Contents lists available at ScienceDirect

Separation and Purification Technology

ELSEVIER

Separation of nadolol racemates by high pH reversed-phase fixed-bed and simulated moving bed chromatography

Rami S. Arafah [a,b], António E. Ribeiro [a,b], Alírio E. Rodrigues [c], Luís S. Pais [a,b,*]

[a] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[b] Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[c] Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials, Associate Laboratory LSRE-LCM, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal

ARTICLE INFO

ABSTRACT

The separation of nadolol racemates under high pH reversed-phase using both the fixed-bed (FB) and the simulated moving bed (SMB) preparative chromatographic techniques is reported after the previous published work [1] where the Waters XBridge C18 adsorbent and an ethanol:water:diethylamine solvent mixture were validated to allow the separation of the multicomponent feed mixture composed by four nadolol stereoisomers into two pure racemates (two pairs of enantiomers).

In this work, the experimental preparative separations using one commercial fixed-bed preparative HPLC Azura system equipped with one sole column of preparative dimensions (30 mm ID × 250 mm L) and one lab-scale SMB apparatus (the FlexSMB-LSRE pilot unit) equipped with six semi-preparative columns (19 mm ID × 100 mm L) are presented. Both systems use the Waters XBridge C18 adsorbent of 10 μm particle diameter. The screening of the mobile phase composition elected the 30:70:0.1 (v/v/v) ethanol:water:diethylamine solvent mixture to perform both FB and SMB preparative operations.

A large set of experimental, modelling and simulation results are presented, including pulses, measurement and modelling of the adsorption equilibrium isotherms, and its validation through breakthroughs measurements. The modelling and simulation steps allowed the prediction and the optimization of both the FB and SMB operating conditions.

For FB, using a feed concentration of 9 g/L of an equimolar mixture of the two nadolol racemates, both were recovered almost pure (at least 99.9 %), with a global system productivity of 3.06 g_{feed}/(Lbed.hr) and a solvent consumption of 4.21 $L_{solvent}$/g_{feed}. For SMB, the pilot unit's pressure drops limits imposed a maximum internal flow-rate of only 5 mL/min and, for a nadolol feed concentration of 2 g/L, both racemates were recovered 100 % pure, with a system productivity of 0.13 g_{feed}/(Lbed.hr) and a solvent consumption of 6.19 $L_{solvent}$/g_{feed}. Additional simulation results showed that a SMB preparative unit can perform the 9 g/L nadolol racemate separation with a system productivity of 3.61 g_{feed}/(Lbed.hr) and a solvent consumption of only 1.95 $L_{solvent}$/g_{feed} using the same average internal flow-rate as in FB operation. Even better SMB productivities can still be obtained using the same feed or solvent flow-rates as in FB operation if the internal SMB flow-rates are allowed and not limited by the system pressure drop.

The experimental results presented in this work validate the strategy of separating a four nadolol stereoisomers mixture into two pure nadolol racemates, each one composed by a pair of nadolol enantiomers, using an achiral C18 adsorbent through FB and SMB chromatographic techniques. Each nadolol racemate can later be purified into pure nadolol stereoisomers using standard binary chiral FB and SMB chromatography. In this way, this works introduces a real and experimental solution for the complete multicomponent preparative separation of the four nadolol stereoisomers.

---

* Corresponding author at: Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal.
  E-mail address: pais@ipb.pt (L.S. Pais).

1. Introduction

The continuous interest for the commercialization of safer and more efficient drugs together with the more and more restrictive international legislation is pushing the pharmaceutical industry in the direction of the development and production of chiral optically pure form pharmaceutical drugs [2,3]. Enantioselective synthesis, also called asymmetric synthesis, followed by a crystallization process, is the traditionally method to obtain single pure form enantiomers [4,5]. Often, the synthesis involves several consecutive and specific reactions directed to the production of one target single molecule, imposing a time-consuming process. As, in industrial operations, time-consuming processes are associated with high costs, alternative approaches are welcome. The two main alternatives are the indirect chromatographic resolution of a racemic mixture using chiral derivatization reagents to obtain one pure form enantiomer and the direct chromatographic resolution using a chiral adsorbent that allows the simultaneous production of the two enantiomers [6,7].

High performance liquid chromatography using chiral stationary phases is a long-time established technique to perform the analytical separation and quantification of chiral compounds. Simulated moving bed (SMB) technology is an approach already accepted and adopted in the pharmaceutical industry for the large-scale production of chiral drugs. SMB technology allows, in a shorter period, the availability of both the single enantiomers presented in a binary mixture, essential in the initial stages for the development of a pharmaceutical chiral drug. This can be confirmed by the increasing number of SMB preparative and industrial units already installed in the pharmaceutical industry worldwide. Some other important advantages also reinforce the success of the SMB technology, as the fact of being a continuous process simulating the counter-current movement of liquid and solid phases and, thus, maximizing the mass transfer driving force and succeeding in more difficult separations. Additionally, as the solvent is recycled and the adsorbent is re-used continuously, the SMB operation introduces a significant improvement in productivity and decrease in solvent consumption, when compared with the traditional fixed-bed chromatographic operation [8].

The SMB concept was first designed and patented in 1961 by Broughton and Gerhold, from UOP Inc., USA, to be used in the petrochemical industry [9]. Later, the SMB technology was adapted and used in new fields like pharmaceutical process development and bioprocessing to perform binary and pseudo-binary separations. This technology proved to have several advantages over the conventional preparative chromatography, being a faster, safer, smaller and even cleaner process [8,10]. Moreover, with the development of new and more stable chiral adsorbents, the design of new operating modes, and the hyphenation with other multicolumn techniques and operation strategies, the original concept of SMB was redesigned to more complex chromatographic allied techniques with the main objective to allow more efficient operations [11–18]. In a recent publication [19], a complete review of these improvements were presented, including dynamic configuration variations (Varicol and Pseudo-SMB), flow-rate modulation (Power Feed, Improved-SMB, Partial-Feed, Partial-Discard and Partial-Withdrawal, Outlet Swing Stream), concentration modulation (ModiCon, Enriched Extract SMB), gradient operation (solvent gradient SMB, temperature gradient SMB), and SMB nonstandard configurations (SMB with reduced number of sections, SMB with extended number of sections, SMB cascades). Other recent studies refer the use of several improved modelling optimization tools and the use of a superstructure allowing more general configurations [20,21]. Despite all these improvements, FB and SMB chromatography still face the challenge of effective preparative separation of multicomponent mixtures.

Nadolol is a nonselective beta-adrenergic receptor antagonist pharmaceutical drug that represents a very interesting case-study of multicomponent chiral separation since it is composed by four stereoisomers, being two pairs of enantiomers. In this way, it introduces the possibility of alternative separation strategies, using different kind of separation sequences and techniques, the use of different packings (chiral and achiral stationary phases), and the correspondent mobile phase optimization at both normal and reversed-phase modes. Only few published works can be found reporting the chiral separation of nadolol stereoisomers. The chiral separation of all its four components was first reported by McCarthy in 1994 [22], but only at analytical scale, using a Chiralpak® AD adsorbent and a solvent mixture of hexane:ethanol:diethylamine. In 2013, Ribeiro *et al.* [23] reported an upgrade in this analytical separation by using the same chiral material but replacing hexane by heptane in the mobile phase composition. This same work reported, for the first time, the SMB preparative separation of the more retained nadolol stereoisomer (component 4) from a feed mixture containing the four nadolol stereoisomers and using a solvent mixture of heptane:ethanol:diethylamine. In 2015, Jermann *et al.* [24] also reported this separation using a three-column intermittent simulated moving bed chromatography (3C-ISMB) with the same solvent mixture and adsorbent. In 2016, Arafah *et al.* [25] presented this pseudo-binary separation using a more efficient immobilized Chiralpak® IA chiral stationary phase and a solvent mixture of methanol:diethylamine. This work proved that the immobilized Chiralpak® IA is a better alternative to the former coated Chiralpak® AD chiral stationary phase, since it extends the range of compatibility between adsorbent and solvent compositions [26–29], promoting new possibilities for the preparative separation of chiral drugs. In 2019, Arafah *et al.* [30] reported an improved nadolol pseudo-binary separation by replacing the pure methanol solvent to an methanol:acetonitrile mixture. In all these previous works [23, 25 and 30], the authors explored the separation of the most active nadolol stereoisomer through chiral FB and SMB chromatography, using Chiralpak® AD and IA adsorbents. Using SMB chromatography, the most retained nadolol species (component 4) was purified and collected at the extract outlet from a quaternary feed mixture, and a second outlet stream (raffinate) was obtained containing the other three nadolol stereoisomers (components 1 + 2 + 3). But, being the final objective the complete separation of all the four nadolol stereoisomers, this initial separation must be followed by other chromatographic operation steps to achieve the separation of the remaining nadolol ternary mixture. This can be achieved with two extra SMB binary operations (SMB cascade) or by a more complex pseudo-simulated moving bed chromatographic technology to separate ternary mixtures in one single operation, like the JO process [13,31].

An achiral adsorbent is not able to separate the enantiomers of a chiral racemic mixture but it can separate pairs of racemates. This was, for the first time, demonstrated by Arafah *et al.* [1] by validating the capacity of an achiral C18 adsorbent to separate the two pairs of nadolol racemates; that is, to separate components (2 + 3), the less retained racemate B, from components (1 + 4), the more retained racemate A. Three different Waters C18 adsorbents (XBridge, Shield and XSelect) were extensively studied using ethanol:water:dietylamine solvent mixtures and at both FB and SMB chromatographic operations. The high basic nature of nadolol (pKa of 9.67) imposes the use of high pH reversed-phase operation (pH values between 11 and 12) and requires adsorbents that allow these extreme conditions. The Waters XBridge C18 adsorbent was selected as the most appropriate for the separation of nadolol racemates using a solvent composition range of 30–40 % ethanol:70–60 %water:0.005 %-0.1 %diethylamine and pH values close to 12.

The design of the complete separation of nadolol stereoisomers asks for a global simulation and experimental methodology considering both the characterization and the optimization of each separation step and its sequences to achieve the four nadolol components in its pure form. The objective of this work is to validate the experimental separation of the nadolol racemates using the high-pH reversed-phase strategy through both FB and SMB chromatographic operations and to evaluate the system performances in terms of productivity and solvent consumption. These new findings at the preparative scale will allow to obtain two

outlet streams of pure nadolol racemates, racemate (2 + 3) and racemate (1 + 4), that will be of utmost importance to study the future chiral separations of component (2) from component (3), and of component (1) from component (4) and, in that way, to achieve a clear definition of the best separation strategy for the complete separation of nadolol stereoisomers and the experimental availability of all its four pure stereoisomers.

2. Experimental

2.1. Reagents

Nadolol quaternary mixture and uracil (used as the non-retained compound) were both obtained from Merck (Darmstadt, Germany). Type I ultrapure water was obtained with a Merck Millipore Direct Q3 UV lab equipment. The basic modifier diethylamine (DEA) and the solvent ethanol (E), both HPLC grade, were obtained from Fluka (Neu Wulmstorf, Germany). All reagents and solvents were used without further purification.

2.2. Material and equipment

The analytical HPLC equipment was assembled with one Smartline 1050 pump with a 10 mL pump head, a Smartline UV detector 2520 set at 270 nm wavelength, and a manual 6-port/3-channel injection valve with a 20 μL loop. The collected samples from FB and SMB operations were analysed using a Waters XBridge C18 column of analytical dimensions (4.6 mm ID × 250 mm L) packed with a 5 μm particle size material.

The preparative HPLC system was equipped with two Smartline 1050 pumps with 50 mL pump heads, a Smartline UV detector 2520 set at 270 nm wavelength, and a manual 6-port/3-channel injection valve with a 1000 μL loop. Seven preparative Waters XBridge C18 columns (19 mm ID × 100 mm L) packed with a 10 μm particle size diameter, named as "SMB columns" were tested in the preparative HPLC system. The hydrodynamic characterization by means of HETP (Height Equivalent to a Theoretical Plate) was performed for all these seven columns. One of these SMB columns was selected and used for preparative pulses and measurement of the adsorption equilibrium isotherms and breakthroughs. Further, and based in the previous work [1], a more accurate screening of the solvent composition was carried using different ethanol:water:0.1 %diethylamine mixtures. This study allowed the selection of the same 30:70:0.1 (v/v/v) ethanol:water:diethylamine solvent composition to perform both FB and SMB operations.

The preparative separation of nadolol racemates was carried out using two apparatus. The SMB operation was carried out using the lab-scale FlexSMB®-LSRE unit, a pilot unit completely built at the LSRE-LCM/FEUP group. The FB operation was performed using a commercial Azura® preparative HPLC system obtained from Knauer.

The SMB unit was operated using six preparative XBridge C18 columns assembled with a [1–2–2–1] configuration for a conventional four-zone SMB. The selected valves system configuration for this unit is similar to the one proposed by Negawa and Shoji [32]. More information about the hardware of the FlexSMB®-LSRE unit can be obtained elsewhere [8,33].

The Azura® preparative HPLC system is equipped with one Waters XBridge Prep C18 column of preparative dimensions (30 mm ID × 250 mm L) packed with the same adsorbent as the SMB columns (10 μm particle size diameter). This system is equipped with two preparative HPLC pumps *P*2.1L model with two 250 mL/min pump heads and one UV/VIS detector UVD2.1L set at 270 nm wavelength. One loop of 10 mL was used for all fixed-bed preparative separations.

3. Results and discussion

3.1. SMB preparative separation

3.1.1. Screening of solvent composition and evaluation of SMB column efficiency

When leading with preparative chromatographic separations (high feed concentrations), the selection of the suitable solute-adsorbent-solvent combination is a complex task. Under these competitive conditions, a trade-off between high selectivity and short retention times must be settled. For preparative separations, high selectivity is not so crucial when compared with the analytical demands; additionally, high solute–solvent solubilities are also of utmost importance. Previous works presented the solubility of nadolol racemates using different type of solvents and it was concluded, as expected, that the solubility increases with the increase of the alcoholic content in the mobile phase [25]. As mentioned before, it was also concluded that the Waters XBridge C18 adsorbent and a 30 %ethanol:70 %water (30E70W) solvent composition is the most promising combination for the preparative separation of nadolol racemates [1]. Also, it must be stressed out that, due to the strong alkaline nature of nadolol (pKa = 9.67) [34,35], and in order to achieve enough resolution, this separation needs a mobile phase with a pH value one to two units above nadolol pKa value [36], leading to a value around 10.7–11.7. It is known that standard C18 absorbents are not compatible with so high pH values. However, as referred by the manufacturer and experimentally observed, an absorbent such as the preparative Waters XBridge C18 is compatible with such high pH values. These pH values can be reached by adding 0.1 to 0.2 % (%v/v) of diethylamine basic modifier to a 30–40 %ethanol:70–60 %water mobile phase composition.

A large set of preliminary studies were carried out to validate the solvent composition. Several pulses of nadolol were performed using a Waters XBridge C18 column (19 mm ID × 100 mm L) packed with C18 absorbent with a particle size diameter of 10 μm. These experiments were performed using the preparative HPLC system, using a 2 g/L of nadolol feed solution prepared in the same solvent. A flow-rate of 5 mL/min and an injection volume of 1000 μL were used.

Some of the most representative experimental results are presented in Fig. 1 for three ethanol:water:0.1diethylamine compositions, 30E70W, 35E65W and 40E60W.

These results confirm that the 30E70W composition represents a good situation in terms of resolution and confirm the previous published results for nadolol racemates retention using this adsorbent [1].

The same Waters XBridge C18 column was used to measure the influence of the superficial velocity, $U_0 = Q/A$, upon the Height Equivalent to a Theoretical Plate, $HETP = \sigma^2 L/\mu_1^2$ (in the former equations, Q is the flow-rate, A the column section area, L the column length, σ the peak variance and μ_1 the first moment of the chromatographic peak). The HETP results obtained for 30E70W and 40E60W compositions are presented in Fig. 2. These results show that, when using 40E60W, the HETP values remain constant (around 30 μm) for the two racemates as the flow-rate increases. For the 30E70W, the HETP value increases with the increase of the flow-rate (around 40 to 60 μm). This means a better chromatographic efficiency when using the 40E60W, being more significant at high flow-rates. However, this must be confirmed by measurements of the adsorption behaviour using high nadolol concentrations.

3.1.2. Measurement of the adsorption equilibrium isotherms

The experimental measurement of the competitive adsorption equilibrium isotherms was carried out using the adsorption–desorption static method and then validated by a dynamic method of frontal chromatography [37,38]. A detailed description of the applied methodology for modelling and fitting of competitive adsorption equilibrium isotherms can be found in previous works [39,40]. The measurements were carried
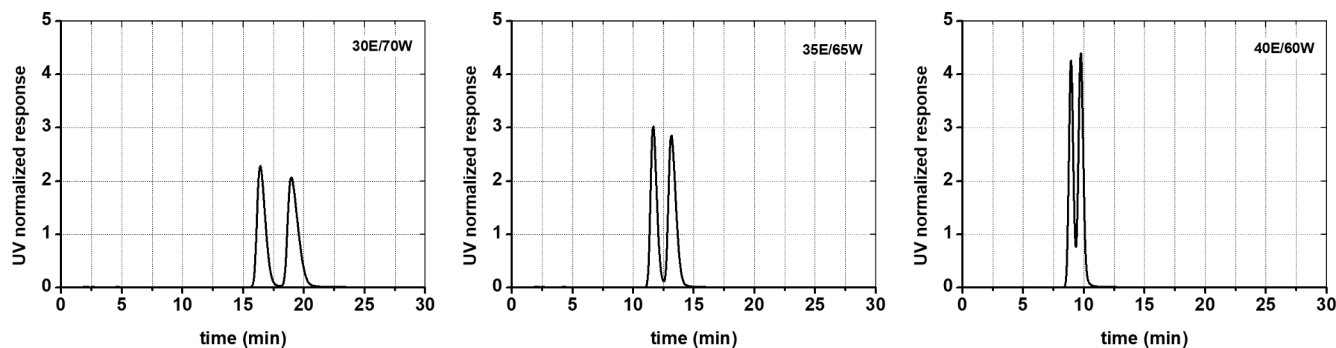
Fig. 1. Pulse experiments of nadolol using three different ethanol: water:0.1diethylamine solvent compositions: 30E70W, 35E65W and 40E60W. $C_F^T = 2$ g/L; loop of 1000 μL; Q = 5 mL/min.
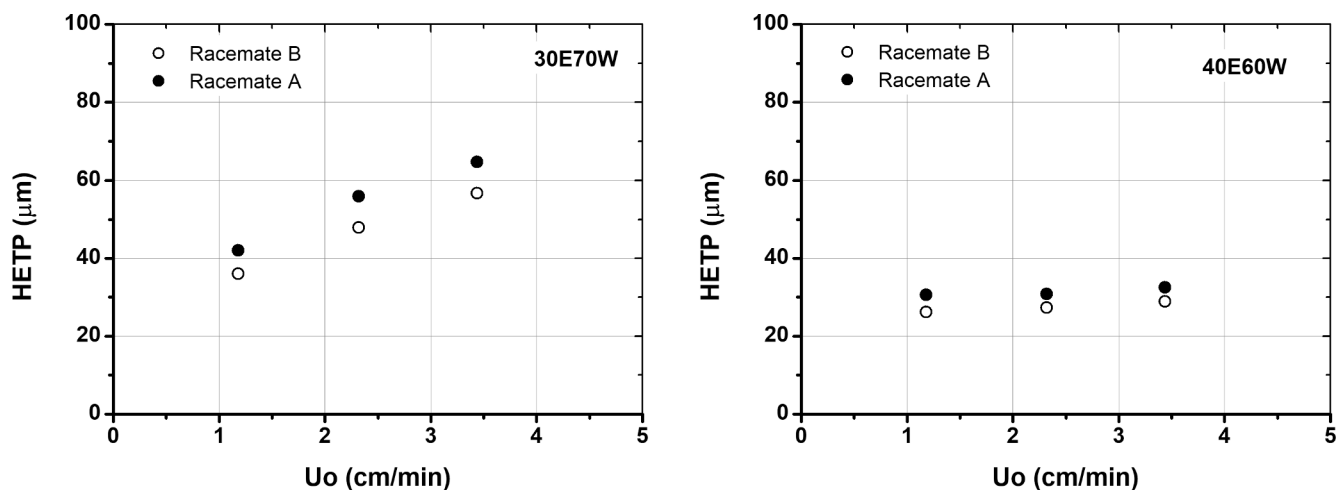

Fig. 2. HETP as a function of superficial velocity (U_0) for a Waters XBridge C18 preparative column (19 mm ID × 100 mm L) and a mobile phase composition of 30 % ethanol:70 %water:0.1 % diethylamine (left) and of 40 %ethanol:60 %water:0.1 % diethylamine (right). Open and closed circles for the less and the more retained nadolol racemate, respectively. $C_F^T = 2$ g/L; loop of 1000 μL; Q = 5, 10 and 15 mL/min.

out for the 30E70W and the 40E60W mobile phase compositions and different isotherm models were tested to describe the experimental data. The competitive linear + Langmuir model was found to better describe the experimental adsorption behaviour using the 30E70W composition and the competitive Langmuir model was found to better describe for the 40E60W composition. The selectivity versus feed concentration plots justifies the use of different models: the 40E60W shows a constant selectivity, predicted by the Langmuir model, while the 30E70W composition shows a decrease of selectivity with the increase of the feed concentration and a more complex linear + Langmuir model is needed to predict it. Table 1 presents the model equations and the estimated parameters using the Levenberg-Marquardt algorithm for both solvent compositions.

The experimental results (points) and the predicted adsorption behaviour (lines) obtained for the 30E70W and 40E60W mobile phase compositions are presented in Fig. 3. The left plot presents the adsorption equilibrium isotherm and the right plot the A/B selectivity behaviour. The selectivity plots clearly justify the use of different adsorption models: the 40E60W shows a constant selectivity, predicted by the Langmuir model, while the 30E70W composition shows a decrease of selectivity with the increase of the feed concentration and a more complex linear + Langmuir model is needed to predict it.

These results show that, for high feed concentrations such as 10 g/L ($C_B^F = C_A^F = 5g/L$), there are no significant differences in selectivity for both mobile phase compositions (1.12 for 40E60W and 1.13 for 30E70W). Nevertheless, the 40E60W composition allows higher adsorption isotherm linearity for high feed concentrations, that may be

Table 1
Selected adsorption equilibrium isotherm and estimated parameters obtained by fitting the experimental data to the competitive linear + Langmuir and Langmuir models. Average room temperature of 23 °C. Nadolol racemate A is more retained than racemate B.

Mobile Phase Composition	Theoretical Model	Estimated Parameters
30E70W	**Linear + Langmuir** $$q_B^* = mC_B + \frac{Qb_BC_B}{1 + b_BC_B + b_AC_A} ; q_A^* = mC_A + \frac{Qb_AC_A}{1 + b_BC_B + b_AC_A}$$	$m = 1.3694$ $Q = 31.02$ g/L $b_B = 9.877 \times 10^{-2}$ L/g $b_A = 1.258 \times 10^{-1}$ L/g ($SQ = 0.3245$; $SD = 0.2326$)
40E60W	**Langmuir** $$q_B^* = \frac{Qb_BC_B}{1 + b_BC_B + b_AC_A} ; q_A^* = \frac{Qb_AC_A}{1 + b_BC_B + b_AC_A}$$	$Q = 99.56$ g/L $b_B = 1.534 \times 10^{-2}$ L/g $b_A = 1.725 \times 10^{-2}$ L/g ($SQ = 0.1287$; $SD = 0.1356$)

q_i^* and C_i are, respectively, the solid and the liquid concentrations of racemate i ($i = A$, B), and m, Q, b_B and b_A are the adsorption equilibrium isotherm parameters. SQ is the sum of square of the residues and SD is the corrected standard deviation.
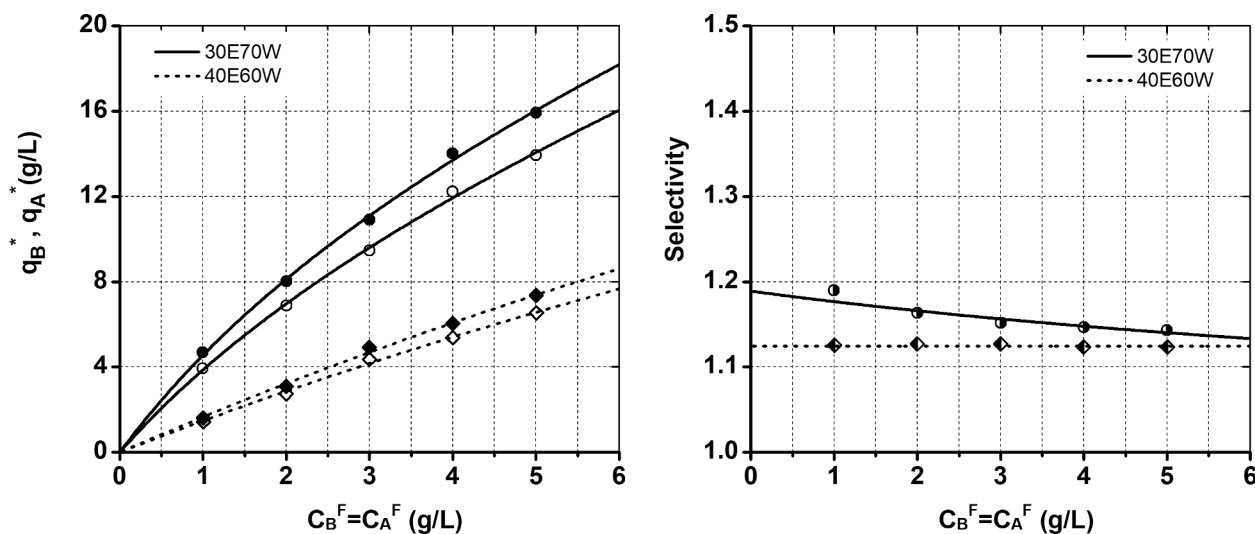
Fig. 3. Comparison between experimental (points) and model (lines) results for the adsorption equilibrium isotherms of nadolol racemates using two solvent compositions: 30 %ethanol:70 %water (solid lines) and 40 %ethanol:60 %water (dashed lines). Left figure presents the adsorption equilibrium isotherms and its fitting to a linear + Langmuir (30E70W) and to a Langmuir (40E60W) models. Right figure presents the selectivity of racemates A/B as a function of the nadolol feed concentration.

advantageous for the preparative separation.

3.1.3. Fixed-bed behaviour (frontal chromatography)

The experimental measurement of the fixed-bed adsorption behaviour is a crucial step to validate the previous selected competitive adsorption equilibrium isotherm models. When leading with preparative non-linear frontal chromatography, the modelling of the saturation and regeneration behaviours can be carried out using the linear driving force (LDF) model to describe the mass transfer resistance inside the adsorbent particle. The model equations used for fixed-bed chromatography (breakthrough) using the LDF model can be found elsewhere [40,41]. The simulation of the fixed-bed adsorption behaviour requires the estimation of the mass transfer resistance coefficient (k) and the Peclet number (Pe) that accounts with the axial dispersion effect. The mass transfer coefficient was estimated by fitting the model to the experimental data using a 10 g/L of nadolol feed solution. The Peclet number was estimated with the same experimental methodology and a 10 g/L of uracil solution, used as the non-retained compound. The

estimated values of Pe = 5000 and k = 5 s^{-1} show very good agreement with the experimental data, representing a situation of low axial dispersion and resistance to mass transfer, and validates the high column efficiency shown previously by the HETP experiments.

The experimental results (points) and the predicted adsorption behaviour (lines) obtained with the selected adsorption isotherms models for 30E70W and 40E60W solvent compositions are both presented in Fig. 4. There is a very good agreement between experimental (points) and simulation (lines) using the linear + Langmuir and Langmuir adsorption equilibrium isotherm models for saturation and regeneration steps for both the 30E70W and the 40E60W compositions. These results are a good validation of the selected models and reinforce previous conclusions for the adsorption equilibrium isotherm measurements. Also shows that the 40E60W composition, notwithstanding the decrease in selectivity, presents a decrease in the roll-up phenomena, a decrease in the retention and less dispersion (tailing) for the two racemates in the regeneration step. Knowing that the use of a solvent composition with high alcoholic content will also promote nadolol
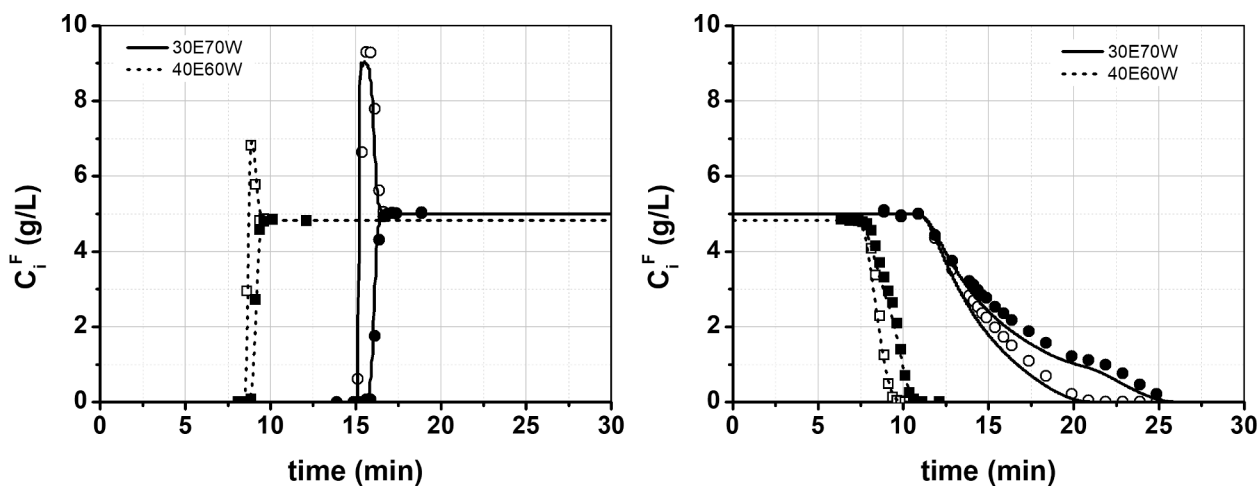

Fig. 4. Saturation (adsorption; left) and regeneration curves (desorption; right) for a 10 g/L nadolol feed concentration and 30 %ethanol:70 %water (30E70W) and 40 %ethanol:60 %water (40E60W) mobile phase compositions. Comparison between experimental (points; circles for 30E70W and squares for 40E60W) and simulation results (lines; solid for 30E70W and dashed for 40E60W). Flow-rate: 5 mL/min. Model parameters: ε = 0.4, Pe = 5000, k = 5 s^{-1}, and the linear + Langmuir (for 30E70W) or Langmuir (for 40E60W) model parameters.

solubility, all these behaviours may have a positive impact in the productivity and solvent consumption at the preparative scale.

3.1.4. Regions of complete separation for SMB operation

The performance of the SMB preparative separation process for both the 30E70W and the 40E60W solvent compositions are compared using the so called Triangle Theory [42,43]. This methodology considers the ideal counter-current operation, assumes that the mass transfer resistances and axial dispersion can be considered negligible, and that the adsorption equilibrium is described by the Langmuir or the linear + Langmuir models. More precise models can be used when dealing with more restrictive situations, where both axial dispersion and mass transfer resistances cannot be negligible [12,44].

The performance of the SMB operation is normally predicted by defining the region of complete separation. Within this region, both extract and raffinate outlet streams are collected pure. The region is obtained by plotting γ_{III} as a function of γ_{II}, where $\gamma_j = v_j/u_s$ is the ratio between the interstitial velocities of the liquid and the solid in section j of the equivalent true moving bed concept (counter-current operation). The vertex of this region represents geometrically the furthest point from the diagonal $\gamma_{III} = \gamma_{II}$ and so, it represents the optimal operation conditions in terms of productivity and solvent consumption still achieving complete separation (100 % pure racemates at both the extract and the raffinate outlet streams). Fig. 5 presents the predicted regions of complete separation using nadolol feed concentrations of 2 and 10 g/L for both the 30E70W and the 40E60W mobile phase compositions.

As expected, the increase in the feed concentration leads to higher productivities but to smaller triangle separation regions and, consequently, more harder experimental SMB operating conditions. In this case, the 40E60W presents very small complete separation areas, meaning that the SMB unit will be quite more difficult to operate using this composition.

3.1.5. SMB performance parameters

The SMB operation can be characterized in terms of performance parameters, namely, purity, recovery, productivity and solvent consumption. For the pseudo-binary separation of nadolol racemates it is expected that racemate A, the more retained racemate, to be completely recovered and collected in the extract, while the less retained racemate B to be completely recovered and collected in the raffinate stream.

The extract purity (PU_X, %) is defined as the ratio between the mean

concentration of the more retained racemate A and the sum of the mean concentrations of the two racemates in the extract stream. Similarly, the raffinate purity (PU_R, %) is defined as the ratio between the mean concentration of the less retained racemate B and the sum of the mean concentrations of the two racemates in the raffinate stream. The recovery in the extract stream (RC_X, %) is defined as the ratio between the amount of the more retained racemate A collected in the extract and the total amount of the same racemate A fed into the system. The recovery in the raffinate stream (RC_R, %) is defined as the ratio between the amount of the less retained racemate B obtained in the raffinate and the total amount of the same racemate B fed into the system. Inside a SMB complete separation region both purities and both recoveries are 100 %, as $\overline{C}_B^X = 0$ and $\overline{C}_A^R = 0$, as well as $\overline{C}_A^X = Q_F C_A^F/Q_X$ and $\overline{C}_B^R = Q_F C_B^F/Q_R$.

The system productivity of racemate A (PR_A, g/Lbed.hr) is defined as the amount of the more retained racemate A recovered in the extract stream per total volume of bed and per time. Similarly, the system productivity of racemate B (PR_B, g/Lbed.hr) is defined as the amount of the less retained racemate B recovered in the raffinate stream per total volume of bed and per time. A global system productivity can also be defined as the sum of these two amounts. Inside a SMB complete separation region, as purities and recoveries are 100 %, this global system productivity is also equal to the amount of nadolol racemates fed into the system per total volume of bed and per time, $PR = Q_F(C_A^F + C_B^F)/V_T$.

The solvent consumption (SC, $L_{solvent}$/g) is defined as the ratio between the total amount of solvent fed into the system (in eluent and feed) and the total amount of nadolol racemates recovered in the extract (racemate A) and in the extract (racemate B) streams. As well, inside a SMB complete separation region, as purities and recoveries are 100 %, solvent consumption is also equal to the ratio between the total amount of solvent fed into the system (in eluent and feed) and the amount of nadolol racemates fed into the system, $SC = (Q_E + Q_F)/\left[Q_F(C_A^F + C_B^F)\right]$ or $SC = (Q_E + Q_F)/(PR \times V_T)$.

The SMB performance parameters are summarized in Table 2. Fig. 6 presents the prediction of SMB productivity and solvent consumption for the two solvent compositions, as a function of the nadolol feed concentration, for a maximum flow-rate of 20 mL/min in section I, a safety margin of 20 % in both sections I and IV, and at the vertex points of the correspondent complete separation regions. The differences in the productivity for 40E60W and 30E70W naturally arise from the different adsorption equilibrium isotherms, presented in Fig. 3. As expected, (lower ethanol content), the 30E70W solvent composition presents higher adsorption and less linearity of the adsorption isotherms, leading
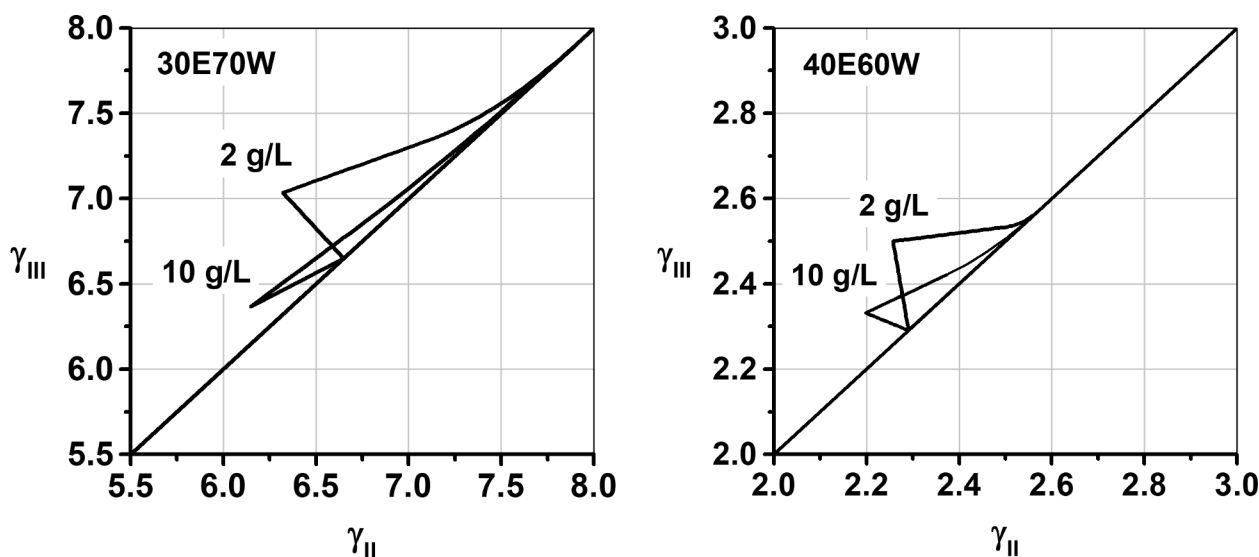

Fig. 5. SMB regions of complete separation for the pseudo-binary separation of nadolol racemates for 30E70W (left plot) and 40E60W (right plot) solvent compositions and for nadolol feed concentrations of 2 and 10 g/L.

Table 2

SMB performance parameters. A represents the more retained racemate to be recovered in the extract and B represents the less retained racemate to be recovered in the raffinate stream.

Extract Purity $PU_X(\%)$	$PU_X = \dfrac{\overline{C}_A^X}{\overline{C}_A^X + \overline{C}_B^X}$
Raffinate Purity $PU_R(\%)$	$PU_R = \dfrac{\overline{C}_B^R}{\overline{C}_A^R + \overline{C}_B^R}$
Extract Recovery $RC_X(\%)$	$RC_X = \dfrac{Q_X \overline{C}_A^X}{Q_F C_A^F}$
Raffinate Recovery $RC_R(\%)$	$RC_R = \dfrac{Q_R \overline{C}_B^R}{Q_F C_B^F}$
Global Recovery $RC(\%)$	$RC = \dfrac{Q_X \overline{C}_A^X + Q_R \overline{C}_B^R}{Q_F (C_A^F + C_B^F)} = \dfrac{RC_X + RC_R}{2}$ if $C_A^F = C_B^F$
Productivity of racemate A $PR_A(\text{g/Lbed.hr})$	$PR_A = \dfrac{Q_X \overline{C}_A^X}{V_T}$
Productivity of racemate B $PR_B(\text{g/Lbed.hr})$	$PR_B = \dfrac{Q_R \overline{C}_B^R}{V_T}$
Global Productivity $PR(\text{g/Lbed.hr})$	$PR = \dfrac{Q_X \overline{C}_A^X + Q_R \overline{C}_B^R}{V_T} = PR_A + PR_B$
Solvent Consumption $SC(\text{L}_{\text{solvent}}/\text{g})$	$SC = \dfrac{Q_E + Q_F}{Q_X \overline{C}_A^X + Q_R \overline{C}_B^R}$

to vertex points closer to the diagonal $\gamma_{III} = \gamma_{II}$ when dealing with high feed concentrations. This is clearly perceptible by comparing the evolution of the complete separation regions shown in Fig. 5, and justifies the SMB productivity for 30E70W to reach a lower value and a plateau for lower feed concentrations when compared with 40E60W (Fig. 6). Basically, Fig. 6 shows that there are no significant differences in SMB productivity and solvent consumption for a nadolol feed concentration of 2 g/L but, for high feed concentrations, such as 10 g/L, the 40E60W composition presents better SMB productivity performance, despite its smaller separation region and, so, harder SMB operating conditions.

3.1.6. SMB operating conditions

The experimental SMB preparative pseudo-binary separation of the nadolol racemates was performed using a 2 g/L feed solution. For this feed concentration, similar performance parameters are predicted for the 30E70W and the 40E60W solvent compositions. Avoiding the small region of complete separation associated with 40E60W, the 30E70W was the selected solvent composition to perform the SMB separation. The system was initially stabilized in order to identify the maximum allowed flow-rate in section I due to pressure drop limitations. Since this section represents the maximum local pressure inside the unit, a maximum pressure drop of 75 bar was imposed in the lab-scale

FlexSMB®-LSRE unit, corresponding to a flow-rate in section I of $Q_I^* = 5 mL/min$. This represents a very low experimental maximum flow-rate, due to the hardware characteristics of the pilot unit, the use of a 10 μm adsorbent, and the high viscosity of the mobile phase. Even knowing that, under these conditions, very low productivity will be obtained, the system was run to validate the SMB separation methodology.

The critical values of γ_I and γ_{IV} were fixed using $\gamma_I = \frac{1-\varepsilon}{\varepsilon} K_A \beta$ and $\gamma_{IV} = \frac{1-\varepsilon}{\varepsilon} K_B / \beta$, where K_i is the initial slope of the adsorption equilibrium isotherm ($K_i = m + Qb_i$ for the linear + Langmuir model and $K_i = Qb_i$ for the Langmuir model), and β is the safety margin, considered 20 %, that is, $\beta = 1.2$. The switching time value was fixed using $t^* = \frac{\varepsilon V_c}{Q_I^*}(\gamma_I + 1)$ giving $t^* = 23.79 min$. The final SMB operating conditions were then defined using the separation region obtained using the Triangle Theory methodology. A pair of $(\gamma_{II}; \gamma_{III})$ values inside the triangle was chosen and the corresponding internal SMB flow-rates Q_j^* in sections II, III and IV were estimated using $Q_j^* = \frac{\varepsilon V_c}{t^*} \gamma_j^*$ ($j = II, III, IV$), where $\gamma_j^* = \gamma_j + 1$. The inlet, outlet and recycle flow-rates were then calculated through $Q_E = Q_I^* - Q_{IV}^*$; $Q_X = Q_I^* - Q_{II}^*$; $Q_F = Q_{III}^* - Q_{II}^*$; $Q_R = Q_{III}^* - Q_{IV}^*$; $Q_{REC} = Q_{IV}^*$. Table 3 summarizes the initial experimental operating conditions used for the SMB operation. Fig. 7 shows the separation region (triangle) predicted for the 30E70W solvent composition using a feed concentration of 2 g/L and the proposed $\gamma_{III} \times \gamma_{II}$ operating point (open circle) that represents the initial SMB operating conditions presented in Table 3.

3.1.7. SMB experimental operation and performance

During the SMB experimental operation, both raffinate and extract outlet streams were collected and weighted at the end of each full cycle (six rotations) in order to monitor the total recovered mass. In addition,

Table 3

Initial operating conditions used for the SMB preparative separation of nadolol racemates.

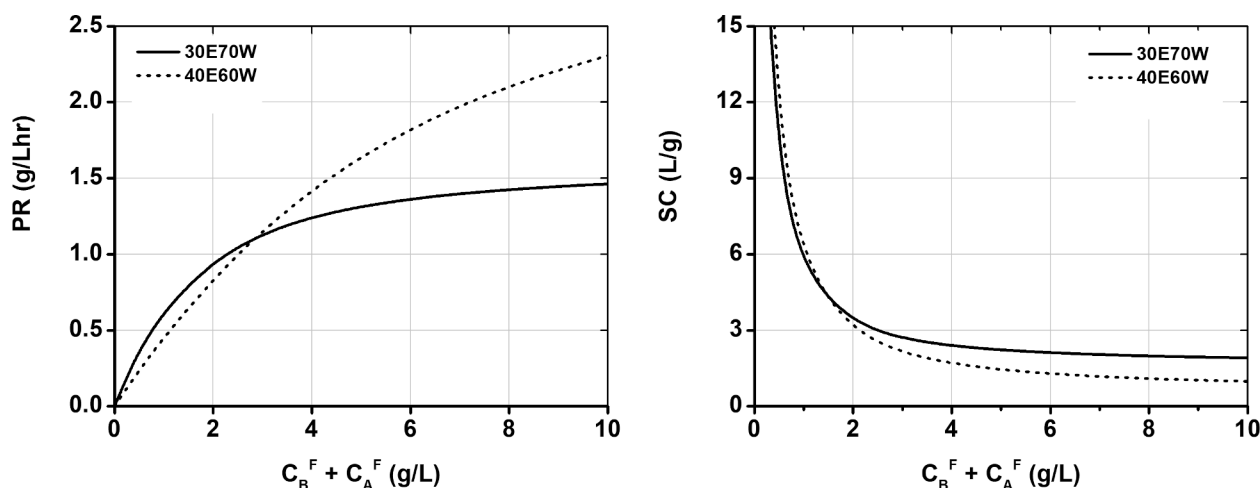| *Column, packing parameters and feed concentration*		
$N_j = [1, 2, 2, 1]$	$d_P = 10 \mu m$	
$D_C = 1.9 cm$	$\varepsilon = 0.4$	
$L_C = 10 cm$	$C_F^T = 2 g/L$	
SMB initial operating conditions		
$\gamma_I = 9.4891$	$Q_I^* = 5.00 mL/min$	$Q_E = 1.88 mL/min$
$\gamma_{II} = 6.5000$	$Q_{II}^* = 3.58 mL/min$	$Q_X = 1.42 mL/min$
$\gamma_{III} = 7.0000$	$Q_{III}^* = 3.81 mL/min$	$Q_F = 0.24 mL/min$
$\gamma_{IV} = 5.5416$	$Q_{IV}^* = 3.12 mL/min$	$Q_R = 0.70 mL/min$
$t^* = 23.79 min$		$Q_{REC} = 3.12 mL/min$

Fig. 6. Prediction of SMB productivity (left) and solvent consumption (right) for the two solvent compositions, as a function of the nadolol feed concentration. Solid line for the 30E70W, dashed line for the 40E60W solvent composition.
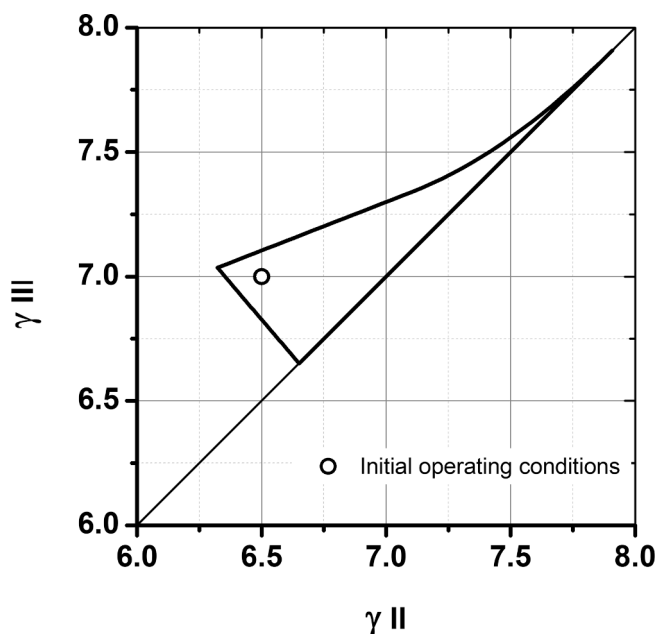
Fig. 7. SMB region of complete separation (triangle) and experimental SMB operating point (open circle) for the pseudo-binary separation of nadolol racemates using a feed concentration of 2 g/L and a 30 %ethanol:70 %water solvent composition.

two Coriolis flow-meters were used, one positioned at the extract outlet stream to measure the correspondent extract flow-rate, another located immediately before section II to measure the flow-rate inside this section. These experimental measurements allow the validation of the SMB operating conditions and the accurate flow-rates observed inside and outside the unit. All samples were analysed by analytical HPLC using the same solvent composition and the same Waters XBridge C18 adsorbent but with a particle size diameter of 5 μm. The cycle steady state was considered to be achieved when the average concentrations at the extract and the raffinate outlet streams do not vary more than 5 % after ten full cycles, and both the recoveries and the purities of outlet streams were validated to be no less than 99.9 %. At that moment, the SMB internal concentration profiles were measured by collecting samples at 50 %, 75 % and 95 % of each switching time, by means of a six-port valve located at the end of section IV.

The average racemate concentrations at the extract and the raffinate products were measured and the performance parameters of recovery, purity, productivity and solvent consumption were calculated using equations presented in Table 2. A summary of the experimental SMB

Table 4
Experimental SMB operating conditions and results for the separation of a 2 g/L nadolol feed concentration and a 30 %ethanol:70 %water solvent composition.

Experimental SMB operating conditions		
$Q_E = 2.05 mL/min$	$Q_I^* = 5.05 mL/min$	$\gamma_I = 9.5932$
$Q_X = 1.34 \frac{mL}{min}$	$Q_{II}^* = 3.71 mL/min$	$\gamma_{II} = 6.7824$
$Q_F = 0.18 \frac{mL}{min}$	$Q_{III}^* = 3.89 mL/min$	$\gamma_{III} = 7.1599$
	$Q_{IV}^* = 3.00 mL/min$	$\gamma_{IV} = 5.2930$
$Q_R = 0.89 \frac{mL}{min}$		
$Q_{REC} =$		
$3.00 \frac{mL}{min}$		
$t^* = 23.79 min$		
Outlet concentrations and performance parameters		
$\overline{C}_A^X = 0.1343 g/L$	$PU_X = PU_R = 100\%$	$PR = 0.13 g/(L.hr)$
$\overline{C}_B^R =$	$RC_X = RC_R = 100\%$	$SC = 6.19 L/g$
$0.2022 g/L$		

operating conditions and results can be found in Table 4.

Fig. 8 presents the experimental (points) and the predicted (lines) transient evolution (24 full cycles) of the nadolol racemate concentrations in the extract and raffinate outlet streams. Fig. 9 presents the experimental (points) and predicted (lines) cycle steady state internal concentration profiles in the six columns of the SMB unit (measured at cycles 20 and 22). The internal concentration profiles presented in Fig. 9 show two small plateaus near the extract and raffinate outlets that are due of the dead volumes of the FlexSMB-LSRE unit, taken in account in the simulation software. Despite the operational difficulties due to high pressure drops and low internal flow-rates, Figs. 8 and 9 show a very good agreement between the experimental results and the predictions using the transient SMB model. This experiment also validates the capacity to complete separate (100 % purities and recoveries) the two nadolol racemates using an achiral C18 adsorbent by SMB chromatography.

3.2. FB preparative separation

The use of fixed-bed (FB) preparative separation was also validated and characterized for the separation of nadolol racemates. Different ethanol:water:diethylamine solvent compositions were studied (solvent screening). The amount of diethylamine added in each solvent mixture was established in order to achieve a pH value near 12, referred by the column manufacturer as the upper safety limit for the C18 adsorbent, but ensuring its capacity to resolve the nadolol racemates with a pKa of 9.67. Additionally, the fixed-bed flow-rate was chosen in order to operate with a system pressure drop around 150 bar. Six ethanol:water: diethylamine solvent compositions were tested using a nadolol feed concentration of 9 g/L. All feed solutions were prepared using the same solvent composition used as mobile phase. The Azura preparative HPLC system was equipped with one Waters XBridge preparative OBD C18 column (dimensions of 30 mm ID × 250 mm L) and a 10 mL feed injection loop.

3.2.1. FB performance parameters

The FB performance is determined based in a equivalence with the SMB operation taking into consideration the fractions of pure racemates recovered and the waste(s) fraction(s) in each run. For each injection, the less retained racemate B is recovered in the first product fraction (fraction B), while the more retained racemate A is recovered in the second product fraction (fraction A), ensuring the minimum purity standards. Between the first and the second product fractions there is a waste fraction containing both racemates A and B.

The purity of each FB product fraction, PU_A and PU_B (%), is calculated as the ratio between the concentration of the desired racemate and the total concentration of both racemates in that fraction. The recovery of each racemate, RC_i (%), is defined as the ratio between the amount of that racemate collected in its fraction and the total amount of that racemate fed into the system. As in FB operation the product fractions are not collected continuously, as in SMB operation, a global recovery, RC (%), can be defined as the ratio between the total amount of racemates A and B collected in the desired product fractions (fraction A and B, respectively) and the total amount of racemates A and B fed into the system. Contrary to SMB operating inside its complete separation region, FB does not usually operates with 100 % recovery since that would impose a baseline separation of the two racemates. In that way, FB operation usually has a waste fraction between the two pure product fractions. If both product fractions are pure and considering that racemates A and B are detected the same way by the UV equipment, the global recovery can also be experimentally calculated using the chromatogram areas of each fraction solution, that is, $RC = \frac{A_B + A_A}{A_B + A_w + A_A}$, where A_B, A_w and A_A are the chromatogram areas of the less retained racemate B fraction, of the waste W fraction and of the more retained racemate A fraction, respectively.
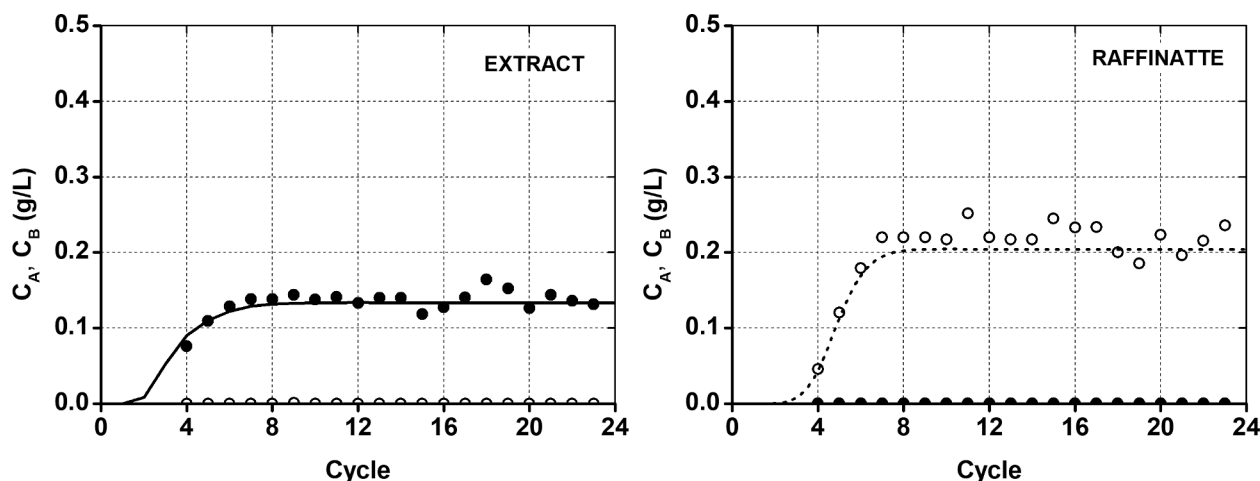
Fig. 8. Experimental (points) and predicted (lines) transient evolution (24 full cycles) of the nadolol racemate concentrations in the extract and raffinate outlet streams. Closed circles for the more retained racemate A, open circles for less retained racemate B. Model parameters of $\varepsilon = 0.4$, Pe = 5000, k = 5 s^{-1}, and the linear + Langmuir isotherm model parameters as in Table 1.
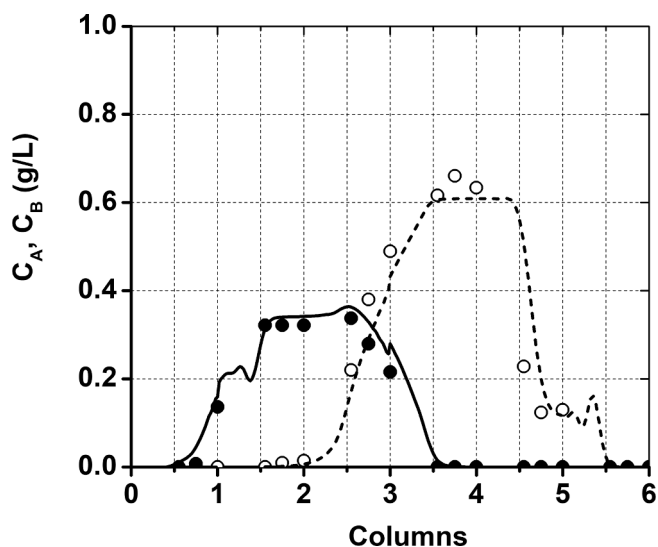

Fig. 9. Experimental (points) and predicted (lines) internal concentration profiles of the nadolol racemates at cycle steady state. Closed circles for the more retained racemate A, open circles for less retained racemate B. Model parameters of $\varepsilon = 0.4$, Pe = 5000, k = 5 s^{-1}, and the linear + Langmuir isotherm model parameters as in Table 1.

The system productivity of each racemate (PR_i, g/Lbed.hr) is defined as the amount of that racemate recovered in its product fraction per total volume of bed and per time. A global system productivity can also be defined as the sum of these two amounts. The solvent consumption (SC, L$_{solvent}$/g) is defined as the ratio between the total amount of solvent fed into the system and the total amount of racemates recovered in its product fractions. The FB performance parameters are summarized in Table 5.

It can also be defined the equivalent inlet and outlet flow-rates for the FB operation: the feed flow-rate as $Q_F = V_{inj}/\Delta t_{inj}$, the eluent flow-rate as $Q_E = Q - Q_F$, the raffinate flow-rate as $Q_R = (\Delta t_{f_B}/\Delta t_{inj})Q$, the extract flow-rate as $Q_X = (\Delta t_{f_A}/\Delta t_{inj})Q$, and a waste flow-rate as $Q_W = (\Delta t_{f_W}/\Delta t_{inj})Q$, where Δt_{f_j} is the collecting time period of the fraction j, $\Delta t_{f_B} + \Delta t_{f_W} + \Delta t_{f_A} = \Delta t_{inj}$ and $Q = Q_E + Q_F = Q_R + Q_W + Q_X$. Using these equivalent flow-rates, the FB performance equations presented in Table 5 can be re-written the same way as the performance equations for SMB operation presented in Table 2.

Table 5
FB performance parameters. A and B represent the more and the less retained racemate, respectively.

Purity of fraction A PU_A(%)	$PU_A = \dfrac{\overline{C}_A^{f_A}}{\overline{C}_A^{f_A} + \overline{C}_B^{f_A}}$
Purity of fraction B PU_B(%)	$PU_B = \dfrac{\overline{C}_B^{f_B}}{\overline{C}_A^{f_B} + \overline{C}_B^{f_B}}$
Recovery in fraction A RC_A(%)	$RC_A = \dfrac{V_{f_A}\overline{C}_A^{f_A}}{V_{inj}C_A^F}$, with $V_{f_A} = Q\Delta t_{f_A}$
Recovery in fraction B RC_B(%)	$RC_B = \dfrac{V_{f_B}\overline{C}_B^{f_B}}{V_{inj}C_B^F}$, with $V_{f_B} = Q\Delta t_{f_B}$
Global Recovery RC(%)	$RC = \dfrac{V_{f_A}\overline{C}_A^{f_A} + V_{f_B}\overline{C}_B^{f_B}}{V_{inj}(C_A^F + C_B^F)} = \dfrac{RC_A + RC_B}{2}$ if $C_A^F = C_B^F$
Productivity of racemate A PR_A(g/Lbed.hr)	$PR_A = \dfrac{V_{f_A}\overline{C}_A^{f_A}}{V_T\Delta t_{inj}} = RC_A\dfrac{V_{inj}C_A^F}{V_T\Delta t_{inj}}$
Productivity of racemate B PR_B(g/Lbed.hr)	$PR_B = \dfrac{V_{f_B}\overline{C}_B^{f_B}}{V_T\Delta t_{inj}} = RC_B\dfrac{V_{inj}C_B^F}{V_T\Delta t_{inj}}$
Global Productivity PR(g/Lbed.hr)	$PR = PR_A + PR_B = \dfrac{V_{f_A}\overline{C}_A^{f_A} + V_{f_B}\overline{C}_B^{f_B}}{V_T\Delta t_{inj}} = RC\dfrac{V_{inj}(C_A^F + C_B^F)}{V_T\Delta t_{inj}}$
Solvent Consumption SC(L$_{solvent}$/g)	$SC = \dfrac{Q\Delta t_{inj}}{V_{f_A}\overline{C}_A^{f_A} + V_{f_B}\overline{C}_B^{f_B}} = \dfrac{1}{PR}\dfrac{Q}{V_T}$

C_i^F is the feed concentration of racemate i, $\overline{C}_i^{f_j}$ is the average concentration of racemate i in product fraction j, V_{inj} is the feed injection volume, Δt_{inj} is the injection time interval (time between two successive feed injections), V_{f_j} is the volume of product fraction j, Δt_{f_j} is the collecting time period of product fraction j, V_T is the volume of the FB column, and Q is the FB flow-rate.

3.2.2. FB experimental operation and performance

The experimental results obtained for three consecutive pulses using six different ethanol:water:diethylamine (E:W:DEA) compositions are presented in Fig. 10 and the correspondent FB performance parameters can be found in Table 6. For each run, the injection time interval (time between two successive feed injections) was adjusted (reduced) till eliminating the need for a second waste fraction between the elution of two consecutive feed bands and to ensure that the column is continuously used to separate the two racemates. This means that, for each feed injection, there is only one waste fraction between the two product fractions, ensuring the desired purities. The fraction of the more retained racemate is then contiguous to the fraction of the less retained racemate coming out from the next injection.

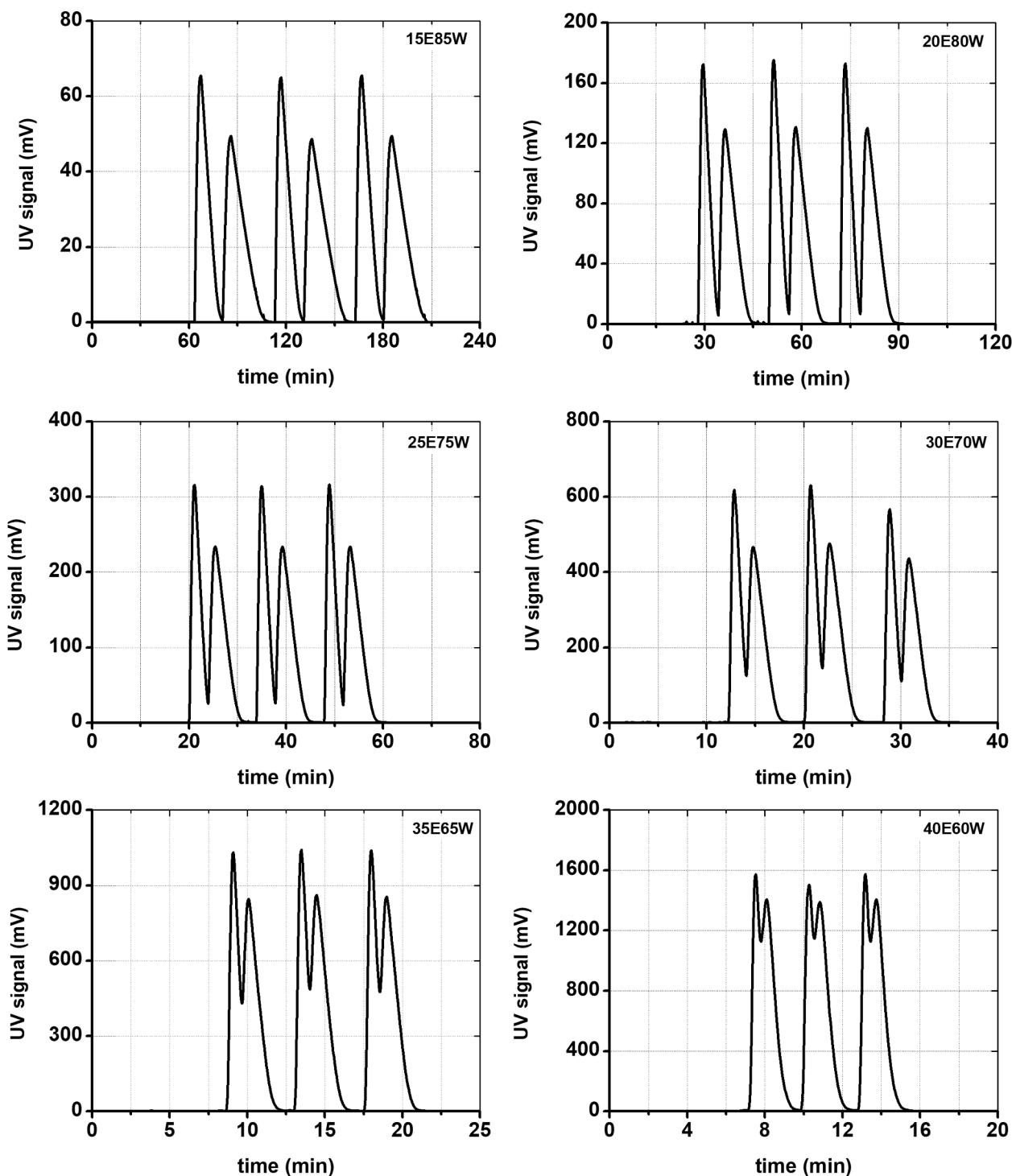As expected, increasing the ethanol content in the solvent mixture

Fig. 10. Experimental FB chromatograms of three consecutive pulses of nadolol racemates using different ethanol:water solvent compositions. Nadolol feed concentration of 9 g/L, injection volume of 10 mL. Flow-rate and injection time interval according to Table 6.

significantly decreases the retention time, together with a decrease in the baseline resolution. The best solvent composition arises from the trade-off between retention time (and time between two successive injections) and the recovery of pure fractions, ensuring a minimum purity standard. The experimental results presented in Table 6 show that the higher productivity and lower solvent consumption are obtained for a solvent composition between 30 and 35 %ethanol:70–65 %water (runs 4 and 5). Fig. 11 presents the HPLC analysis of the first, waste and second fractions collected at run 4. Considering the first feed injection period, the first fraction (product fraction B) was experimentally collected from

11.8 to 13.3 min, the waste fraction (mixture of racemates A and B) was collected from 13.3 to 15.3 min, and the second fraction (product fraction A) from 15.3 to 18.9 min. The first fraction is 100 % pure racemate B and the second fraction is 99.9 % pure racemate A.

Figs. 12 and 13 present the effect of solvent composition (ethanol content in a ethanol:water mixture) in flow-rate, injection time interval (time between two successive feed injections), recovery, productivity and solvent consumption of the FB operation.

The results presented in Fig. 12 clearly show that the increase of the ethanol content (in the range of 15 to 40 % of ethanol in water) leads to a

Table 6

Experimental FB performance parameters for different ethanol:water solvent compositions.

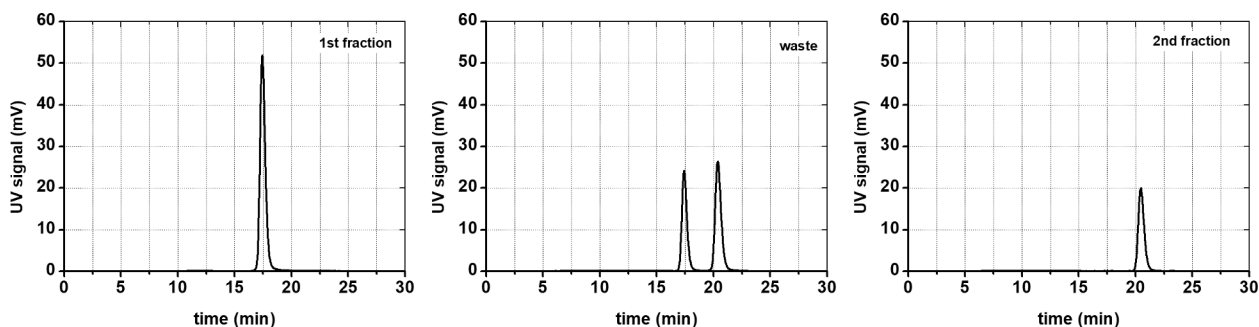| RUN	E:W:DEA %(v/v/v)	pH	Q(mL/min)	ΔP (bar)	Δt_{inj}(min)	RC(%)	PU_B(%)	PU_A(%)	PR(g/L.hr)	SC(L/g)
1	15:85:0.30	11.9	54	154	47.4	96.1	100	99.4	0.62	29.6
2	20:80:0.25	11.8	50	153	18.5	94.3	100	99.7	1.56	10.9
3	25:75:0.15	11.8	42	155	12.5	89.7	100	99.9	2.19	6.50
4	30:70:0.15	11.9	38	153	7.1	60.8	100	99.9	2.62	4.93
5	35:65:0.15	11.9	35	154	4.8	40.7	100	99.8	2.59	4.59
6	40:60:0.15	11.9	32	153	3.8	17.7	100	99.8	1.42	7.63

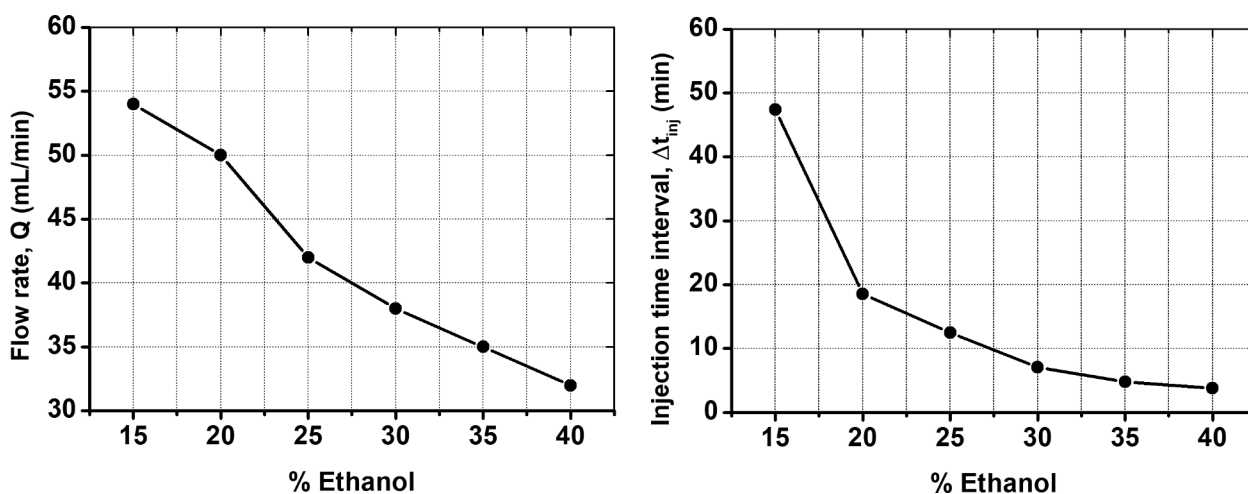**Fig. 11.** HPLC analysis of the three collected fractions of run 4.

Fig. 12. Effect of the ethanol content in the flow-rate (left plot) and in the injection time interval (time between two consecutive feed injections, right plot) for the FB preparative separation of nadolol racemates.
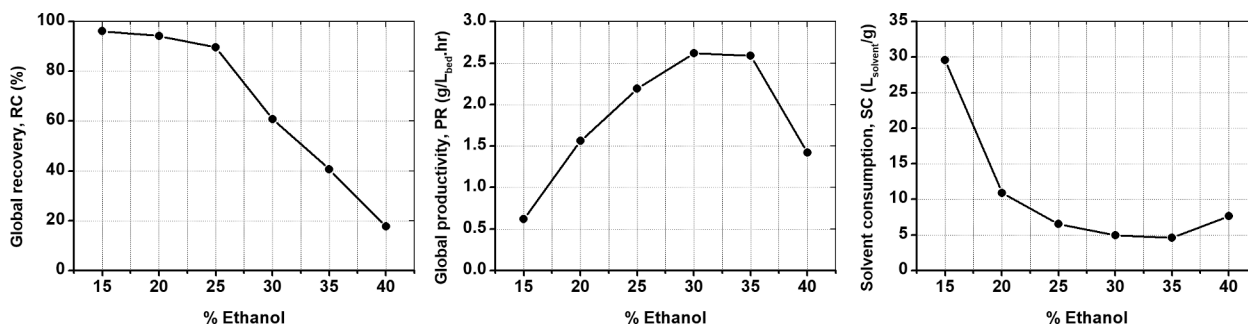

Fig. 13. Effect of the ethanol content in recovery (left plot), system productivity (middle plot) and solvent consumption (right plot) for the FB preparative separation of nadolol racemates.

significant decrease in the operating flow-rate due to the increase of solvent viscosity and system pressure drop (in order to keep system pressure drop around 150 bar). The increase of the ethanol content also decreases the injection time interval (the time between two consecutive feed injections) due to the significant decrease in retention. This effect explains the significant increase of system productivity and the decrease in solvent consumption as showed in Fig. 13. However, the decrease in retention also implies an important decrease in resolution and, consequently, a significant decrease in recovery. For ethanol contents higher than 25 % FB recovery starts to decrease significantly. In this way, a trade-off between retention and recovery (resolution) must be achieved. For the FB preparative separation of nadolol racemates, a good compromise is obtained using a 30 to 35 % of ethanol content when both productivity and solvent consumption are optimized.

3.2.3. Validation of the estimated adsorption equilibrium isotherm model and kinetic data for FB simulation

The equilibrium and kinetic data estimated using one SMB column were validated in the FB preparative column. Although presenting different dimensions (19 mm ID × 100 mm L versus 30 mm ID × 250 mm L) both columns are packed with the same C18 material (Waters XBridge C18 adsorbent of 10 μm particle size diameter). The validation was carried out for the FB operation using the solvent composition of 30 % ethanol:70 %water (Fig. 10, run 4), since this was the solvent composition used in the SMB column experiments, including the measurement of the adsorption equilibrium isotherms, breakthrough experiments and estimation of mass transfer resistance and axial dispersion. The same adsorption model parameters were used to predict the three consecutive pulses in the FB preparative column. The results are presented in Fig. 14 and show a very good agreement between the experimental and simulated chromatograms, validating the use of these model parameters to predict both the SMB and the FB operations.

3.2.4. Optimisation of FB operation

The FB operation was optimised by simulation for the selected mobile phase composition (30 %ethanol:70 %water) and using the model parameters validated in the previous section. The flow-rate was fixed to 38 mL/min considering the maximum system pressure drop around 150 bar, and the total feed concentration to 9 g/L considering it close to the solubility limit. The optimisation was then carried out by studying the effect of the feed injection volume, V_{inj}, in the FB performance parameters, ensuring both product fractions A and B 99.99 % pure. Figs. 15
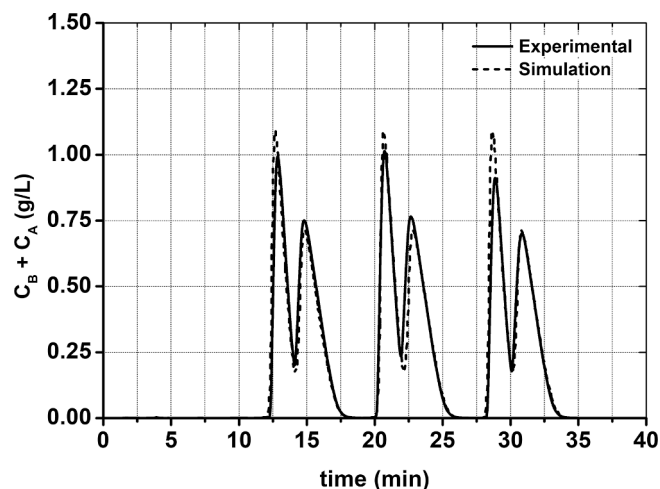
and 16 show how the feed injection volume influences the injection time interval (time between two consecutive feed injections), the equivalent flow-rates (as defined in Section 3.2.1), the recovery, system productivity and solvent consumption.

Contrary to SMB, FB operation with high feed injection volumes (equivalent to high feed flow-rates) implies big waste fractions and low recoveries. It should be underlined that the presence of a waste fraction (low recoveries) represents the waste of part of the feed or the need of its reuse as feed, increasing the cost of the separation process. For the FB separation of nadolol racemates under the conditions studies, Figs. 15 and 16 show that the productivity of racemates A and B are higher for feed injection volumes between 10 and 20 mL (roughly, 6–12 % of the column volume). As the recoveries significantly decrease for feed injections volumes higher than 5 mL, a 10 mL injection volume can be chosen as the best choice to achieve high productivities with acceptable recoveries. Additionally, a 10 mL feed injection volume also presents a solvent consumption close to the minimum. Fig. 17 presents the simulated chromatograms of nadolol racemates and purities under these best FB operating conditions and the indication of the successive product and waste fractions. In short, nadolol feed is injected in the FB column each $\Delta t_{inj} = 7.10 min$ and B, W and A fractions are collected at the time periods of $t_{f_B} \in [11.70 + 7.10(n_i - 1); 13.45 + 7.10(n_i - 1)]$, $t_{f_W} \in [13.45 + 7.10(n_i - 1); 15.00 + 7.10(n_i - 1)]$, and $t_{f_A} \in [15.00 + 7.10(n_i - 1); 18.80 + 7.10(n_i - 1)]$, respectively, and where $n_i = 1, 2, 3, \cdots$ is the number of the feed injection.

In resume, this simulation study shows that a 10 mL feed injection volume represents a good compromise by obtaining high productivity (87.5 % of the maximum using 20 mL) and acceptable recovery (71.2 %), as well as low solvent consumption (114.2 % of the minimum using 20 mL). Despite the 20 mL feed injection volume allows maximum productivity and minimum solvent consumption, it presents a very low recovery (44.6 %). Comprehensively, 100 % recovery is obtained only when using a very low feed injection volume (less than 1 mL) where productivity is very low and with very high solvent consumption. It should be underlined that a more precise optimisation procedure of the FB operation would need the definition of an objective function taking into account the cost of FB operation, particularly the cost of feed and solvent, and the cost of feed and solvent reuse.

3.3. SMB and FB performance comparison

The performance values obtained experimentally in this work for the SMB operation may mislead the reader when selecting the better technology. It is well known that the continuous SMB has advantages when compared to the discontinuous FB operation, namely the continuous injection of feed and the simulated counter-current contact between the solid and the liquid phases that maximizes the mass transfer driving force, leading to an increase of the system productivity and a decrease of the solvent consumption. Additionally, SMB operating at complete separation regions presents full recoveries of the injected feed (100 %), while FB operation presents better system productivities with the presence of waste fractions and low recoveries. It must be clarified that the SMB and FB experimental results obtained in this work cannot be directly compared. As referred before, due to the specific hardware limitations of the lab-scale SMB-LSRE unit, the use of a 10 μm C18 adsorbent material and ethanol:water solvents imposed a maximum flow-rate of 5 mL/min, leading to low productivity performance. To establish a possible comparison, the simulation of the SMB operation can be carried out under equivalent conditions to the optimised FB operation.

The following equivalences are used:

(i) The same total adsorbent amount (column volume) is used in both situations: for FB, $D = 3$ cm, $L = 25$ cm; for SMB, as it is composed by six columns, simulation uses the same diameter and

Fig. 14. Experimental (solid line) and simulated (dashed line) FB chromatograms of three consecutive pulses of a 9 g/L nadolol feed solution, using a 30:70 ethanol:water solvent composition (run 4 of Fig. 11). Model parameters of ε = 0.4, Pe = 5000, k = 5 s^{-1}, and the linear + Langmuir isotherm model parameters as in Table 1.
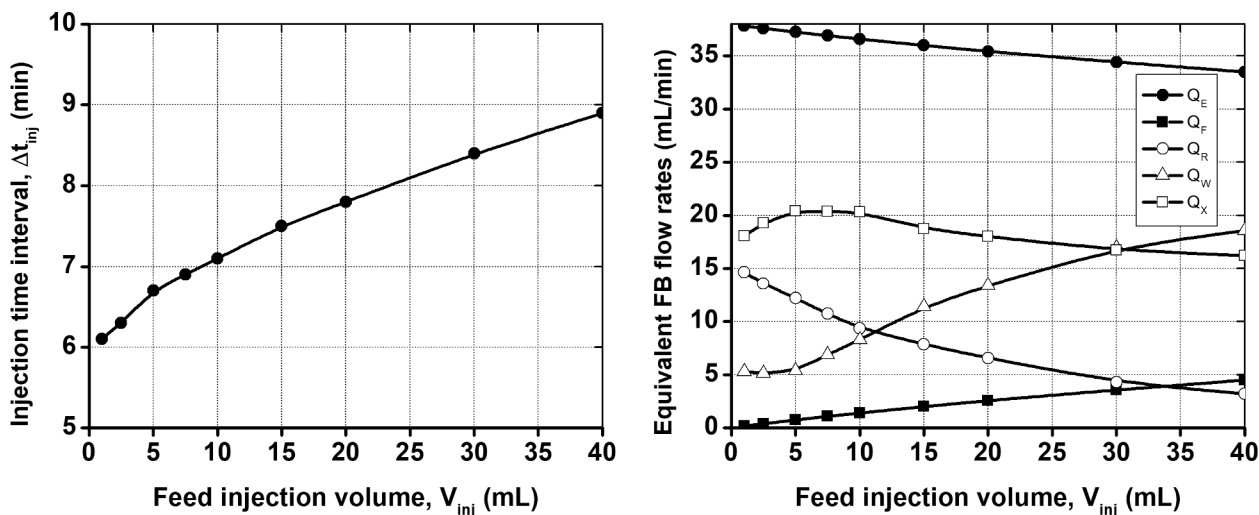
Fig. 15. Effect of the feed injection volume in the injection time interval (time between two consecutive feed injections, left plot) and in the equivalent flow-rates (right plot) for the FB preparative separation of nadolol racemates.
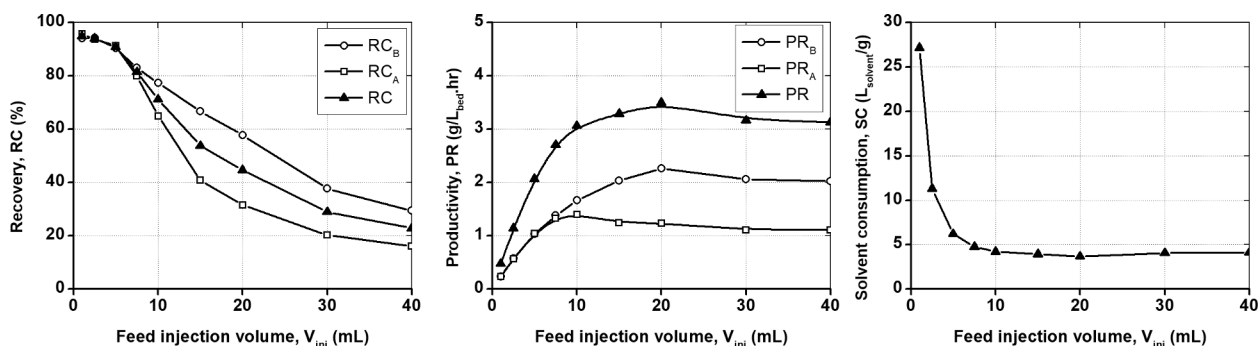

Fig. 16. Effect of the feed injection volume in recovery (left plot), system productivity (middle plot) and solvent consumption (right plot) for the FB preparative separation of nadolol racemates.
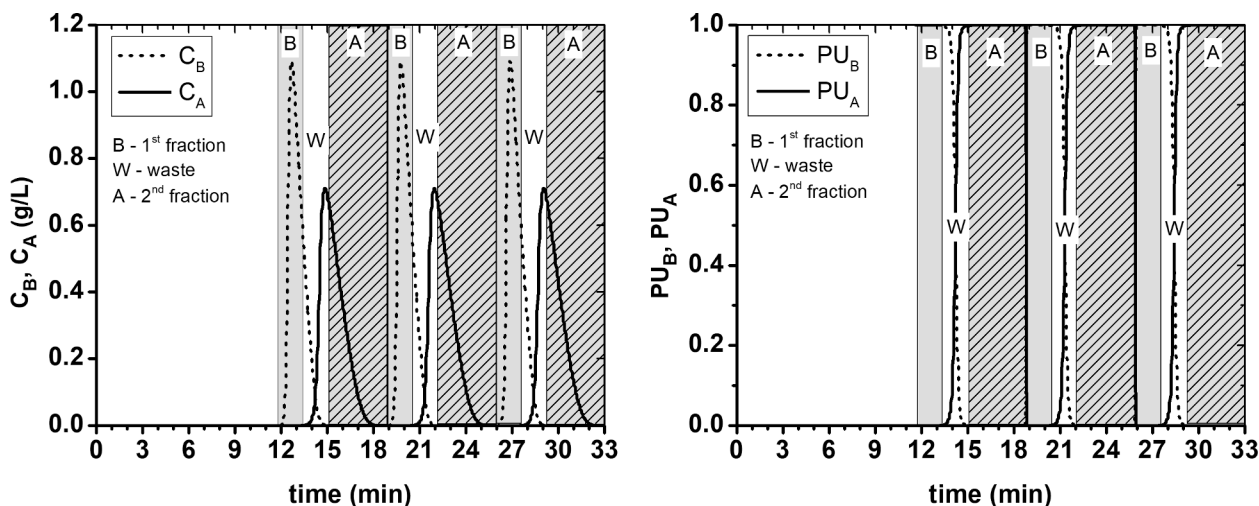

Fig. 17. Simulated FB chromatograms of three consecutive pulses (left plot, dashed line for the less retained racemate B, solid line for more retained racemate A) and purities of B and A (right plot, dashed line for purity of B, solid line for purity of A) of a 9 g/L nadolol feed solution, using a 30:70 ethanol:water solvent composition. Flow-rate of 38 mL/min, feed injection volume of 10 mL, and injection time interval of 7.1 min. Model parameters of $\varepsilon = 0.4$, Pe = 5000, k = 5 s^{-1}, and the linear + Langmuir isotherm model parameters as in Table 1.

a length of one sixth, that is, $Dc = 3$ cm, $Lc = 25/6$ cm. SMB operation uses a 1–2–2–1 configuration (six columns).

(ii) The critical values of γ_I and γ_{IV} for SMB operation are fixed the same way as previously, through $\gamma_I = \frac{1-\varepsilon}{\varepsilon} K_A \beta$ and $\gamma_{IV} = \frac{1-\varepsilon}{\varepsilon} K_B / \beta$, using $\beta = 1.2$ (see Section 3.1.6). It should be underlined that the use of a safety margin of 20 % represents a secure situation of non-contamination at SMB sections I and IV that can lead to lower productivity and higher solvent consumption performances. It means that, using lower β values, could still ensure complete separation with higher productivity and lower solvent consumption.

(iii) The SMB operating conditions are defined using the complete separation region considering the Triangle Theory methodology, that is, the pair of $(\gamma_{II}; \gamma_{III})$ values are considered at the vertex point of the 9 g/L triangle.

(iv) The SMB flow-rates can be established considering different equivalence situations:

(a) The flow-rate at SMB section I, Q_I^* (maximum internal flow-rate in SMB operation), is equal to the FB flow-rate, Q^{FB};

(b) The flow-rate at SMB section I, Q_I^*, is defined in order to keep the same average flow-rate for both FB and SMB operations, that is, $< Q^* > = \left(Q_I^* + 2Q_{II}^* + 2Q_{III}^* + Q_{IV}^* \right) / 6 = Q^{FB}$ (SMB operates with a 1–2-2–1 configuration);

(c) The SMB feed flow-rate, Q_F, is equal to the equivalent FB feed flow-rate (same throughput; that is, the same mass of nadolol fed into the system per unit time);

(d) The solvent used is SMB operation, $Q_E + Q_F$, is equal to the solvent used in FB operation, Q^{FB}.

Table 7
FB and SMB operating conditions and performance parameters for the separation of nadolol racemates. Solvent composition of 30:70 ethanol:water, 9 g/L nadolol feed solution, model parameters of Pe = 5000; k = 5 s^{-1}; ε = 0.4, and linear + Langmuir model parameters as in Table 1.

FB operation	SMB operation				
Column dimensions					
$D = 3cm$	$D_c,$ cm	3			
$L = 25cm$	L_c, cm	25/6			
	N_j	[1, 2, 2, 1]			
FB-SMB equivalence:	$Q_I^* = Q^{FB}$	$< Q^* > = Q^{FB}$	$Q_F = Q_F^{FB}$	$Q_E + Q_F = Q^{FB}$	
Operating conditions					
$V_{inj} = 10mL$	γ_I	9.4891			
$\Delta t_{inj} = 7.1 min$	γ_{II}	6.1555			
	γ_{III}	6.3945			
	γ_{IV}	5.5416			
	t^*, min	3.2519	2.3836	1.9989	1.2979
Flow-rates, Q(mL/min)					
$Q = 38.000$	Q_I^*	38.000	51.842	61.820	95.210
$Q_E = 36.592$	Q_E	14.301	19.511	23.266	35.832
$Q_F = 1.408$	Q_F	0.866	1.181	1.408	2.169
$Q_R = 9.366$	Q_R	3.090	4.216	5.027	7.742
$Q_W = 8.296$	–	–	–	–	–
$Q_X = 20.338$	Q_X	12.077	16.476	19.647	30.259
Performance parameters: PU(%), RC(%), PR(g/(L.hr)), SC(L/g)					
$PU_B = 99.99$	PU_R	100			
$PU_A = 99.99$	PU_X	100			
$RC_B = 77.32$	RC_R	100			
$RC_A = 64.98$	RC_X	100			
$RC = 71.15$	RC	100			
$PR = 3.062$	PR	2.646	3.609	4.304	6.629
$SC = 4.213$	SC	1.947			

(v) The SMB switching time is calculated through $t^* = \frac{\varepsilon V_C}{Q_I^*}(\gamma_I + 1)$ and the other SMB internal flow-rates are calculated using $Q_j^* = \frac{\varepsilon V_C}{t^*}\gamma_j^* (j = II, III, IV)$, where $\gamma_j^* = \gamma_j + 1$. The other inlet and outlet flow-rates are calculated through $Q_E = Q_I^* - Q_{IV}^*$; $Q_X = Q_I^* - Q_{II}^*$; $Q_F = Q_{III}^* - Q_{II}^*$; $Q_R = Q_{III}^* - Q_{IV}^*$.

The simulated SMB results are compared with the ones obtained by simulation of the FB operation under optimised operating conditions as presented in the previous section. Table 7 summarizes the FB and the SMB operating conditions and the performance parameters estimated by simulation.

All SMB runs present the same γ_j values (ratios between fluid and solid interstitial velocities in the equivalent true moving bed concept), ensuring the operation at optimised conditions, i.e., at the $(\gamma_{II}; \gamma_{III})$ vertex point of the complete separation triangle, and ensuring thorough cleaning of solid and liquid in sections I and IV. The SMB switch time interval, t^* (time between the switch of the inlet and outlet stream positions at SMB operation, and related to the solid velocity in the equivalent true moving bed concept), is defined depending on the SMB liquid internal flow-rates and in order to keep constant the γ_j values. In that way, solvent consumption is constant for all SMB runs, as it depends on the feed concentration and the ratio $(\gamma_I - \gamma_{IV})/(\gamma_{III} - \gamma_{II})$. Ensuring complete separation, the system productivity increases with the increase of the feed flow-rate. Being the feed concentration and the γ_j values constant, also the product $PR \times t^*$ is constant.

Table 7 shows the main advantages of SMB operation when compared with FB chromatography. The SMB optimised operating conditions are achieved for full recovery of the feed, while that do not occurs with the optimised FB operation. This means that SMB is a cleaner process and that FB operation introduces the waste or the need to reuse the waste fraction, increasing the cost of the operation particularly when the cost of feed or feed reuse are considerable. SMB also presents significant lower values of solvent consumption (in this study, 46 % of the solvent consumption at FB operation), as the unit includes sections I and IV to continuously clean and reuse solvent and adsorbent.

Considering SMB and FB operating with the same throughput, that is, the same mass of feed per unit time, $\left(Q_F C_{A+B}^F \right)^{SMB} = \left(Q_F C_{A+B}^F \right)^{FB}$, SMB achieves a system productivity 40 % higher than FB operation. Considering SMB and FB operating with the same inventory of solvent used per unit time, $(Q_E + Q_F)^{SMB} = (Q_E + Q_F)^{FB} = Q^{FB}$, SMB presents a system productivity 2.2 times higher than FB. Nevertheless, it should be pointed out that, under these circumstances, SMB operates with higher internal flow-rates, which introduces the challenge to use the appropriate hardware (columns, pumps, valves, tubing and fittings) for system pressure drop. In fact, preparative and industrial SMB and FB apparatus use larger column diameters (low length to diameter ratios) and larger adsorbent particle size materials.

4. Conclusions

This work presented extensive experimental and simulation results concerning the separation of nadolol racemates by fixed-bed (FB) and simulated moving bed (SMB) achiral preparative chromatography. The two alternative separation processes were carried out using a Waters XBridge C18 10 μm adsorbent and were both optimised in terms of solvent composition and pH (screening of ethanol:water:diethylamine mixtures) considering the need of an high pH reversed-phase operation due to the high basic nature of nadolol. Both processes presented similar better solvent compositions of 30–40 %ethanol in water and 0.10–0.15 % of diethylamine basic modifier to ensure a pH close to 12.

The optimisation of both the SMB and the FB operating conditions was carried out through simulation, based on equilibrium (adsorption isotherms) and kinetic (mass transfer resistance and axial dispersion)

data, estimated by frontal (breakthrough) chromatography. The competitive Langmuir or linear + Langmuir models proved to well describe the adsorption equilibrium behaviour and the C18 adsorbent used allowed high column efficiency with low mass transfer resistances and axial dispersion. In this way, the optimisation of SMB operating conditions can be safely carried out using the Triangle Theory methodology, although more precise simulation strategies can be used to follow the transient evolution of SMB operation or the presence of column and equipment dead volumes.

The experimental operation of both SMB and FB processes was implemented for the separation of a nadolol quaternary mixture into two pure racemates and well predicted by simulation. Additional simulation results were carried out to optimise the FB operation by studying the effect of the feed injection volume in its operating conditions and performance parameters.

SMB and FB operations were compared in terms of its performance parameters of recovery, system productivity and solvent consumption, and using different equivalence conditions, namely, to operate with the same feed mass flow-rate (throughput), the same solvent flow-rate, and the same internal flow-rates, potentially limited by system pressure drop. SMB globally presents better operating performances than FB, being a cleaner process by allowing a more efficient use of the adsorbent and solvent. Nevertheless, this work also emphasises the need of productive and industrial SMB apparatus working at low pressure drops which can be achieved by efficient flow distributors for columns with larger diameters and/or efficient larger particle size adsorbent materials.

Together with the former work [1], these two publications fully validate a novel strategy for the complete separation of nadolol stereoisomers by using a first achiral separation step with a C18 adsorbent to effectively produce pure nadolol racemates from the original quaternary nadolol mixture. The pure products obtained in this work can now be further purified by using standard chromatographic binary chiral steps to separate each racemate into its pure enantiomers. In this way, this work presents for the first time a successful and efficient way to complete separate a multicomponent nadolol feed into its four enantiomers at SMB and FB preparative scales.

CRediT authorship contribution statement

António E. Ribeiro: Software, Writing - original draft, Writing - review & editing. **Alírio E. Rodrigues:** Supervision, Resources. **Luís S. Pais:** Supervision, Conceptualization, Resources, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Acknowledgements

References

[1] R. Arafah, A. Ribeiro, A. Rodrigues, L. Pais, Separation of nadolol racemates by high pH reversed-phase preparative chromatography, Sep. Pur. Technol. 233 (2020), 116018.

[2] S. Branch, International Regulation of Chiral Drugs, Wiley-VCH, Weinheim, Germany, 2001.

[3] J. Rekoske, Chiral Separations, AIChE J. 47 (2001) 2–5.

[4] S. Andersson, S. Allenmark, J. Biochem. Biophys. Methods 54 (2002) 11–23.

[5] D. Kozma. Optical Resolutions via Diastereomeric Salt Formation. CRC Press, Taylor & Francis Group, ISBN-13: 978-1-4200-4260-3 (2002) 9-40.

[6] E. Francotte. Enantioselective chromatography: a real alternative to enantioselective synthesis? In: G. Subramanian, (Ed.), Proceedings of the EUROTECH98 Preparative and Process Scale Separations, Cambridge, England, 1998.

[7] E. Francotte, Enantioselective chromatography as a powerful alternative for the preparation of drug enantiomers, J. Chromatogr. A 906 (2001) 379–397.

[8] A. Rodrigues, C. Pereira, M. Minceva, L. Pais, A. M. Ribeiro, A. Ribeiro, M. Silva, N. Graça, J. Santos. Simulated Moving Bed Technology: Principles, Design and Process Applications. Butterworth-Heinemann, Elsevier, ISBN 978-0-12-802024-1, 2015.

[9] D. Broughton, C. Gerhold, Continuous sorption process employing fixed-bed of sorbent and moving inlets and outlets, US (1961) 2985589.

[10] R. Nicoud, Recent advances in industrial chromatographic processes, in: R.-M. Nicoud (Ed.), Recent Advances in Industrial Chromatographic Processes, Nancy, France, 1997, pp. 4–5.

[11] L. Pais, A. Rodrigues, Design of simulated moving bed and Varicol processes for preparative separations with a low number of columns, J. Chromatogr. A 1006 (2003) 33–44.

[12] A. Rodrigues, L. Pais, Design of SMB chiral separations using the concept of separation volume, Sep. Sci. Technol. 3 (2004) 245–270.

[13] L. Pais, V. Mata, A. Rodrigues, Simulated moving bed and related techniques, in: G. Cox (Ed.), Preparative Enantioselective Chromatography, Blackwell Publishing, Oxford, 2005, pp. 176–204.

[14] P. Gomes, M. Minceva, L. Pais, A. Rodrigues, Advances in simulated moving bed chromatographic separations, in: G. Subramanian (Ed.), Chiral Separation Techniques: a Practical Approach, 3rd ed., Wiley-VCH, Weinheim, 2006, pp. 181–202.

[15] P. Gomes, A. Rodrigues, Outlet Streams Swing (OSS) and MultiFeed operation of simulated moving beds, Sep. Sci. Technol. 42 (2007) 223–252.

[16] A. Seidel-Morgenstern, L. Keßler, M. Kaspereit, New developments in simulated moving bed chromatography, Chem. Eng. Technol. 31 (2008) 826–837.

[17] L. Rajendran, G. Paredes, M. Mazzotti, Simulated moving bed chromatography for the separation of enantiomers, J. Chromatogr. A 1216 (2009) 709–738.

[18] P. Gomes, M. Minceva, A. Rodrigues, Simulated moving bed technology: old and new, Adsorption 12 (2006) 375–392.

[19] R. Faria, A. Rodrigues, Instrumental aspects of Simulated Moving Bed chromatography, J. Chromatogr. A 1421 (2015) 82–102.

[20] G. Agrawal, B. Sreedhar, Y. Kawajiri, Systematic optimization and experimental validation of ternary simulated moving bed chromatography systems, J. Chromatogr. A 1356 (2014) 82–95.

[21] G. Agrawal, Y. Kawajiri, Full superstructure for multi-objective of multi-column chromatography for ternary separations, Chem. Eng. Technol. 38 (2015) 177–182.

[22] J. McCarthy, Direct enantiomeric separation of the four stereoisomers of nadolol using normal-phase and reversed-phase high-performance liquid chromatography with Chiralpak AD, J. Chromatogr. A 685 (1994) 349–355.

[23] A. Ribeiro, A. Rodrigues, L. Pais, Separation of Nadolol Stereoisomers by Chiral Liquid Chromatography at Analytical and Preparative Scales, Chirality 25 (2013) 197–205.

[24] S. Jermann, M. Meijssen, M. Mazzotti, Three column intermittent simulated moving bed chromatography: 3. Cascade operation for center-cut separations, J. Chromatogr. A 1378 (2015) 37–49.

[25] R. Arafah, A. Ribeiro, A. Rodrigues, L. Pais, Separation of Nadolol Stereoisomers Using Chiralpak IA Chiral Stationary Phase, Chirality 28 (2016) 399–408.

[26] T. Kubota, C. Yamamoto, Y. Okamoto, Preparation of chiral stationary phase based on immobilization of cellulose 3,5-dimethylphenylcarbamate derivatives on silica gel, Chirality 15 (2003) 77–82.

[27] T. Zhang, C. Kientzy, P. Franco, A. Ohnishi, Y. Kagamihara, H. Kurosawa, Solvent versatility of immobilized 3,5-dimethylphenylcarbamate of amylose in enantiomeric separations by HPLC, J. Chromatogr. A 1075 (2005) 65–75.

[28] T. Zhang, M. Schaeffer, P. Franco, Optimization of a Ca-sensitizing drug on an immobilized polysaccharide-based chiral stationary phase - Case study with a preparative view, J. Chromatogr. A 1083 (2005) 96–101.

[29] T. Zhang, P. Franco, D. Nguyen, R. Hamasaki, S. Miyamoto, A. Ohnishi, T. Murakami, Complementary enantiorecognition patterns and specific method optimization aspects on immobilized polysaccharide-derived chiral stationary phases, J. Chromatogr. A 129 (2012) 178–188.

[30] R. Arafah, A. Ribeiro, A. Rodrigues, L. Pais, Improving the performance of nadolol stereoisomers' preparative separation using Chiralpak IA by SMB chromatography, Chirality 31 (2019) 62–71.

[31] N. Graça, L. Pais, A. Rodrigues, Separation of ternary mixtures by pseudo-simulated moving-bed chromatography: separation region analysis, Chem. Eng. Technol. 38 (2015) 2316–2326.

[32] M. Negawa, F. Shoji, Simulated Moving Bed System, US Patent 5 456 825, 1995.

[33] P. Gomes, M. Zabkova, M. Zabka, M. Minceva, A. Rodrigues, Separation of Chiral Mixtures in Real SMB Units: The FlexSMB-LSRE, AIChE J 56 (2010) 125–142.

[34] X. Wang, C. Ching. Liquid Chromatographic Retention Behavior and Enantiomeric Separation of Three Chiral Center β-Blocker Drug (Nadolol) Using Heptakis (6-Azido-6-deoxy-2, 3-di-O-phenylcarbamolyted) β-Cyclodextrin Bonded Chiral Stationary Phase, Chirality 14 (2002) 798-805.

[35] M. Meloun, S. Bordovská, Benchmarking and validating algorithms that estimate pKa values of drugs based on their molecular structures, Anal. Bioanal. Chem. 389 (2007) 1267–1281.

[36] L. Snyder, J. Kirkland, J. Glajch, Pratical HPLC Method Development, 2nd Ed., Wiley-Interscience, New York, 1997, pp. 292–349.

[37] R.M. Nicoud, A. Seidel-Morgenstern, Simulated Moving Beds, Basics and Application, Institut National Polytechnique de Lorraine, Nancy, 1993.

[38] A. Seidel-Morgenstern, Experimental determination of single solute and competitive adsorption isotherms, J. Chromtogr. A 1037 (2004) 255–272.

[39] A. Ribeiro, N. Graça, L. Pais, A. Rodrigues, Optimization of the mobile phase composition for preparative chiral separation of flurbiprofen enantiomers, Sep. Purif. Technol. 68 (2009) 9–23.

[40] A. Ribeiro, P. Gomes, L. Pais, A. Rodrigues, Chiral Separation of Flurbiprofen Enantiomers by Preparative and Simulated Moving Bed Chromatography, Chirality 23 (2011) 602–611.

[41] A. Ribeiro, P. Gomes, L. Pais, A. Rodrigues, Chiral Separation of Ketoprofen Enantiomers by Preparative and Simulated Moving Bed Chromatography, Sep. Sci. Technol. 46 (2011) 1726–1739.

[42] G. Storti, M. Mazzotti, M. Morbidelli, S. Carra, Robust design of binary countercurrent adsorption separation processes, AIChE J. 39 (1993) 471–492.

[43] M. Mazzotti, G. Storti, M. Morbidelli, Optimal operation of simulated moving bed units for nonlinear chromatographic separations, J. Chromatogr. A 769 (1997) 3–24.

[44] D. Azevedo, A. Rodrigues, Design of a simulated moving bed unit in the presence of mass-transfer resistances, AIChE J. 45 (1999) 956–966.