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Optimization of the mobile phase composition for preparative chiral separation of flurbiprofen enantiomers

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ABSTRACT

This work presents the experimental and simulation results obtained for the optimization of the mobile phase composition for the preparative separation of flurbiprofen enantiomers by liquid chromatography using an amylose-based chiral stationary phase (Chiralpak AD). The experimental work carried out includes solubility and adsorption isotherm measurements and pulse and breakthrough experiments under preparative conditions. The simulation work predicts the operation of a simulated moving bed (SMB) system for the separation of flurbiprofen enantiomers to compare the productivity and solvent consumption performances, for the different mobile phase compositions and using the experimental data obtained. This paper presents a new and different case study (flurbiprofen) of the one recently reported by the authors (ketoprofen enantiomers [A. Ribeiro, N. Graça, L. Pais, A. Rodrigues, Preparative separation of ketoprofen enantiomers: choice of mobile phase composition and measurement of competitive adsorption isotherms, Sep. Purif. Technol. 61 (2008) 375–383]), to clearly show that the optimization of the mobile phase composition for preparative chiral separation requires an individualized study, since different results are obtained even for enantiomers systems of the same family.

1. Introduction

Flurbiprofen [(R,S)-2-(2-fluoro-4-biphenyl)-propionic acid], a 2-arylpropionic acid derivative (profen), is a chiral non-steroidal anti-inflammatory drug (NSAID) (Fig. 1). This drug has a pharmacological action similar to other drugs of this class, such as, ketoprofen, ibuprofen or naproxen. This chiral drug is still being marketed worldwide as a racemic mixture, although the increasing number of published studies referring that R(−)-Flurbiprofen and S(+)-Flurbiprofen pure enantiomers have distinct pharmacological activities. In this way, the chiral resolution of flurbiprofen enantiomers can promote the development of two new therapeutic drugs which have distinct profiles and/or which are more pharmacologically safe.

Currently, there is a strong interest in enantioseparation of profens, mainly the flurbiprofen enantiomers. This interest is based on the fact that his R enantiomer has been referred as a promoter of efficient inhibition on the development of varied forms of cancer in human beings [2], such as, the prostate cancer [3–5] and the colon cancer [6,7]. The most recent application area of flurbiprofen enantiomers has been described in numerous clinic and pharmacological reports, in which the R enantiomer is referred as an important hypothetical drug used to minimize the progression of Alzheimer disease [8–11].

Liquid chromatography is now the most accepted method for chiral separations, not only in the direct way, using chiral stationary phases, but also in the indirect way, by using chiral derivatizing reagents [12]. Examples of flurbiprofen enantiomers separation, using a Chiralpak AD stationary phase as chiral selector, can be found in the literature. Patel et al. used a hexane/isopropyl alcohol/trifluoracetic acid (90/10/0.05%, v/v) mobile phase composition and obtained a selectivity value of 1.7 [13,14]. A similar mobile phase composition (95/5/1) was used by Booth and collaborators, using the same stationary phase [15]. At a preparative scale, some examples can be found on both the enzymatic resolution of R-Flurbiprofen [16–18] and S-Flurbiprofen [19–21] enantiomers. However, as far as our knowledge, there are no published studies related to the preparative separation of flurbiprofen enantiomers by liquid chromatography.

The objective of this paper is to study the effect of mobile phase composition on the preparative separation of flurbiprofen enantiomers by chiral liquid chromatography. Experimental results obtained for different mobile phase compositions will be presented and discussed, including solubility measurements, elution (pulses) and frontal (breakthroughs) chromatographic experiments, and the measurement of the equilibrium binary adsorption isotherms.

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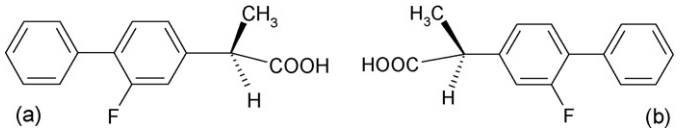

Fig. 1. Chemical structure of flurbiprofen enantiomers: (a) S(+)-Flurbiprofen and (b) R(−)-Flurbiprofen.

Afterwards, the comparison of the performance of the preparative separation of flurbiprofen enantiomers is carried out, using the predictions for fixed bed and simulated moving bed operation.

2. Experimental

All the analysis were performed on a Jasco HPLC system with an UV-1575 multiwavelength detector set at 260 nm, equipped with a preparative cell (1.0 mm). Two chiral chromatographic columns were used with the same adsorbent material (Chiralpak AD, Daicel Chemical Industries Ltd., Japan) and the same dimensions (250 mm L × 4.6 mm ID). These two columns have different particle size: one column, with a particle size of 10 μm, was used for analytical purposes (measurement of enantiomers concentrations); the other, with a particle size of 20 μm, was used in the preparative chromatographic experiments (adsorption–desorption steps, pulses and breakthrough experiments). It must be pointed out that a particle size of 20 μm is normally used for preparative separations, including SMB operation.

The measurements of the binary adsorption isotherms were carried out at 23 and 35 °C, using an Eldex CH-150 column oven and a thermostatic water bath for solvents. Analytical grade racemic flurbiprofen was purchased from Merck (Darmstadt, Germany). Methanol, ethanol and n-hexane (Fluka, Buchs, Switzerland) were all HPLC grade. Trifluoracetic acid (TFA) was spectrophotometric grade. If nothing is said in contrary, all mobile phase compositions used in this work include 0.01% of the TFA modifier. For example, 1000 mL of a 10% ethanol/90% n-hexane mixture is prepared adding 100 mL ethanol, 900 mL n-hexane and 100 μL of TFA.

The gravimetric method for solubility measurements and the adsorption–desorption method used in the experimental determination of binary adsorption isotherms are described in the previous work [1]. In the adsorption–desorption method, the preparative column is saturated with a large amount of feed solution (racemic mixture), with known concentration of both enantiomers, until equilibrium is achieved. The column is then completely regenerated with eluent. The eluted volume, resulting from this desorption step, is collected and analyzed, in order to measure each enantiomer concentration. A mass balance will allow the evaluation of each enantiomer concentration retained in the particle, in equilibrium with its known concentration in the feed solution. The entire adsorption isotherm measurement will require a set of adsorption–desorption experiments, using different feed concentrations. The concentration of each flurbiprofen enantiomer in the feed (racemic) and eluted solutions was evaluated by HPLC, equipped with the analytical column described before.

3. Modeling, correlation and simulation

3.1. Binary adsorption equilibrium data

After experimental determination, the fitting of the adsorption measurements to an adsorption isotherm model is advised in order to allow the simulation and prediction of the adsorption behavior and a better understanding of the overall chromatographic separation process. In this study, it is presented the binary adsorption equilibrium data of Chiralpak AD/enantiomer mixtures (R(−)Flurbiprofen and S(+)Flurbiprofen). The adsorption equilibrium measurements yield data of adsorbed phase concentrations of R(−)Flurbiprofen and S(+)Flurbiprofen as functions of their solution phase concentrations. These data were fitted with three relatively simple binary isotherm equations, Langmuir and its modified versions (see Table 1 for the expressions). The isotherm parameters obtained are listed in Table 1 as well. These isotherm model parameters can be estimated using a Levenberg–Marquardt algorithm for the minimization of the sum of squares of the residues, SQ (see Table 1, Eq. (7)) or, in order to compare models with a different number of parameters, the standard deviation, SD (see Table 1, Eq. (8)).

The system selectivity and its dependence on both enantiomer concentrations can be evaluated by $\alpha = (q_2^*/C_2)/(q_1^*/C_1)$, where q_i^* is the concentration of enantiomer i retained in the particle, in equilibrium with its concentration in the liquid phase, C_i. The subscript $i = 1, 2$, represents, respectively, the less (R(−)-Flurbiprofen) and the more (S(+)-Flurbiprofen) retained enantiomer.

3.2. Simulation of fixed bed and SMB operation and performance

The simulation of fixed bed operation under non-linear preparative conditions can be carried out by using a linear driving force model. Model equations include the mass balance equations, the equilibrium isotherms models, the initial and the boundary conditions, and can be found in the previous work [1].

The simulation of SMB operation and performance was carried out by using the findings published by Morbidelli and co-workers, who developed a complete design of the binary countercurrent separation processes by SMB chromatography in the frame of equilibrium theory, assuming that mass transfer resistances and axial dispersion are negligible, and that the adsorption equilibria can be described through a variable selectivity modified Langmuir isotherm [22]. The SMB performance can be evaluated by defining the complete separation regions and through the performance parameters of productivity and solvent consumption. A separation region is the area of possible SMB internal flow rates that allows 100% pure products (pure extract, only containing the more retained enantiomer; and pure raffinate, only containing the less retained enantiomer). The performance parameters are evaluated at the vertex of each separation region, since it represents the best operating conditions in terms of system productivity and solvent consumption for a given feed concentration:

$$\mathrm{Pr} = \frac{\varepsilon}{N_c t^*}(\gamma_3 - \gamma_2)(C_1^F + C_2^F) \tag{9}$$

$$\pi = \mathrm{Pr}\frac{N_c t^*}{\varepsilon} = (\gamma_3 - \gamma_2)(C_1^F + C_2^F) \tag{10}$$

$$\mathrm{SC} = \frac{1}{C_1^F + C_2^F}\left(1 + \frac{\gamma_1 - \gamma_4}{\gamma_3 - \gamma_2}\right) \tag{11}$$

where ε is the bed porosity, N_c the total number of columns in the SMB unit, t^* the switch time interval, $\gamma_j = v_j/u_s$ the ratio between fluid and solid interstitial velocities in section j of the equivalent true moving bed operation, and C_i^F the feed concentration of enantiomer i.

Although it represents a simplified approach (no axial dispersion or mass transfer resistances and equivalence to the ideal true moving bed operation), the equilibrium theory model allows a straightforward prediction of SMB performance and is very useful for comparative studies as the one carried out in this work (to compare the performances obtained for different mobile phase compositions). For more information concerning SMB modeling and simulation, through the equilibrium theory and other more precise SMB models, see Refs. [22–29].