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Comparison of volume and concentration overloadings in preparative enantio-separations by supercritical fluid chromatography

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ABSTRACT

The adsorption isotherms of both naproxen enantiomers were measured on a (R,R)-Whelk-O1 chiral adsorbent from a mobile phase made of liquid carbon dioxide and methanol, using frontal analysis. These isotherms were used to model the competitive adsorption behavior of a racemic mixture of naproxen enantiomers. Their overloaded elution band profiles were calculated numerically using these isotherm data. The injected volume and the injected concentration of the samples of racemic mixture were carried out simultaneously, using two objective functions. The maximization of the production rate and of the product of the recovery yield and the production rate were performed. Based on the optimum points obtained, the two objective functions were compared for several recovery yield constraints in a range of injected volumes and concentrations. The consequences of choosing a separation design focused on the degree of band overlapping are discussed based on the results of these optimizations.

1. Introduction

The use of supercritical fluid chromatography is now affecting markedly the preparative purification of enantiomers and considerably changing the development of this field [1,2]. It permits an important decrease of the cycle time and a significant increase of the column loadability; the combination of these effects results in an important increase of the production rates of this purification process, which now exceeds that of traditional liquid chromatographic techniques based on the use of aqueous solutions of organic modifiers as the mobile phase [3,4]. The decrease in the amount of solvent needed and the lower production cost have a high impact on the economy of the process and some environmental consequences.

Computer assisted optimization algorithms are needed to fully realize the maximum possible production rate and the optimal performance of preparative chromatographic systems. Without them, the empirical development of a separation process that uses a compressible supercritical fluid as the mobile phase may take weeks or months before it can be considered as optimized. Even then, engineers are unable to guarantee the successful achievement of this optimum. The requirements for conducting a successful numerical optimization of a preparative separation are a basic knowledge of the thermodynamics of adsorption, the availability of the properties of the sample components and mobile phase accurately determined in supercritical fluids and methods of isotherm determination suitable for use in compressible mobile phases [5,6].

The methods available for the numerical optimization of preparative chromatographic processes in HPLC have been developed and extensively used [7,8]. They require knowledge of the isotherm of the compounds involved and of the system properties. Using appropriate algorithms, the optimum particle size, retention factors and values of the system parameters in large scale chromatographic purifications can be determined [9–11]. However, the optimization of preparative enantio-separations may require a more complex treatment in supercritical fluid chromatography [2,3,12–14].

The goal of this study was to determine the optimum values of the injection volume and sample concentration in an enantio-separation carried out by supercritical preparative chromatography. Two different objective functions were used to optimize the selected experimental system. The optimum values of the production rate and of the product of the production rate and the recovery yield were compared for the purification of the naproxen enantiomers. The results are discussed for different recovery yield constraints and scale-up approaches.

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2. Theory

2.1. Frontal analysis (FA)

The adsorption isotherms of the naproxen enantiomers were determined by single step frontal analysis. The adsorbed concentration at equilibrium with the mobile phase concentration was calculated by using the following well-known equation modified for compressible mobile phases [8,15]:

$$q_i = \frac{C_i(t_{R,F,i} \times \widetilde{F_V} - t_{R,0,i} \times \widetilde{F_V} - V_0)}{V_{ads}} \quad (1)$$

where q_i and C_i are the adsorbed and the mobile phase concentrations at equilibrium of the ith compound, respectively; $t_{R,F,i}$ and $t_{R,0,i}$ are the retention times of the front when the column is connected or is replaced with a zero-volume connector, respectively; V_0 is the column void volume, determined by the weight difference method [16]; $\widetilde{F_V}$ is the average volumetric flow rate, which reflects the density drop of the mobile phase along the column. It can be estimated from the mass flow rate F_m of the mobile phase and its average density $\widetilde{\rho}$:

$$\widetilde{F_V} = \frac{F_m}{\widetilde{\rho}} \quad (2)$$

2.2. Isotherm models

The surface of a selective chiral adsorbent is heterogeneous by nature. It has at least two different types of adsorption sites. The chiral sites interact more strongly and selectively with one of the two enantiomers in the sample mixture than with the other one and are responsible for the selective separation and the high retention of the one enantiomer that gives these interactions. Non-specific sites bind both enantiomers with the same energy since they cannot distinguish the chemical natures of the enantiomers. The retention and adsorption of the enantiomers can be described by the following bi-Langmuir equation [17]:

$$q_1 = \frac{q_{s,1}b_{s,1}C_1}{1 + b_{s,1}C_1} + \frac{q^\star_{s,2}b^\star_{s,2}C_1}{1 + b^\star_{s,2}C_1} \quad (3)$$

where $q_{s,1}$ and $b_{s,1}$ are the saturation capacity and the adsorption equilibrium constant of adsorption on the non-specific sites. $q^\star_{s,2}$ and $b^\star_{s,2}$ are the saturation capacity and the adsorption equilibrium constant of adsorption on the selective chiral sites. The higher retention of one of the two enantiomers is due to its stronger specific interactions with the chiral centers. In this case Eq. (3) can be written:

$$q_2 = \frac{q_{s,1}b_{s,1}C_2}{1 + b_{s,1}C_2} + \frac{q^\star_{s,2}b^\star_{s,3}C_2}{1 + b^\star_{s,3}C_2} \quad (4)$$

where $b^\star_{s,3}$ is the adsorption equilibrium constant of adsorption of the enantiomer most adsorbed on the selective chiral sites.

When the adsorption isotherms are determined for either enantiomer separately, the competitive adsorption of these compounds when their mixture is injected onto the column can be estimated by fitting simultaneously Eqs. (3) and (4) to the two sets of isotherm data points. During this simultaneous nonlinear fit, $q_{s,1}$, $q^\star_{s,2}$ and $b_{s,1}$ are fitted as common parameters for both compounds. $b^\star_{s,2}$ and $b^\star_{s,3}$ are fitted to the two enantiomers separately since they interaction energy differs significantly with the specific adsorption sites.

2.3. Numerical calculation of the high concentration bands

The equilibrium–dispersive (ED) model was used to describe the chromatographic process of separation of the enantiomers [8]. The ED model is the simplest realistic model of non-linear chromatography. It is also the model most often used to model the preparative separation of compounds of moderate molecular weight eluted at high concentrations. When the kinetics of mass transfer resistance in the column is small or moderate, the ED model provides usually satisfactory predictions of the elution profiles of chromatographic bands and requires short calculation times. For component i, the mass balance equation of the ED model is:

$$\frac{\partial C_i}{\partial t} + u\frac{\partial C_i}{\partial z} + F\frac{\partial q_i}{\partial t} = D_{a,i}\frac{\partial^2 C_i}{\partial z^2} \quad (5)$$

where t and z are the time elapsed from the beginning of the sample injection and the distance travelled by the molecules inside the column, respectively. u is the mobile phase velocity and F the phase ratio. $D_{a,i}$ is the apparent dispersion coefficient of component i. Since the ED model assumes instantaneous equilibrium between the stationary and the mobile phases, the adsorbed phase concentrations q_i are directly derived from the competitive adsorption isotherm model for the less and the more retained enantiomers:

$$q_1 = \frac{q_{s,1}b_{s,1}C_1}{1 + b_{s,1}C_1 + b_{s,1}C_2} + \frac{q^\star_{s,2}b^\star_{s,2}C_1}{1 + b^\star_{s,2}C_1 + b^\star_{s,3}C_2} \quad (6)$$

$$q_2 = \frac{q_{s,1}b_{s,1}C_2}{1 + b_{s,1}C_2 + b_{s,1}C_1} + \frac{q^\star_{s,2}b^\star_{s,3}C_2}{1 + b^\star_{s,3}C_2 + b^\star_{s,2}C_1} \quad (7)$$

Eqs. (6) and (7) were used to calculate the elution profiles of high concentration samples of the naproxen enatiomers using the Rouchon algorithm [18].

3. Experimental

3.1. Chemicals

The enantiomers (R)- and (S)-naproxen (at a purity of 98% each) were purchased from Sigma–Alrich (St. Louis, MO, USA). Pure CO_2 was obtained from Airgas (Knoxville, TN, USA). HPLC grade methanol was purchased from Fisher Scientific (Fair Lawn, NJ, USA).

3.2. Instrumentation

A supercritical fluid chromatograph from JASCO (Hachioji, Japan) was used. This instrument was equipped with a PU-2080-CO2 carbon dioxide pump, two PU-1580 modifier pumps, a CO-2060 Plus column oven, an AS-2059-SF Plus autosampler with a 20 μL loop, a MD-2010 Plus Multiwavelength detector, and a BP-1580-81 back pressure regulator. A dynamic mixer of small volume (250 μL, model MX-2080-32) was connected to three tubing inlets and one outlet. The system was controlled by ChromNAV software (Hachioji, Japan).

In all the experiments reported here, the mass flow rate of CO_2 was measured with a mini CORI-FLOW instrument (Model No. M13-ABD-11-0-S, Serial No. B11200776A) from Bronkhorst High-Tech B.V. (Ruurlo, NL). The accuracy of this device is ±(0.2% of the read value + 0.5 g/h) and the sensitivity 0.01 g/min. This mass flow meter was installed between the CO_2 cylinder and the pump. The pressure drop along the flow meter was less than 1 bar and its installation did not affect the behavior of the instrument.

The long term flow rate accuracy of the modifier pumps were tested by pumping pure methanol for 1 h at the same flow rate as used during the measurements and weighing the amount of pumped methanol. The deviation between the set and the actual flow rates of the modifier was less than 0.5%. The online mass flow meter was used to monitor the flow accuracy of the carbon dioxide pump during the measurements. The volumetric flow rate was calculated from the mass flow data and the density value of the mobile