ± 1 °C). The column was eluted with mobile phase (50 mL hexane), which was put into a 500 mL round-bottom flask at the start of the run and was heated. The mobile phase vapor traveled up the distillation arm, and flooded into the column housing the silica gel and the NPLF. The condenser ensured that any mobile phase vapor condenses and drips back down into the column. The column was slowly eluted with the mobile phase at controlled flow rate and temperature. After predetermined time, the flask that contained the desired extract was removed and replaced immediately by another flask that contained 50 mL fresh mobile phase so that the total amount of mobile phase remained the same as in the beginning of the run.

After removing the mobile phase, each fraction was analyzed by TLC and high-temperature gas chromatography (HTGC). The flow rate of mobile phase vapor depends on the heat input from the heater and was predetermined (data not shown). Fig. 2 shows the flow chart of separation of squalene and FASEs from the NPLF of SODD. The 1st fraction, which

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