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Automated post-collection concentration for purified preparative fractions via solid phase extraction

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Abstract

Recent advancements in preparative HPLC have improved and streamlined compound purification. However, fraction evaporation remains a bottleneck within the process. An alternative to fraction evaporation is to remove the water and reduce the overall volume of the collection by trapping the fraction onto a solid phase extraction (SPE) cartridge. This method (as opposed to analytical applications involving SPE) works by collecting and then diluting the fraction(s), passing the fraction(s) through a SPE, drying the SPE with nitrogen and ultimately eluting the concentrated fraction(s) in a small amount of 100% organic solvent. An appreciable breakthrough is not observed using this method. In addition, recovery from the SPE for the tested compounds rosmarinic acid and carvacrol, two naturally occurring antioxidants in oregano, was found to be 95–98% for a 100 mg injection via preparative HPLC purification at 50 mg/compound.

Keywords: LC-UV-SPE; Solid phase extraction; Fraction enrichment; Preparative chromatography; Fraction concentration

1. Introduction

The methodology of preparative chromatography is the predominate means by which compounds that are currently used in pharmaceutical and biotechnology research are purified. However, this technique introduces problems that plague researchers. The purified target compound is often present in several collected fraction tubes and/or in a large volume of low volatility solvent that needs to be removed by a time consuming evaporation step. Mobile phase modifiers used for optimization of chromatography (e.g., TFA: trifluoroacetic acid) concentrate during the evaporation process, potentially resulting in hydrolysis of functional groups (e.g., esters, amides) and product degradation. An alternative to evaporation of collected fractions is to apply the fractions to pre-conditioned solid phase extraction cartridges to absorb and concentrate the purified compound in the fraction. During a preparative chromatographic purification, fractions are collected based on predetermined criteria (e.g., volume, time, peak level, peak slope and mass). In this technique, the liquid handler automatically dilutes the collected fractions of interest in aqueous solvent. The diluted fraction is then passed through the SPE allowing total absorption of the purified compound onto the cartridge. The SPE cartridge is dried with nitrogen from the liquid handler prior to the small volume elution of the purified compound with 100% organic solvent. Alternatively, the compound may remain absorbed on the SPE for storage and elution at a later time based on predetermined compound stability. Elution of the purified compound from the SPE will allow for rapid evaporation without requiring a temperature increase, thus eliminate concerns associated with heat and mobile phase modifiers that can degrade the target compound. Several investigations have been published involving the use of SPE cartridges and trace enrichment cartridges (TEC) to concentrate purified or isolated compounds under analytical chromatographic conditions [1–3]. There have even been reports implementing HPLC columns to trap and concentrate the purified compounds [4–5]. The implementation of SPE to concentrate and eliminate the bottleneck associated with the evaporation of preparative collected fractions has proven to be a very effective alternate method. Rosmarinic acid and carvacrol, two natural compounds isolated from oregano that exhibit antioxidant properties, were chosen to evaluate this technique. Distinct polarities of the chosen compounds allowed basic evaluation of the technique through a common reversed-phase gradient: 10–95% organic mobile phase.

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2. Experimental

2.1. Instrumentation

Gilson (Middleton, WI) GX-281, Gilson UV–vis 155 Detector, Gilson 322-H2 pump, Phenomenex (Torrance, CA) Luna 5 μm C-18(2), 100A, 21.20 mm × 150 mm, PN 00F-4252-P0.

2.2. Reagents

Burdick & Jackson (Muskegon, MI) DMSO, acetonitrile, methanol HPLC grade; Barnstead (Dubuque, IA) NANOpure water; Aldrich (Milwaukee, WI) trifluoroacetic acid, spectrophotometric grade, rosmarinic acid, carvacrol.

2.3. Collection system

Gilson code 307 rack with Gilson positive pressure caps for 6 mL SPE cartridges: Phenomenex (Torrance, CA) Strata, C-18E (55 μm, 70A) 1 g/6 mL, 20/rack, 15 mL × 45 mL elution vials (4 mL).

Mobile phase solutions were prepared in 20 liter carboys to which the appropriate amount of TFA was added to achieve 0.1% (*v/v*) solutions for both the aqueous (water) and organic (acetonitrile) mobile phase. The HPLC flow rate was set to 30 mL/min, using a linear gradient from 10 to 95% organic component. Equilibration time at the start and end of the run was 1 min. This adjustment from high organic to initial conditions should be based on the researcher's specific system (plumbing geometry, column type and size, etc.).

2.4. Experimental compounds

Concentrated solutions of rosmarinic acid and carvacrol were dissolved in DMSO and then diluted with methanol to achieve a 100 mg/mL concentrated solution. A 1.0 mL sample containing a 1:1 mixture of the concentrated compounds rosmarinic acid and carvacrol was injected for an on column loading of 50 mg of each compound. Fractions were collected based on a peak level setting of 10% above baseline with a 3 mL volume setting per fraction (tube) collected. On the basis of either predetermined criteria or visual observation the sample list was populated with the selected fractions for subsequent concentration via SPE cartridges. The SPE cartridges were conditioned prior to fraction loading based on the manufacture's recommendations: 15 mL of methanol followed by 15 mL of water. Determination of the volume required to dilute the fractions for the purpose of weakening the elution strength of the fraction solvent was based on the presumption that a 3 mL fraction was 100% organic mobile phase, therefore, a 1:5 mL dilution with aqueous solvent is required based on prior experimental data [6]. The diluted fractions were passed through the SPE cartridges allowing the compound within the fraction to absorb and concentrate onto the sorbent. The cartridges were evaporated with nitrogen (2–3 min/SPE cartridge). The compound was eluted from the SPE cartridge in 3 mL of acetonitrile yielding a concentrated compound in a volatile organic. The entire process was automated under the control of Gilson TRILUTION® LC Software (version 1.3), including subsequent data collection and reporting operations.

3. Results

The preparative chromatogram shown in Fig. 1 represents the separation of rosmarinic acid (R_t 2.96) and carvacrol (R_t 5.81). The collection criterion was based on a peak level set at 10% above baseline. The selected fractions were diluted and loaded on pre-conditioned SPE cartridges. Each 5 mL of diluted fraction passing through the SPE was collected; from each collected eluent a 1 mL injection was analyzed via HPLC to determine compound breakthrough. Overlaid chromatograms (five collections) for both rosmarinic acid and carvacrol show no significant breakthrough (Figs. 2 and 3). The data is consistent with several independent intra-day and inter-day runs. The SPE cartridges containing the absorbed compound were dried with nitrogen. The concentrated compound absorbed on the SPE was eluted with 3 mL of organic solvent. The data for a 1 mL injection of the eluted compound is shown in Figs. 4 and 5; the samples were

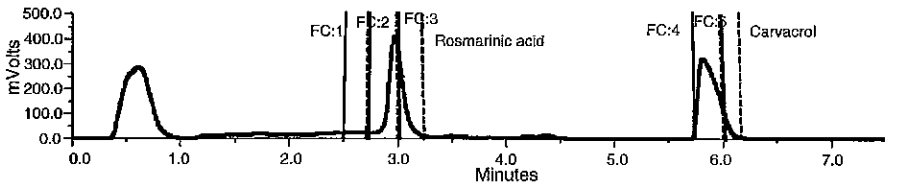

Fig. 1. Purification of rosmarinic acid and carvacrol. 50 mg of each compound on column with fraction collection.

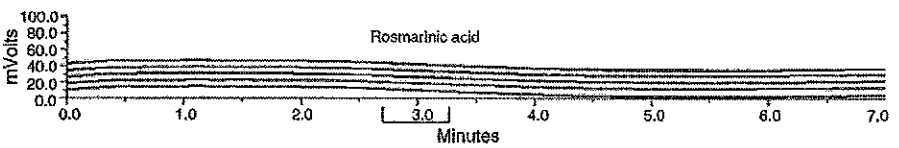

Fig. 2. Overlaid chromatograms of rosmarinic acid for the diluted fraction passing through the SPE cartridge, bracketed elution window identified for rosmarinic acid indicating no appreciable breakthrough.

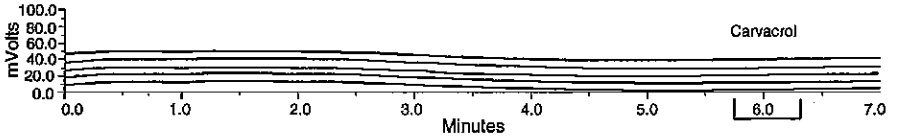

Fig. 3. Overlaid chromatograms of carvacrol for the diluted fraction passing through the SPE cartridge, bracketed elution window identified for carvacrol indicating no appreciable breakthrough.

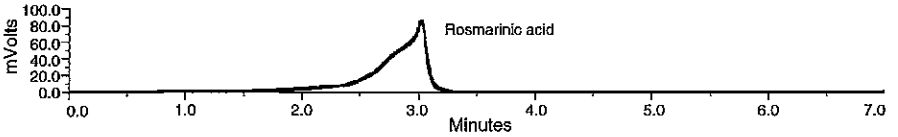

Fig. 4. 1 mL injection from the 3 mL acetonitrile eluent, rosmarinic acid.

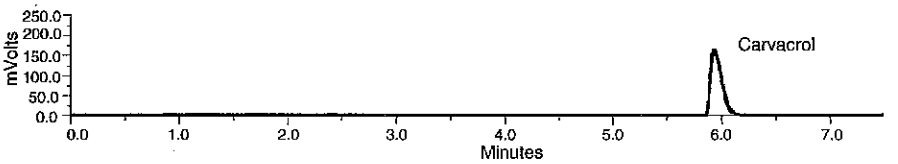

Fig. 5. 1 mL injection from the 3 mL acetonitrile eluent, carvacrol.

not dried or reconstituted prior to analysis. Recoveries for rosmarinic acid were 95 ± 5.0% and for carvacrol were 98 ± 0.5%. Retention capacity for the 1 g/6 mL SPE cartridge (50 mg of solute) is consistent with the literature; SPE cartridges retain a mass of solute that is equivalent to 5% of the sorbent mass [7].

The SPE cartridges were also assessed as a storage/transport device for the concentrated fractions. After the SPE cartridges were loaded and dried, they were stored and protected from light for 1 week. The concentrated fraction was eluted from the SPE cartridge with 3 mL of organic. Analysis and recoveries for a 1 mL injection of the eluted compound is consistent with the data presented in Figs. 4 and 5. The compounds, rosmarinic acid and carvacrol, are known to be stable. It is important to note that the reactivity and stability of any compound should be thoroughly tested prior to relying on this technique to yield pure compound from the fraction. The automated system can process 20 fractions within 2 hr.

4. Discussion

Automated post-collection and concentration of purified preparative fractions by SPE has obvious advantages compared to direct fraction solvent evaporation, namely automation, increased throughput and operation at room temperature. In addition, on-line fraction trapping via HPLC columns or SPE cartridges presents limitations. In the case of post-separation on-line HPLC trapping columns, there are a finite number of columns that can be realistically plumbed into the system, thus limiting the number of fractions per run and requiring regeneration of the trapping columns. Collection directly onto SPE cartridges offers the potential for sample loss via break through. In both cases, a dilution pump set at a flow rate of at least five times greater than the chromatographic flow rate, is required prior to the collection column in order to obtain sufficient reduction in solvent elution strength. The increase in total flow and pressure can cause a myriad of additional problems; such as breakthrough, sample loss, flow cell breakage and excessive column pressure.

The technique presented in this application is a unique alternative to concentrating fractions from a preparative HPLC purification. SPE cartridges are available in a variety of phases, supports, permeability, loads and sizes; thus offering many options and possibilities. In addition, reusing the SPE cartridges is under evaluation, with the ultimate goal of reducing consumable cost. Also, investigations are proceeding that utilize this technique employing larger SPE cartridges (sorbent mass) for gram quantity concentration of purified compound.

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