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Short communication

Facile optimization for chromatographic separation of liquiritin and liquiritigenin

Shaoyan Wang*, Min Guo, Jingxiang Cong, Shoujiang Li

School of Chemical Engineering, University of Science & Technology Liaoning, Anshan 114051, China

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ABSTRACT

A reversed phase chromatographic system, composed of a stationary phase of C_{18} silica gel (ODS, 20 μm) and a mobile phase of ethanol/water, was used to separate liquiritin and liquiritigenin in the raw material of flavonoids. The linear adsorption isotherm and the equilibrium-dispersive model were adopted to approximatively describe the chromatographic separation behaviors of liquiritin and liquiritigenin in the raw material under different column temperatures, ethanol contents and flow rates of the mobile phase, sample concentrations and feeding times. Combined with orthogonal design, the ED model was used to optimize the chromatographic separating conditions, the corresponding experimental result with a good agreement was obtained and the overload separation was realized.

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1. Introduction

Licorice is one of the oldest and most popular herbal medicines in the world. It shows a variety of pharmacological activities, including antiulceric, antiinflammatory, antispasmodic, antioxidative, antiallergic, antiviral, antidiabetic, anticancer, antidepressive, hepatoprotective, expectorant, memory enhancing and hypnotic effects, which are attributed to the definite chemical components [1].

For the separation of the active components of licorice mixture, an effective and traditional technology has been preparative chromatography with different solid support such as C_{18} reversed-phase, macroporous resin and silica gel [2–5]. In order to achieve an acceptable production rate at reasonable cost, the separating conditions of preparative chromatography need to be optimized [6,7]. If a suitable mathematical model is available, the optimization of some operating conditions will be carried out by simulated calculation without the troublesome experiments [8–13].

In general, preparative chromatography is a complex phenomenon to model. The fundamental approach to this problem consists in writing the differential mass balance equations for all the mixture components, and considering simultaneously the thermodynamics of phase equilibrium and the kinetics of phase transfers. The nonlinearity of isotherms, the coupling of mass balance equations, of the kinetics of mass transfers and between the thermodynamic and the kinetic effects of the different components should be taken into account for an accurate model. For a mixture such as natural product with multiple unknown components, it is impossible to obtain the accurate adsorption isotherms and chromatographic models. A considerable simplification of the model with negligible loss of accuracy can be achieved by making some simple assumptions [14].

Flavonoid is one kind of the active components in licorice. It contains liquiritin, isoliquiritin, liquiritigenin, isoliquiritigenin, as well as some unique compounds [1]. In this paper, C_{18} reversed-phase preparative chromatography was used to separate liquiritin and liquiritigenin in a flavonoid mixture. Based on the experimental elution profile of the raw material, the linear adsorption isotherm of a single component of interest and the artificial, numerical diffusion of the equilibrium-dispersive (ED) model used for handling the kinetics and thermodynamics of phase equilibrium at the same time, we predicted the chromatography behaviors of liquiritin and liquiritigenin in the raw material and optimized their separating conditions by simulated calculation.

2. Experimental

2.1. Materials and reagents

The raw material of flavonoids with mass percentage of 19.54% liquiritin and 8.38% liquiritigenin was purchased from Yiteng Pharmaceutical Company (Inner Mongolia, China). The standard samples of liquiritin and liquiritigenin were provided by Cdmust Biotech Company (Chengdu, Sichuan, China). The solvents including methanol (chromatographic grade) used for HPLC analysis, ethanol (analytical grade) used for preparative chromatography and the solute uracil used as a non-retained tracer were purchased from the Chemical Reagent Factory of Anshan (Liaoning, China).

* Corresponding author. Tel.: +86 412 5929106; fax: +86 412 5928009.
E-mail address: aswsy64@163.com (S. Wang).

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2.2. HPLC analysis

Analysis was performed using a HPLC system (Agilent 1200LC, U.S.A.) with an ODS column (250 mm × 4.6 mm, 5 μm, Agilent, U.S.A.) at 25 °C, a mobile phase of methanol and water (45/55, v/v) at 1.0 ml/min, an absorbance wavelength at 276 nm.

2.3. Separation with preparative chromatography

Preparative chromatography apparatus was mainly assembled by two HPLC pumps (LC-10AT, Shimadzu, Japan) for eluting and feeding, a column (200 mm × 10 mm, i.d.) packed with ODS silica gel (20 μm diameter, Beijing Jinouya, China) by slurry-packing technique, and an ultraviolet detector (SPD-10A, Shimadzu, Japan) with a chromatography data system (N2000, Zhejiang University, China).

Five grams of flavonoids powder was immersed in 100 ml ethanol, homogenized by ultrasound for 1 h and filtrated to form the raw material stock solution. This solution was diluted according to the ratio of ethanol and water in the mobile phase and filtrated to form the feeding solution. For a sample load within 0.3 mg liquiritin, the detection limit at 276 nm, the elution profile was obtained on-line, and beyond this limit, the elution profile was obtained by off-line HPLC detection of the fractions of per 1.5 ml intervals at the outlet of the preparative column.

2.4. Adsorption isotherm and chromatographic model for optimization

The linear adsorption isotherm and equilibrium-dispersive model were adopted to approximatively describe the chromatographic separation behaviors of liquiritin and liquiritigenin in the raw material, which can be written as Eqs. (1) and (2).

$$f_i = G_i C_i \tag{1}$$

$$\frac{\partial C_i}{\partial t} + F\frac{\partial f_i}{\partial t} + u\frac{\partial C_i}{\partial x} = D_a\frac{\partial^2 C_i}{\partial x^2} \tag{2}$$

Here $i = 1$ stands for liquiritin and $i = 2$ for liquiritigenin, C_i and f_i, are the concentrations of component i in the mobile and the stationary phases, respectively, G_i is the adsorption constant of the component i, u the mobile phase linear velocity, F the phase ratio, x and t are the position and time, respectively.

The column dead time t_0 was obtained by the uracil elution profile with the mobile phase of ethanol/water (35/65, v/v), and the mean column porosity ε and phase ratio F detected at different flow rates (0.5–4.5 ml/min) were 0.667 and 0.500, respectively.

G_i was calculated according to formula $t_{Ri} = t_0(1 + FG_i)$, where t_{Ri} is the retention time in the elution profile.

The upwind scheme was used to solve Eq. (2) and the artificial, numerical diffusion coefficient D_a can account for the experimental diffusion $D_L = Hu/2$ (H is the height equivalent to a theoretical plate) [14,15]. Here, H was fixed at 0.008 cm according to H–u curve of liquiritigenin, H was in the range of 0.007–0.009 cm when u was in the range of 1–9 cm/min.

Combined with orthogonal design, the ED model was used to optimize chromatographic separating conditions. The experiment was carried out under the simulated optimal condition.

3. Results and discussion

3.1. Analysis of the stock solution of flavonoids

The HPLC analysis chromatogram of the stock solution of flavonoids raw material in Fig. 1 showed that the purities of liquiritin and liquiritigenin were 32.27% and 41.17%, respectively,

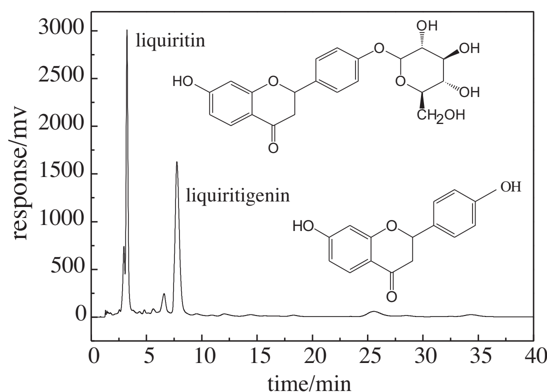

Fig. 1. HPLC analysis chromatogram of the stock solution of flavonoids raw material.

which indicated the main components of flavonoids raw material were liquiritin and liquiritigenin. The liquiritin and liquiritigenin concentrations of this stock solution were 9.77 and 4.19 mg/ml, respectively. According to the ratio of ethanol and water in the mobile phase, when the stock solution was diluted to form a feed solution of which the liquiritin concentration was greater than 0.50 mg/ml, the solution became turbid. Therefore, 0.50 mg/ml was taken as the maximum injection concentration.

3.2. Simulated calculation under different mobile phase compositions and column temperatures

Dominated by the mobile phase composition and column temperature, adsorption coefficient G_i is a thermodynamic parameter, which determines the equilibrium or transfer of the solute from the mobile phase to the stationary phase.

When liquiritin and liquiritigenin concentrations in the feed solution ($C_{1,F}$ and $C_{2,F}$) were 0.20 and 0.072 mg/ml, respectively, mobile phase flow rate (Q) was 2 ml/min, injection time (t_p) 0.16 min, column temperature 20 °C, the experimental elution profiles with the different ethanol contents in the mobile phase were obtained and shown in Fig. 2(a) (solid lines) which indicated that, with the decrease of ethanol content the separation ability increased, but the mobile phase consumption increased and the separation time got longer.

When $C_{1,F} = 0.10$ mg/ml, $C_{2,F} = 0.036$ mg/ml, $Q = 2$ ml/min, $t_p = 0.16$ min, ethanol/water (35/65, v/v) was used as the mobile phase, the experimental elution profiles with different column temperatures were obtained and shown in Fig. 2(b) (solid lines) which indicated that the separation factor α ($\alpha = G_1/G_2$) nearly did not change with the temperature, and the effect of column temperature was more weaker than that of mobile phase composition. Therefore, the separation of liquiritin and liquiritigenin was performed at room temperature (20 °C).

All these experimental elution profiles obtained under the different mobile phase compositions and column temperatures could be simulated by the ED model, with good agreement, as shown in Fig. 2(a) and (b) (dotted lines).

3.3. Simulated calculation under different flow rates of the mobile phase and different sample loads

Adsorption coefficient G_i is a fixed value under a certain temperature and a certain mobile phase composition.

When ethanol content in mobile phase was 35%, column temperature 20 °C, $C_{1,F} = 0.20$ mg/ml, $C_{2,F} = 0.072$ mg/ml, $Q = 2$ ml/min, $t_p = 0.16$ min, the experimental elution profile obtained was shown in Fig. 2(a2) (solid line), and the adsorption coefficients of liquiritin and liquiritigenin measured were 1.61 and 7.61, respectively. Based

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