# Separation of nadolol racemates by high pH reversed-phase preparative 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, Bragança, Portugal
[b] Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials, Associate Laboratory LSRE-LCM, School of Technology and Management, Polytechnic Institute of Bragança, Bragança, Portugal
[c] Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials, Associate Laboratory LSRE-LCM, Faculty of Engineering, University of Porto, Porto, Portugal

ARTICLE INFO

ABSTRACT

The separation of nadolol racemates under high pH reversed-phase preparative chromatography is presented for the first time.

Three Waters C18 adsorbents (XBridge, Shield and XSelect) are compared for the separation of nadolol racemates using ethanol:water:diethylamine solvent mixtures. Experimental and simulation results are presented to compare the separation performances at preparative scale using both the fixed-bed and the simulated moving bed operations.

The Waters XBridge C18 adsorbent and an ethanol:water:diethylamine solvent mixture are selected as a good option for the separation of nadolol racemates. The validated methodology allows the separation of a multi-component nadolol feed mixture composed by four stereoisomers into two pure racemates (two pairs of enantiomers). This work introduces the potential of using an initial achiral separation step in the global strategy for the complete multicomponent separation of the four nadolol stereoisomers.

## 1. Introduction

Analytical and preparative liquid chromatography went through a revolution in several technological fields, including adsorbent types, packing modelling and synthesis. Interesting reviews about particle packed and monolithic columns, and HPLC column technology can be found in literature [1,2]. Currently, a very large number of manufacturers are still focused in the development of new high performance liquid chromatography (HPLC), ultrahigh-pressure liquid chromatography (UHPLC), supercritical fluid chromatography (SFC) and hydrophilic interaction chromatography (HILIC) packing types that could increase several performance parameters for a wide range of applications, from the pharmaceuticals, biological samples and environment pollutants, among others. New chromatographic columns were recently presented by several companies present in Pittcon and other similar events [3].

C18 adsorbents are recently being used for different applications in fixed-bed [4-7] and simulated moving bed (SMB) [8,9] preparative separations. Waters manufacturer (USA) introduced three different types of C18 stationary phases, namely, the XBridge C18, the XBridge

Shield RP18 and the XSelect CSH C18 [10,11]. These columns are designed with the Ethylene Bridged Hybrid (BEH), the Optimum Bed Density (OBD) and the Charge Surface Hybrid (CSH) technologies. BEH technology synthesis creates particles prepared from two high purity monomers, tetraethoxysilane and bis(triethoxysilyl)ethane, resulting in a pH-resistant and mechanically-strong particle. OBD technology was specially developed for preparative separations to effectively improve the packed-bed stability and column lifetime by eliminating the bed collapse. The combination of both manufacturing technologies is referred by the manufacturer to ensure a direct scalability from analytical to preparative liquid chromatography separations. The XBridge C18 column uses a trifunctional C18 as ligand type and the pH range is 1–12. The Shield RP18 uses a monofunctional embedded polar group (carbamate) that "shields" the silica residual silanol surface from highly basic analytes, improving significantly the peak shape and resolution. The XSelect CSH C18 adsorbent uses a trifunctional C18 ligand type and is referred to improve chromatographic selectivity with the increase of solvent's hydrophobicity. These two last adsorbents can both be used in the pH range between 1 and 11. A wide number of published results in analytical HPLC using XBridge C18 [12-17], XBridge Shield RP18 [18-

24] and XSelect CSH C18 [25-27] can be found in literature. All three packings generally use alcohol:water or acetonitrile:water solvents, often with an acid or basic modifier (typically 0.1%).

Nadolol is a pharmaceutical drug which belongs to the class of nonselective beta-adrenergic receptor antagonists (β-blocker). This compound is world widely prescribed for the relief of some cardiovascular diseases, such as congestive heart failure, hypertension, ischemic heart disease (angina pectoris) and certain arrhythmias [28]. Despite the more restrictive legislation for the use of safer and more efficient drugs, together with some studies referring that only one of the four stereoisomers (RSR-nadolol) is responsible for the therapeutic effect, the nadolol is still commercially marketed as a mixture of two racemates; a total of four stereoisomers [29,30]. Moreover, the medical prescription of this chiral drug is associated with some risks, such as, abdominal pains, depression and cardiovascular failure, among others. In this way, the complete separation of the multicomponent mixture of nadolol stereoisomers has clear benefits for its future pharmaceutical use and applications.

Asymmetric organic synthesis and racemate resolution methods are two alternative strategies to obtain pure enantiomers [31]. Despite the large amounts of enantiomer provided by the first strategy, asymmetric synthesis is also time consuming and has the disadvantage of producing only one pure enantiomer. Taking into account that, in the earlier stages of drug discovery processes, only few quantities are necessary, racemate resolution can be the more appropriate choice since it can allow an efficient preparative separation process and provide both pure enantiomers. As referred before, the availability of all the pure enantiomers is an important step to identify new therapeutic principles and possible harmful pharmacological side effects. Among the different resolution methods, direct chromatographic resolution of enantiomers using continuous processes, such as the simulated moving bed (SMB) technology, is nowadays considered an attractive production alternative.

Only few published works can be found concerning the preparative separation of nadolol stereoisomers. The first work of preparative production of the RSR-nadolol pure stereoisomer was published in 2010 by Sung and co-authors. However, the method was based in the resolution via diastereomeric salt formation, followed by preparative HPLC using a JAIGEL-ODS-BP-L 25x500 mm column and methanol:water (84:16) as mobile phase [32]. Wang and Ching [33–35] published the first studies concerning the application of SMB technology to the separation of nadolol stereoisomers. Nevertheless, they used a perphenyl carbamoylated β-cyclodextrin stationary phase which presents a very low saturation capacity and, therefore, weak preparative performances when compared with later developed polysaccharide-derivatives-based chiral phases [36]. In 2013, our group presented the first publication for the experimental separation of the more retained and therapeutic active stereoisomer (RSR-nadolol) using a four zone SMB unit [37]. The preparative SMB columns were packed with coated Chiralpak AD adsorbent and ethanol:hexane:diethylamine (80:20:0.3) was used as solvent. In 2015, Jermann *et al.*, performed the same separation using a 3-column intermittent SMB cascade operation [38]. In 2016 and 2019, Rami *et al.* improved the performance of the SMB separation using the immobilized Chiralpak IA stationary phase and pure methanol and methanol:acetonitrile as solvents [39,40].

The nadolol pharmaceutical drug represents a very interesting case-study of multicomponent chiral separation since it is composed by four stereoisomers, being two pairs of enantiomers. It introduces the possibility of alternative 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. This work proposes a new strategy for the separation of nadolol stereoisomers, using an initial step with C18 achiral adsorbents. C18 material is more common and considerable less expensive than chiral stationary phases. Octadecylsilane phases (ODS or C18 columns) are used in the reversed-

phase mode. In these conditions, the separation of ionised basic compounds poses particular difficulties associated with the detrimental interaction with column silanol groups and overloading, which both result in poor peak shapes [15,41-43]. To overcome these problems, several manufacturers developed new ODS columns allowing high pH and highly aqueous conditions under reversed-phase mode [44-47]. It must be stressed out that, due to the strong alkaline nature of nadolol (pKa = 9.67) [48,49], a good and efficient chromatographic separation of this compound needs a solvent pH two units above the solute's pKa [50,51], resulting in a pH value above 11. An adsorbent such as the Waters XBridge C18 type is compatible with such high pH values, having a maximum allowed value of 12 [52]. Recently, Waters developed other C18 adsorbents, such as the Shield RP18 and the XSelect CSH C18 columns, being these materials able to perform separations using pH values until 11.

In this work, these three types of Waters ODS columns will be tested and compared for the separation of nadolol racemates. The use of a C18 achiral stationary phase for the preparative separation of nadolol racemates is introduced by the first time. Experimental and simulation results, such as, loading pulses, measurement and modelling of adsorption equilibrium isotherms, measurement and simulation of fixed-bed behaviour, and prediction of SMB operation are presented and are useful for the definition of a global strategy for the multicomponent separation of nadolol stereoisomers.

## 2. Experimental

### 2.1. Chemicals

Nadolol, a mixture of the two racemates (four stereoisomers), and uracil (used as non-retained compound for C18 material) were obtained from Sigma-Aldrich (Schnelldorf, Germany). The basic modifier diethylamine (DEA), ethanol (E) and acetonitrile (ACN), all HPLC grade solvents, were obtained from Fluka (Buchs, Switzerland). Ultrapure water with a resistivity value below 18.2 MΩ.cm (Type I) was obtained using a Merck Millipore Direct Q3 UV lab equipment. All reagents and solvents were used without further purification.

### 2.2. Equipment for analytical and preparative chromatography

Two Knauer HPLC systems (Germany) were used to perform elution chromatography, experimental measurements of the adsorption equilibrium isotherms, and breakthroughs experiments.

The analytical experiments were obtained in a Knauer HPLC system equipped with one Smartline 1050 pump with a 10 mL pump head, a manual 6-port/3-channel injection valve with 20 and 100 μL loops, and two detectors in series: a Smartline UV detector 2520 set at 230 or 270 nm wavelengths and a polarimeter detector (Chiralser IBZ, Messtechnik, Germany). Chiralpak IA (Daicel, Japan) was used for analytical chiral separations, and three C18 analytical columns (XBridge C18, XBridge Shield RP18 and XSelect CSH C18) were obtained from Waters (USA) manufacturer. All the four analytical columns have the same dimensions (4.6 mm ID × 250 mm L) and are packed with 5 μm particle size materials. A typical flow-rate of 1 mL/min was used with the analytical HPLC Knauer system.

The preparative experiments were carried out in a Knauer HPLC system equipped with a Smartline UV detector 2520 set at 270 nm wavelength, two Smartline 1050 pumps with 50 mL pump heads, and a manual 6-port/3-channel injection valve with a 1000 μL loop. The preparative reversed-phase separations used a SiliaChrom XT C18 column (20 mm ID × 150 mm L) obtained from Silicycle manufacturer (Canada), and three different C18 columns obtained from Waters manufacturer (19 mm ID × 100 mm L). The Waters preparative columns are packed with the same three materials previously referred (XBridge, Shield and XSelect), but with a particle size diameter of 10 μm. A typical flow-rate of 5 mL/min was used with the preparative
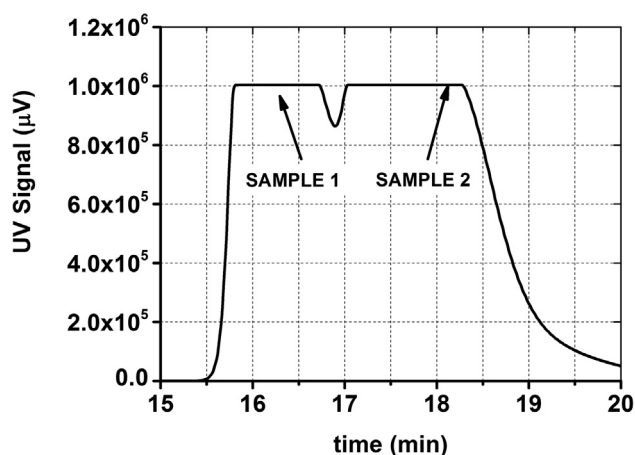
**Fig. 1.** UV response to a pulse of nadolol using a SiliaChrom XT C18 column and a mobile phase composition of ethanol:water:diethylamine (40:60:0.2). Flow-rate of 5 mL/min; $C_F^T = 20$ g/L; injection loop = 1000 μL.

HPLC Knauer system.

## 3. Results and discussion

### 3.1. Identification of nadolol racemates

Achiral C18 material does not directly discriminates chiral solutes. But nadolol is composed by four stereoisomers, being two racemates (two pairs of enantiomers). So, adequate operation using C18 material can distinguish and separate the two nadolol racemates. Additionally, chiral material can separate and identify the nadolol enantiomers of each racemate. A 20 g/L nadolol feed solution was prepared and injected in the SiliaChrom XT C18 preparative column, using a mobile phase of 40:60:0.2 ethanol:water:diethylamine and a 1000 μL loop. The flow-rate was set to 5 mL/min. The obtained result is presented in Fig. 1.

The SiliaChrom XT C18 preparative column succeeded to discriminate the two nadolol racemates. At the top of both peaks (UV saturated-signal plateaus at Fig. 1), two samples were collected. The collecting times were from 16 min and 20 sec to 16 min and 35 sec for sample 1 and from 18 min and 20 sec to 18 min and 35 sec for sample 2. Both collected samples were then analysed in the Knauer HPLC system equipped with the Chiralpak IA analytical column and the UV and polarimeter detectors in series. The results are presented in Fig. 2.

Fig. 2 reinforces that the achiral C18 material can be used to perform the pseudo-binary separation of the two nadolol racemates: the nadolol racemate B (sample 1; mixture of SRR and RSS nadolol enantiomers) from the nadolol racemate A (sample 2; mixture of SRS and RSR nadolol enantiomers). Considering the elution order obtained using the Chiralpak IA chiral stationary phase, sample 1 is a mixture of the two compounds with the intermediate affinity (chiral elution order 2 and 3, racemate B) and sample 2 is a mixture of the less and the more retained compounds (chiral elution 1 and 4, racemate A), as represented in Table 1. Therefore, the reader must note that, using the achiral C18 material, "racemate B" is less retained than "racemate A".

### 3.2. Loading pulses

In a liquid chromatographic separation, the choice of the solvent composition is a crucial step. The solvent selection normally has a remarkable impact on both retention and selectivity. The works published concerning the use of C18 adsorbents for fixed-bed and SMB preparative separations [4–9] use solvent mixtures of water with methanol, ethanol or acetonitrile, and an acid or basic modifier. In this work, the three Waters C18 adsorbents are compared for the separation of nadolol racemates using an ethanol:water solvent mixture with diethylamine as basic modifier. A solvent composition of 30%ethanol:70% water proved to be a good compromise to achieve acceptable pressure drop and selectivity, low retention times and good nadolol solubility for an effective preparative scale separation. Moreover, nadolol is only slightly soluble in water [53] and in acetonitrile [39], so the use of alcohol:water mixtures is mandatory for the preparative separation of nadolol racemates through reversed-phase chromatography. As referred before, due to the strong alkaline nature of nadolol, the baseline separation of nadolol racemates often needs a mobile phase composition having a pH value two units above its pKa value. A pH value near 11 can be reached by adding 0.005% of diethylamine modifier to a 30% ethanol:70%water mobile phase composition. For a pH near 12, a 0.1% of the same modifier is needed.

Fig. 3 presents several loading pulses of 2 and 10 g/L nadolol feed solutions, using a 30%ethanol:70%water solvent composition with a pH of 11 and 12, and for 20, 100 and 1000 μL injection loops. These pulses were carried out using both the analytical and the preparative Waters XBridge, Shield and XSelect columns and to better understand the effect of the increase of the loaded mass. Table 2 presents the results obtained in terms of retention times and chromatographic resolution between the two pairs of nadolol racemates.

For the XBridge C18 material there is a significant difference on the performance of the pulse separation of the nadolol racemates when using pH = 12 instead of pH = 11, at both the analytical and the preparative scales. Particularly, the use of lower pH introduces higher retention, lower resolutions, and higher band broadening. These differences can be justified by pH = 11 to be still close to the nadolol pKa (9.67).

The loss in selectivity at pH = 11 is considerably overcome by using the other Waters C18 materials. Shield RP18 material at pH = 11 presents a separation behaviour closer to the one obtained using XBridge C18 at pH = 12. XSelect CSH C18 material at pH = 11 also presents improved resolution but still maintains higher retention and band broadening. This can justify the preference for Shield RP18 material since it achieves good separation performances using pH = 11 (low retention times and reduced peak tailing), close to ones obtained with XBridge C18 but using pH = 12. Nevertheless, this conclusion should be validated at overloaded conditions (high amounts and concentrations of nadolol) as found at preparative and production scales. This validation can be obtained by measuring the adsorption equilibrium isotherms and through breakthrough experiments.

### 3.3. Measurement, modeling and fitting of adsorption equilibrium isotherms

The determination of the competitive adsorption equilibrium isotherms can be achieved using different experimental methodologies described in literature [55]. The adsorption–desorption method, despite being time consuming and tedious, was selected for this task since it is commonly accepted as the one giving the more accurate data. Considering that the final objective is to work under preparative conditions, the experimental equilibrium data collected through this static method will be then validated using a dynamic method of frontal chromatography by means of fixed-bed breakthrough experiments. A detailed description of this methodology can be found elsewhere [56,57].

The Langmuir model is often used to describe the equilibrium adsorption behaviour. Nevertheless, for the preparative separation of chiral mixtures, this model often fails as it is well known that the selectivity factor decreases with the increase of the chiral species' concentration. In fact, the Langmuir model predicts a constant selectivity over the entire range of concentrations. A practical way to overcome this limitation is to add a linear term to the Langmuir model. For the present work, the following competitive linear + Langmuir (LLG) model was found to better describe the adsorption behaviour, as described in Eq. (1):
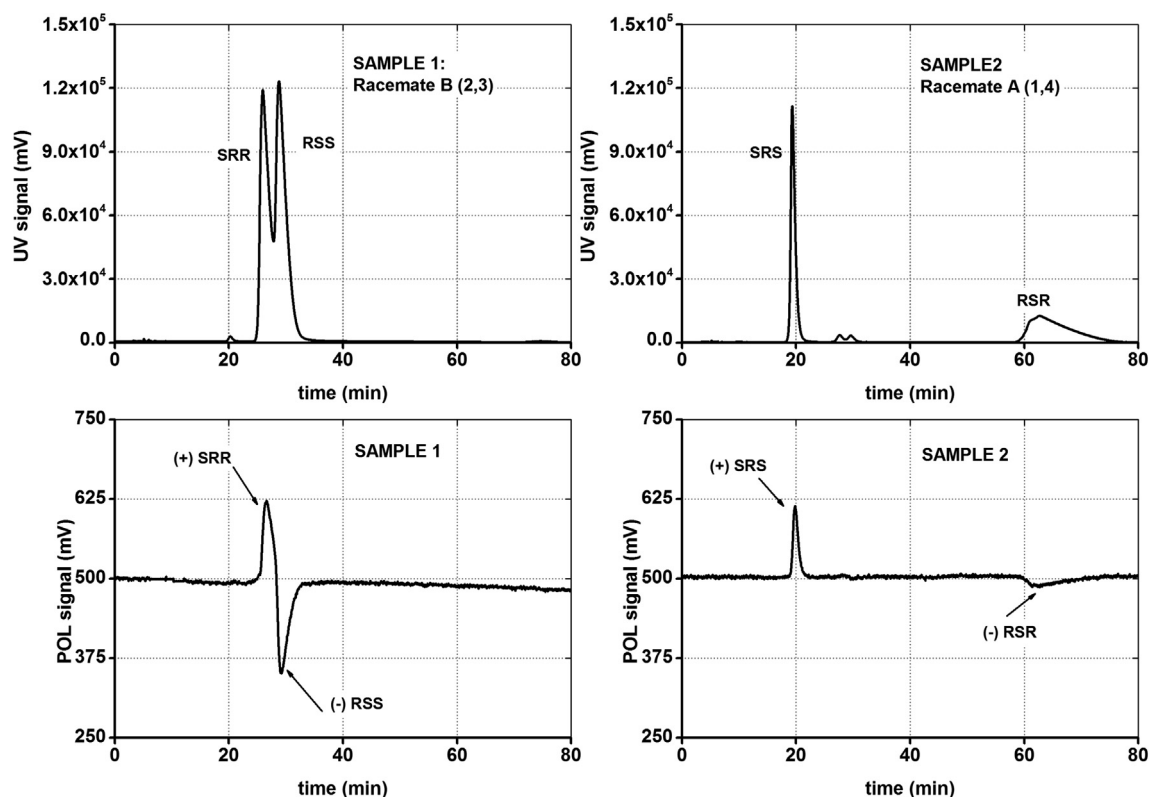
**Fig. 2.** UV (top) and polarimeter (bottom) response to a pulse of each collected sample1 and 2 (see Fig. 1) using a Chiralpak IA analytical column and a mobile phase composition of ethanol:water:diethylamine (40:60:0.2). Flow-rate of 0.5 mL/min; injection loop = 20 μL.

$$q_B^* = mC_B + \frac{Qb_B C_B}{1 + b_B C_B + b_A C_A}; \; q_A^* = mC_A + \frac{Qb_A C_A}{1 + b_B C_B + b_A C_A} \quad (1)$$

where $q_B^*$, $q_A^*$ and $C_B$, $C_A$ are, respectively, the solid and the liquid concentrations of racemates B and A, and $m$, $Q$, $b_B$ and $b_A$ are the adsorption equilibrium isotherm parameters. These parameters were estimated using the Levenberg-Marquardt algorithm for the minimization of the corrected standard deviation, SD, defined as
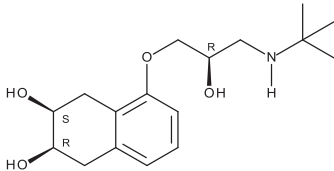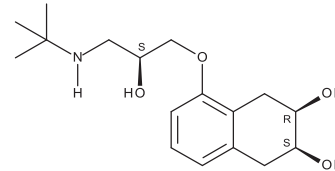
$$SD = \sqrt{\frac{SQ}{M - N}} \quad (2)$$
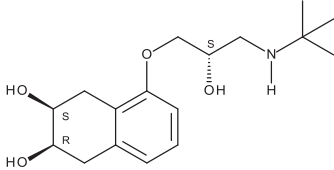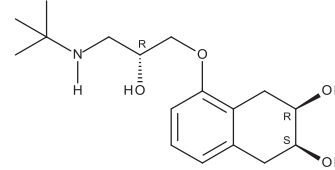
with

$$SQ = \sum_{j=1}^{M/2} [(q_{Bj}^{*T} - q_{Bj}^{*E})^2 + (q_{Aj}^{*T} - q_{Aj}^{*E})^2] \quad (3)$$

being M the number of experimental points, N the number of estimated parameters, and $q^{*E}$ and $q^{*T}$ the experimental and model equilibrium stationary phase concentrations, respectively. The adsorption equilibrium isotherm parameters obtained for the different C18 materials and pH values are presented in Table 3.

**Table 1**

Molecular structure, elution order and optical rotation signal of the four nadolol stereoisomers using Chiralpak AD [37] and Chiralpak IA [39] chiral stationary phases and ethanol-hydrocarbon solvent compositions, and in this work using Chiralpak IA and ethanol:water solvent composition.



**Racemate A**

RSR-Nadolol
Elution order: 4th; optical rotation signal: (−)

SRS-Nadolol
Elution order: 1st; optical rotation signal: (+)

**Racemate B**

RSS-Nadolol
Elution order: 3th; optical rotation signal: (−)

SRR-Nadolol
Elution order: 2nd; optical rotation signal: (+)
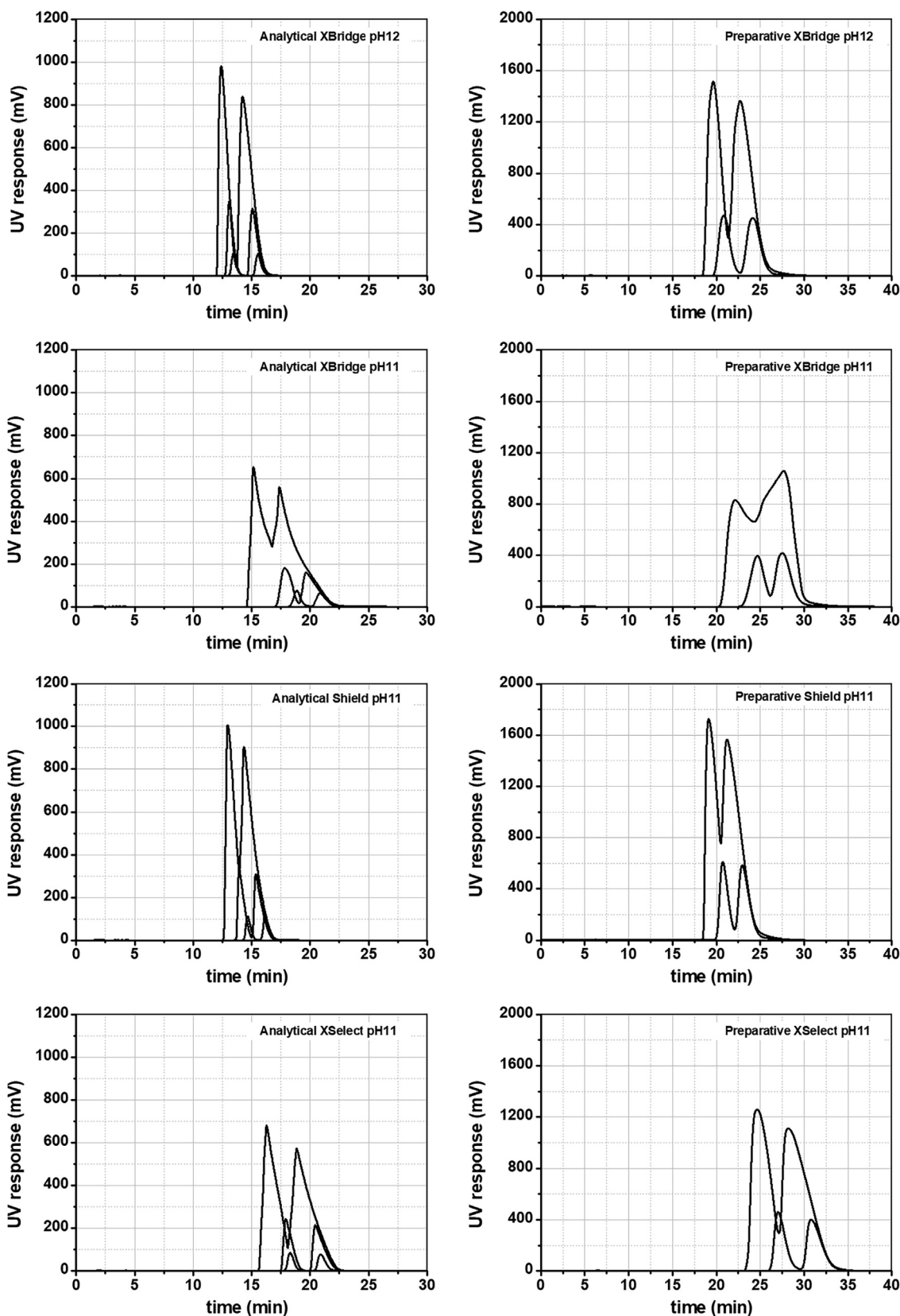
**Fig. 3.** Experimental chromatograms for the separation of nadolol racemates using 30:70:0.1 (pH = 12) and 30:70:0.005 (pH = 11) ethanol:water:diethylamine mobile phase compositions, for the three analytical and preparative Waters C18 columns (XBridge, Shield and XSelect). For analytical columns, feed concentrations of 2 g/L (loops of 20 and 100 μL) and 10 g/L (loop of 100 μL); flow-rate of 0.9 mL/min. For preparative columns, feed concentrations of 2 and 10 g/L (loop of 1000 μL); flow-rate of 5.0 mL/min. UV detection at 270 nm.

**Table 2**
Retention times ($tr_B$ and $tr_A$) and chromatographic resolution ($R_{A,B}$) obtained for loading pulses using both the analytical and the preparative Waters C18 columns.

| Column | Injected mass (mg) | C18 adsorbent, pH | $tr_B$ (min) | $tr_A$ (min) | $R_{A,B}$ [*] |
|---|---|---|---|---|---|
| **Analytical** | **0.04** | XBridge, pH = 12 | 13.45 | 15.55 | 2.47 |
| | | XBridge, pH = 11 | 18.87 | 20.85 | 1.66 |
| | | Shield, pH = 11 | 14.67 | 16.17 | 1.76 |
| | | XSelect, pH = 11 | 18.30 | 20.90 | 2.11 |
| | **0.2** | XBridge, pH = 12 | 13.10 | 15.07 | 1.85 |
| | | XBridge, pH = 11 | 17.83 | 19.65 | 0.91 |
| | | Shield, pH = 11 | 13.93 | 15.35 | 1.34 |
| | | XSelect, pH = 11 | 17.92 | 20.45 | 1.75 |
| | **1** | XBridge, pH = 12 | 12.40 | 14.22 | 1.04 |
| | | XBridge, pH = 11 | 15.15 | 17.38 | 0.75 |
| | | Shield, pH = 11 | 12.90 | 14.35 | 0.79 |
| | | XSelect, pH = 11 | 16.28 | 18.85 | 0.93 |
| **Preparative** | **2** | XBridge, pH = 12 | 20.82 | 24.16 | 1.29 |
| | | XBridge, pH = 11 | 24.68 | 27.51 | 0.88 |
| | | Shield, pH = 11 | 20.72 | 22.94 | 1.21 |
| | | XSelect, pH = 11 | 27.04 | 30.79 | 1.38 |
| | **10** | XBridge, pH = 12 | 19.63 | 22.70 | 0.86 |
| | | XBridge, pH = 11 | 22.15 | 27.69 | n.a. |
| | | Shield, pH = 11 | 19.12 | 21.20 | 0.63 |
| | | XSelect, pH = 11 | 24.66 | 28.20 | 0.71 |

(*) Chromatographic resolution is calculated using $R_{A,B} = \frac{1.18(tr_A - tr_B)}{w_{0.5,A} + w_{0.5,B}}$, where $tr_A$ and $tr_B$ are the retention times (at peak apexes) and $w_{0.5,A}$ and $w_{0.5,B}$ are the peak widths measured at half the peak height [54], being racemate A more retained than racemate B.

**Table 3**
Adsorption equilibrium isotherm parameters obtained for the different Waters C18 preparative columns and pH values (at 23 °C).

| C18 adsorbent, pH | m | Q (g/L) | $b_B$ (L/g) | $b_A$ (L/g) | SD |
|---|---|---|---|---|---|
| XBridge, pH = 12 | 1.3694 | 31.02 | $9.877 \times 10^{-2}$ | $1.258 \times 10^{-1}$ | 0.2326 |
| XBridge, pH = 11 | 1.3594 | 32.19 | $9.667 \times 10^{-2}$ | $1.210 \times 10^{-1}$ | 0.2200 |
| Shield, pH = 11 | 1.3829 | 38.46 | $7.373 \times 10^{-2}$ | $8.529 \times 10^{-2}$ | 0.0423 |
| XSelect, pH = 11 | 0.7947 | 56.92 | $8.150 \times 10^{-2}$ | $9.551 \times 10^{-2}$ | 0.1201 |

The comparison between the experimental data and the adsorption behaviour predicted by the selected LLG model is presented in Fig. 4 for the XBridge C18 preparative column, using an ethanol:water (30:70) solvent composition and for both pH = 12 and pH = 11. The left plot presents the adsorption equilibrium isotherms and the right plot the selectivity behaviour, as a function of the feed concentration. Fig. 5 presents the same comparison for the three different Waters C18 materials using ethanol:water (30:70) solvent composition and pH = 11.

### 3.4. Breakthroughs experiments and simulation

The validation of the adsorption equilibrium isotherm models was carried out through breakthrough experiments and simulation. The breakthrough curve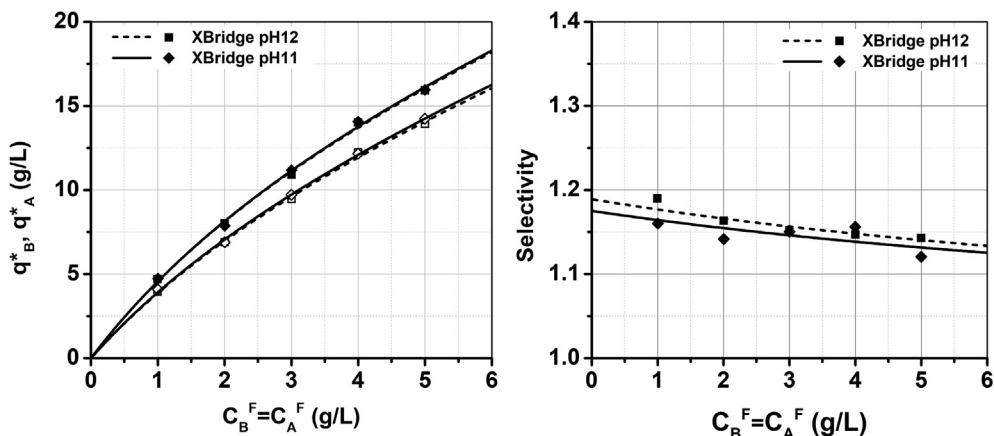s were simulated considering mass balance equations that include axial dispersion and mass transfer resistance through the linear driving force (LDF) model, which proved to be an accurate approximation for the modelling of the mass transfer phenomena [58–60]. The PDECOL package based on the method of orthogonal collocation in finite elements was used to predict the fixed-bed adsorption behavior [61]. The breakthrough experimental data was also used to estimate the kinetic data, i.e., the axial dispersion and the mass transfer resistance parameters. The reader is invited to read previous published work for further information on the simulation of the breakthrough experiments [62,63].

For each breakthrough experiment, several outlet samples were collected at different times of the saturation and regeneration steps. These samples were analyzed by HPLC and compared with the simulated chromatograms. Using uracil as a non-retained compound in the C18 material, an additional experiment was carried out to predict axial dispersion by fitting a model for fixed-bed operation considering homogeneous adsorbent particles to the uracil pulse experimental data. Axial dispersion was predicted through a Peclet number of Pe = 5000. From the breakthrough curves using 10 g/L nadolol feed solutions, a predicted value for the mass transfer coefficient of k = 5 s$^{-1}$ proved to be adequate for all the C18 materials. These values validate the Waters adsorbents as promising for preparative separation as it present negligible axial dispersion and mass transfer resistances. Fig. 6 presents the obtained results using the three Waters C18 materials with pH = 11 or



**Fig. 4.** Comparison between experimental and model results for the adsorption equilibrium isotherms (left) and selectivity (right) for the two nadolol racemates, as a function of their feed concentrations, using ethanol:water (30:70) mobile phase composition with 0.1% diethylamine (dashed line, pH = 12) and with 0.005% diethylamine (solid line, pH = 11), and the XBridge C18 material. Both fittings (lines) use the linear + Langmuir (LLG) competitive model parameters presented in Table 3.
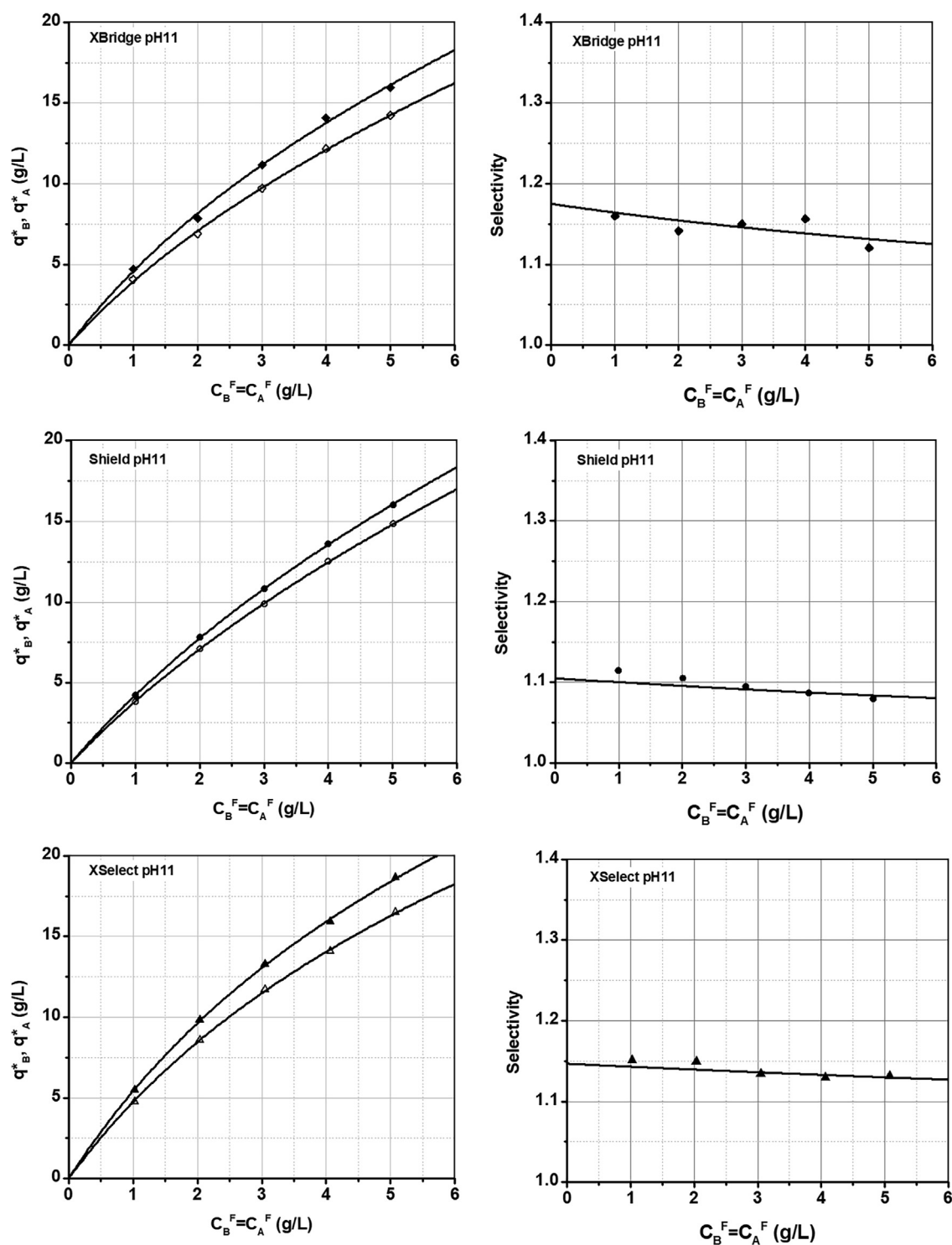
**Fig. 5.** Comparison between experimental and model results for the adsorption equilibrium isotherms (left) and selectivity (right) for the two nadolol racemates, as a function of their feed concentrations, using ethanol:water (30:70) mobile phase composition with 0.005% diethylamine (pH = 11) and the three different Waters C18 preparative columns: XBridge (diamonds, upper row), Shield (circles, middle row) and XSelect (triangles, bottom row). All fittings use the linear + Langmuir (LLG) competitive model parameters presented in Table 3.

pH = 12.

Figs. 4 and 6 show that the significant differences found at pulse chromatography for the XBridge C18 material using pH = 12 versus pH = 11 (see Fig. 3 for the loading pulses) are not observed in the comparison of the adsorption equilibrium isotherms and in the break-through experiments. Both saturation and regeneration curves for pH = 11 and pH = 12 show very similar behaviours, and only a slight

decrease in selectivity is observed for pH = 11. This is a nice and clear example showing that the separation performance under preparative conditions should not be evaluated based on the extrapolation of the results obtained under analytical and linear chromatographic conditions, but needs to be carefully evaluated under those preparative and overloaded conditions.

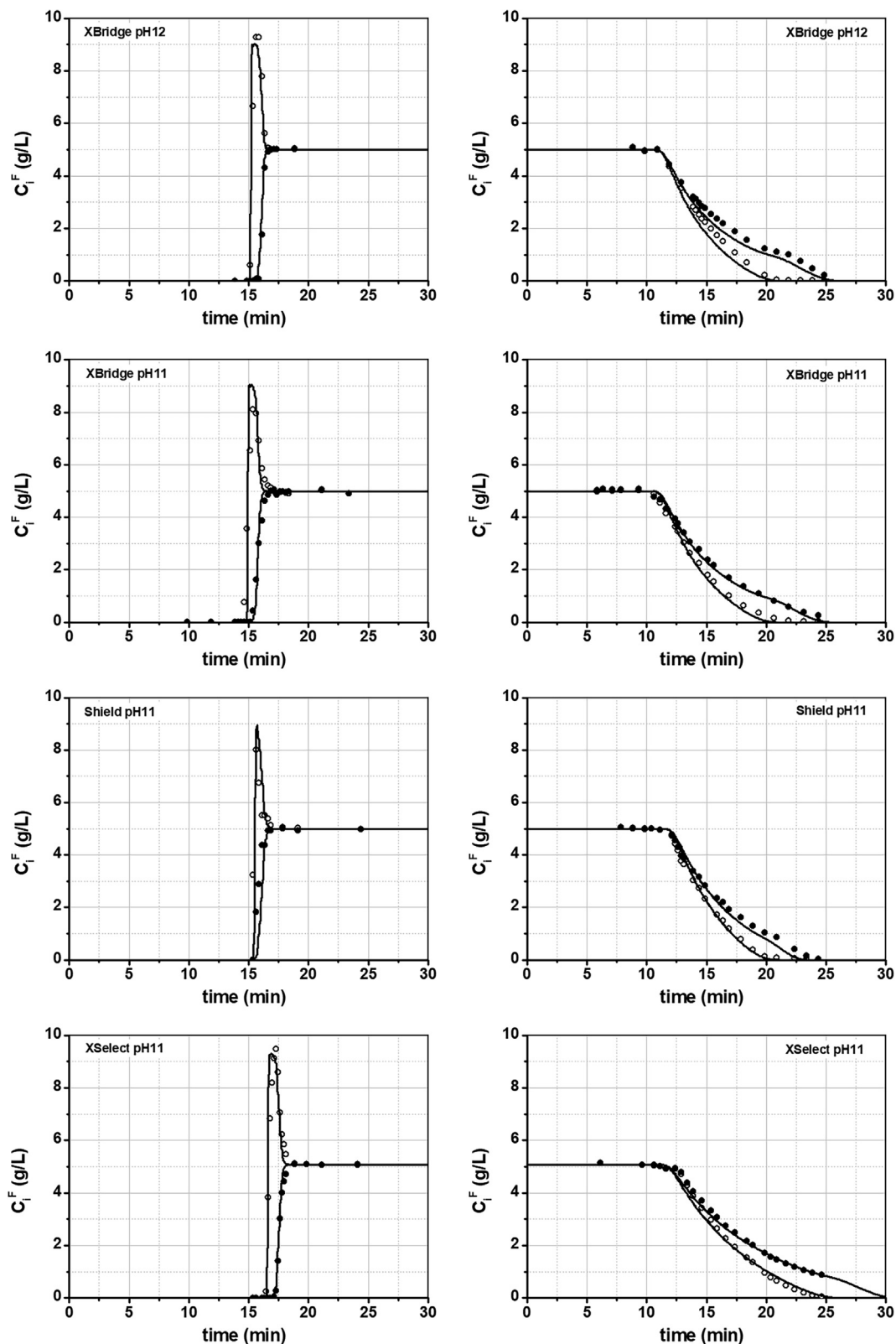Comparing the different Waters C18 materials using pH = 11,

**Fig. 6.** Saturation (adsorption; left plots) and regeneration (desorption; right plots) curves for a 10 g/L nadolol feed solution using XBridge with 30%ethanol/70% water/0.1%diethylamine (pH = 12), and using XBridge, Shield and XSelect with 30%ethanol/70%water/0.005%diethylamine (pH = 11). Comparison between the experimental (points) and the simulation results (lines). Flow-rate of 5 mL/min; model parameters of $\varepsilon$ = 0.4 (bed porosity), Pe = 5000 (Peclet number), and k = 5 s$^{-1}$ (mass transfer coefficient); linear + Langmuir isotherm model parameters as in Table 3.
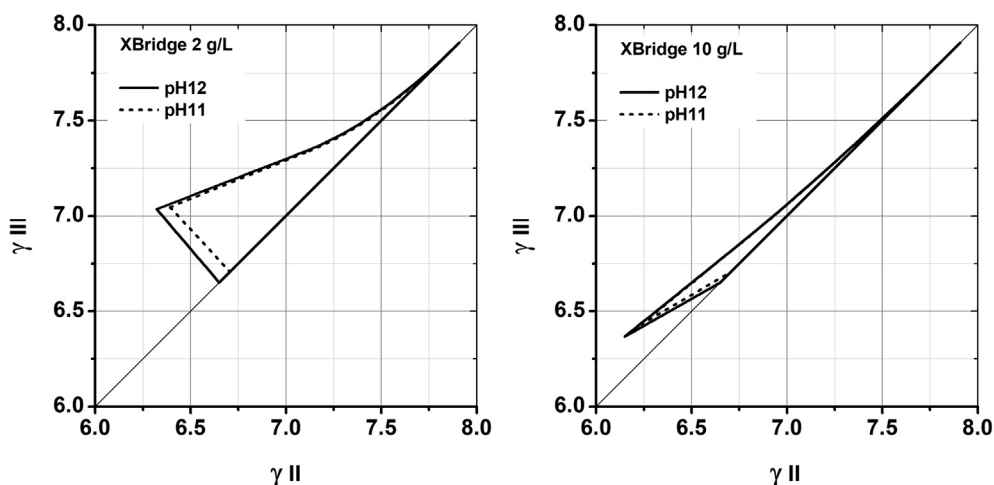
**Fig. 7.** SMB complete separation regions for the separation of nadolol racemates using the XBridge C18 material and ethanol:water (30:70) as mobile phase, with 0.1% diethylamine (pH = 12, solid lines) and with 0.005% diethylamine (pH = 11, dashed lines), for a nadolol feed concentration of 2 g/L (left) and 10 g/L (right). The SMB complete separation regions are predicted considering the equilibrium theory and the linear + Langmuir (LLG) model with the adsorption equilibrium isotherm parameters presented in Table 3.

Figs. 5 and 6 show that Shield presents a considerable loss in selectivity. For Shield material, the less retained nadolol racemate is clearly more adsorbed, while the more retained racemate is also less retained when compared with the behavior found using XBridge at pH = 11. These results for the Shield adsorbent show that the ones obtained at pulse conditions do not maintain at overloaded conditions.

The results obtained for the XSelect material clearly confirm the higher retention behavior (observed in both Figs. 5 and 6) and a selectivity that stands clearly above the Shield material, but slightly below the one obtained with XBridge at pH = 11.

The results presented in Figs. 4–6 and obtained under preparative and overloaded conditions justify the use of the XBridge C18 material for the preparative separation of the nadolol racemates. Although presenting a slight decrease in selectivity using XBridge C18 material with pH = 11 instead of pH = 12, the preparative separation performance using pH = 11 seems to be not significantly affected, ensuring a longer adsorbent lifetime. These results will be further studied and validated in the next section through the simulation of the preparative separation of nadolol racemates by SMB chromatography using the three C18 materials and pH conditions.

### 3.5. SMB modeling and simulation

The comparison of the predicted SMB operation performance is an important additional tool for the evaluation of the preparative separation of nadolol racemates using the three different adsorbent materials studied in this work. The adopted methodology was based in the so-called Triangle Theory, developed the Mazzotti et al. [64], which assumes that mass transfer resistances and axial dispersion are negligible, and that the adsorption equilibria can be described through a modified linear + Langmuir isotherm model. The comparison of the adsorbents' performances is carried out through the prediction of the system productivity and solvent consumption performance parameters at the vertex of the separation regions, defined by the triangles of SMB complete separation. Since a separation region is the area of possible SMB internal flow-rates that allows 100% pure products (pure extract, only containing the more retained nadolol racemate, and pure raffinate, only containing the less retained nadolol racemate), the vertex of each triangle represents the point at the boundary of the separation region most distant from the $\gamma_{III}$- $\gamma_{II}$ diagonal and, therefore, the best operating conditions in terms of SMB system productivity (PR, $g_{target\,product}L_{bed}^{-1}h^{-1}$) and solvent consumption (SC, $L_{solvent}g_{target\,product}^{-1}$), for a given nadolol feed concentration and calculated through

$$PR = \frac{Q_F(C_B^F + C_A^F)}{V_T} = \frac{\varepsilon}{N_C t^*}(\gamma_{III} - \gamma_{II})(C_B^F + C_A^F) \tag{4}$$

and

$$SC = \frac{Q_E + Q_F}{Q_F(C_B^F + C_A^F)} = \frac{1}{(C_B^F + C_A^F)}\left(1 + \frac{\gamma_I - \gamma_{IV}}{\gamma_{III} - \gamma_{II}}\right) \tag{5}$$

where $Q_F$ and $Q_E$ are the SMB feed and eluent external flow-rates, respectively; $C_B^F$ and $C_A^F$ are the feed concentrations of nadolol racemates B and A, respectively; $V_T$ is the total volume of the adsorbent used in the SMB unit; $N_C$ is the number of columns used in the SMB unit; $t^*$ is the SMB switch time interval; and $\gamma_j$ is the ratio between fluid and solid interstitial velocities in section $j$ of the equivalent true moving bed (TMB) operation.

Although it represents a simplified approach, the equilibrium theory model allows a straightforward prediction of the SMB performance and is very useful for comparative studies, particularly under weak axial dispersion and mass transfer resistances, as it is the case in this work.

In SMB operation, both extract and raffinate outlet streams must satisfy the purity and recovery specifications. It is assumed that the more retained species (racemate A) will be completely recovered in the extract stream, while the other less retained components (racemate B) will be completely recovered in the raffinate stream. According to these assumptions, the extract purity (PUX, %) is defined as the ratio between the mean concentration of racemate A (stereoisomers 1 and 4) and the sum of the mean concentrations of the two racemates in the extract stream:

$$PUX = \frac{\langle C_A^X \rangle}{\langle C_A^X \rangle + \langle C_B^X \rangle} \times 100 \tag{6}$$

Similarly, the raffinate purity (PUR, %) is defined as the ratio between the mean concentration of racemate B (stereoisomers 2 and 3) and the sum of the mean concentrations of the two racemates in the raffinate stream:

$$PUR = \frac{\langle C_B^R \rangle}{\langle C_A^R \rangle + \langle C_B^R \rangle} \times 100 \tag{7}$$

A transient SMB model, taking account both axial dispersion and mass transfer resistances, was used to predict the transient evolution of both nadolol racemates' concentrations in the extract and raffinate outlet streams, and the internal concentration profiles at SMB cyclic steady-state. The simulation uses the gPROMS software package from Process System Enterprise (UK). More information concerning SMB modeling and simulation, through the equilibrium theory and other more precise SMB models, can be found elsewhere [65–68].

### 3.5.1. Regions of SMB complete separation

Fig. 7 shows the separation regions obtained for the XBridge adsorbent and for nadolol feed concentrations of 2 and 10 g/L, using an 30:70 ethanol:water solvent composition with pH = 11 and pH = 12. These results show, once more, that there are no significant differences
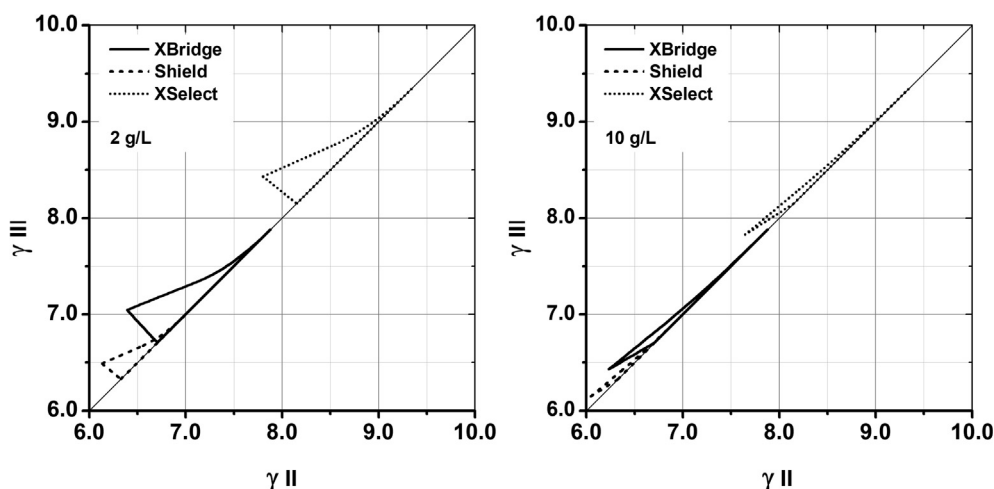
**Fig. 8.** SMB complete separation regions for the separation of the nadolol racemates using the XBridge (solid lines), Shield (dashed lines) and XSelect (dotted lines) C18 materials and ethanol:water:diethylamine (30:70:0.005) as mobile phase (pH = 11), for a nadolol feed concentration of 2 g/L (left) and 10 g/L (right). The SMB complete separation regions are predicted considering the equilibrium theory and the linear + Langmuir (LLG) model with the adsorption equilibrium isotherm parameters presented in Table 3.

in the SMB performance parameters as the dimension of the triangles separation areas are similar. These results confirm that it is feasible to use XBridge C18 material with pH = 11 for a longer column lifetime.

Fig. 8 shows the comparison of the separation regions for the three C18 adsorbents, using the same etanol:water (30:70) solvent with pH = 11 and for 2 g/L and 10 g/L nadolol feed concentrations. The SMB separation regions presented in Fig. 8 are in line with the results obtained for the adsorption equilibrium isotherms and the breakthrough experiments presented in Figs. 5 and 6. XBridge and XSelect present similar size SMB separation regions due to similar selectivity values, although XSelect presents considerable higher operating gamma values due to the higher retention of the nadolol racemates in this adsorbent. Fig. 8 also confirms the results obtained with the Shield RP18 adsorbent. Its SMB separation region is significantly smaller in the entire range of nadolol feed concentrations (2 and 10 g/L) due to the lower selectivity values found previously in Figs. 5 and 6.

### 3.5.2. SMB system productivity and solvent consumption

Fig. 9 presents the predicted SMB system productivity and solvent consumption for the Waters XBridge C18 adsorbent, using an ethanol:water (30:70) solvent composition with pH = 11 and pH = 12, as a function of the nadolol feed concentration. The productivity and solvent consumption parameters were calculated at the vertex of each SMB separation regions considering the equilibrium theory model, the equilibrium adsorption isotherm parameters presented in Table 3, a maximum internal flow-rate of $Q^*_I$ = 20 mL/min at section I of the SMB unit, and safety margins of 20% for the SMB sections I and IV. The simulation results confirm that there is not a strong effect of the pH
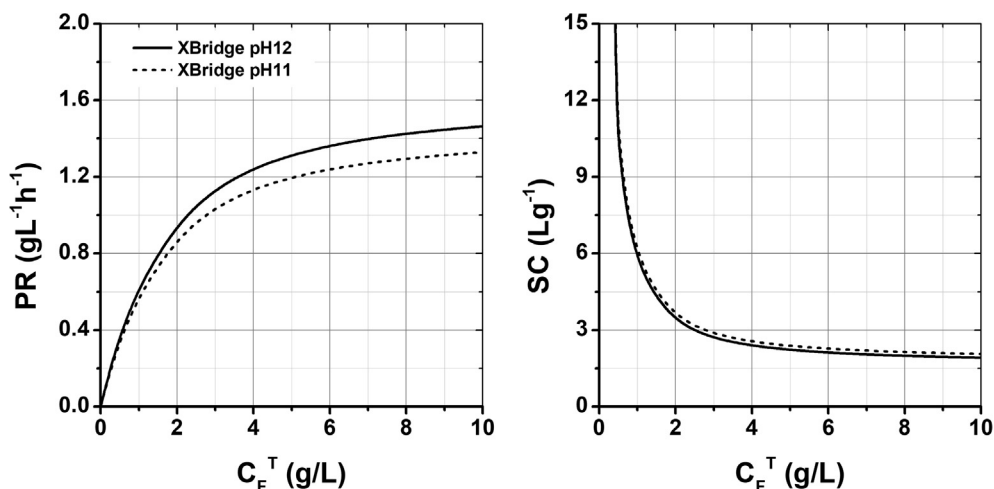
value (pH = 12 versus pH = 11) in the SMB system productivity and solvent consumption obtained using the XBridge C18 adsorbent.

Fig. 10 presents the comparison for the predicted SMB performance parameters for the three Waters C18 adsorbents using the same ethanol:water:diethylamine (30:70:0.005) solvent composition with pH = 11, as a function of the nadolol feed concentration.

From the three studied Waters C18 materials, the XBridge adsorbent presents the better behaviour for both the SMB system productivity and solvent consumption. As explained in the previous results, the lower SMB performances presented by the XSelect and the Shield adsorbents can be justified by the higher retention of the nadolol racemates and by the significant lower selectivity values, respectively. Therefore, the XBridge C18 adsorbent can be considered as the more appropriate material for the preparative separation of nadolol racemates and can operate at pH = 11, avoiding higher pH values (up to 12) and ensuring longer adsorbent lifetime.

### 3.5.3. Prediction of the SMB operation for the preparative separation of nadolol racemates

The simulation of the SMB operation using a conventional four-zone SMB mode with a [1–2–2–1] column configuration was carried for each C18 adsorbent and a nadolol feed concentration of 10 g/L. The SMB model used in simulation took into account both the axial dispersion and the mass transfer resistances, and the linear + Langmuir (LLG) model to describe the adsorption equilibrium isotherms. The model parameters used were the ones raised in the experimental measurements carried out in this work and presented in Table 3 and Figs. 4–6.

Table 4 presents the SMB operating conditions, including the



**Fig. 9.** Predictions of the SMB system productivity (left) and solvent consumption (right) as a function of the nadolol feed concentration for the XBridge C18 adsorbent. Solid line for (30:70:0.1) ethanol:water:diethylamine (pH = 12) and dashed line for (30:70:0.005) ethanol:water:diethylamine (pH = 11). Flow-rate in SMB section I of $Q_I^*$ = 20 mL/min; safety margins of 20% for SMB sections I and IV; linear + Langmuir isotherm model parameters as in Table 3.
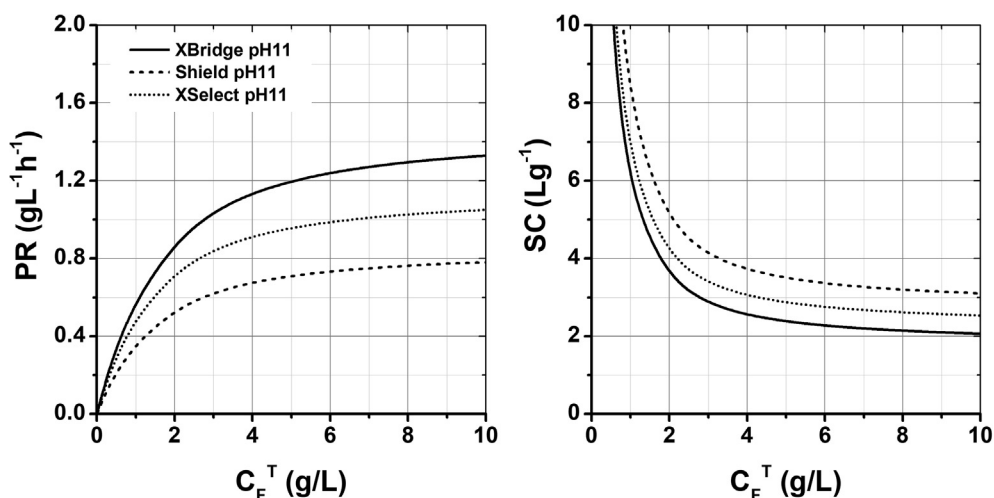
**Fig. 10.** Predictions of the SMB system productivity (left) and solvent consumption (right), as a function of the nadolol feed concentration, for the three Waters C18 adsorbents and using (30:70:0.005) ethanol:water:diethylamine (pH = 11). Solid line for the XBridge, dashed line for Shield and dotted line for XSelect C18 materials. Flow-rate in SMB section I of $Q_I^* = 20$ mL/min; safety margins of 20% for SMB sections I and IV; linear + Langmuir isotherm model parameters as in Table 3.

internal and external SMB flow-rates, the SMB switch time interval, and the corresponding γ values for the equivalent true moving bed (TMB) operation, calculated at the vertex point of each separation region for a 10 g/L nadolol feed concentration (see Fig. 8, right plot). The predictions of the outlet streams purities and the SMB system productivity and solvent consumption parameters are also presented in Table 4, and are in line with the ones presented in Fig. 10. The small contaminations in the extract outlet streams are due to the SMB configuration (6 columns) and the estimated axial dispersion and resistance to mass transfer included in the simulation of the SMB operation.

Fig. 11 shows the predicted transient evolution of the nadolol racemates' average concentration in both the extract and the raffinate outlet streams during the first 25 cycles of SMB operation, for XBridge, Shield and XSelect C18 materials. Also presented are the predicted internal concentration profiles of the two nadolol racemates after the SMB cyclic steady-state was achieved (cycle 25). Fig. 11 again validates the use of the XBridge C18 adsorbent for the separation of nadolol racemates with better SMB system performances than the ones obtained with Shield and XSelect materials for the same solvent and pH conditions.

## 4. Conclusions

The separation of the two nadolol racemates was carried out using

three different Waters C18 adsorbents; the XBridge C18, the Shield RP18 and the XSelect CSH C18, at both analytical and preparative scales.

The identification of all the four nadolol stereoisomers and the capacity of C18 adsorbents to discriminate the two nadolol racemates under reversed-phase chromatography was validated for each C18 adsorbent, using an ethanol:water solvent mixture with diethylamine as basic modifier. The need for a high-pH operation was also identified and justified by the strong alkaline nature of nadolol.

This work presented extensive experimental data for the separation of nadolol racemates at both analytical and preparative scales and shown how crucial is to evaluate the separation performance under the real preparative and overloaded conditions, avoiding the extrapolation of the results obtained under analytical and linear chromatography.

The Waters XBridge C18 adsorbent was concluded to be the better solution for the preparative separation of nadolol racemates, using an ethanol:water: diethylamine (30:70:0.005) solvent composition (pH = 11) and under preparative and simulated moving bed (SMB) chromatography.

The nadolol case-study presented in this work shown that achiral C18 adsorbents can be very useful in the global strategy for multicomponent chiral separations, where initial achiral separation steps can be designed to discriminate racemates and stereoisomers before going to the final enantioseparation steps with chiral stationary phases. In this

**Table 4**
SMB operating conditions and predicted performance parameters for the separation of nadolol racemates using the three different C18 adsorbents and an ethanol:water:diethylamine (30:70:0.005) solvent composition (pH = 11). Nadolol feed concentration of 10 g/L; model parameters of ε = 0.4 (bed porosity), Pe = 5000 (Peclet number), and k = 5 s$^{-1}$ (mass transfer coefficient); linear + Langmuir (LLG) isotherm model parameters as in Table 3. The SMB performance parameters are calculated after achieved the SMB cyclic steady-state (cycle 25).

| C18 adsorbent | Internal flow-rates (mL/min) | External flow-rates (mL/min) | TMB γ values | SMB performance parameters |
|---|---|---|---|---|
| **XBridge** | $Q_I^* = 20.00$ | $Q_E = 7.40$ | $\gamma_I = 9.4579$ | $PUX = 99.4\%$ |
| | $Q_{II}^* = 13.83$ | $Q_F = 0.38$ | $\gamma_{II} = 6.2333$ | $PUR = 100.0\%$ |
| | $Q_{III}^* = 14.21$ | $Q_X = 6.17$ | $\gamma_{III} = 6.4303$ | $PR = 1.33\ gl^{-1}h^{-1}$ |
| | $Q_{IV}^* = 12.60$ | $Q_R = 1.61$ | $\gamma_{IV} = 5.5890$ | $SC = 2.06\ lg^{-1}$ |
| | $t^* = 5.930$ min | $Q_{REC} = 12.60$ | | |
| **Shield** | $Q_I^* = 20.00$ | $Q_E = 6.64$ | $\gamma_I = 8.3937$ | $PUX = 98.4\%$ |
| | $Q_{II}^* = 15.00$ | $Q_F = 0.22$ | $\gamma_{II} = 6.0449$ | $PUR = 100.0\%$ |
| | $Q_{III}^* = 15.22$ | $Q_X = 5.00$ | $\gamma_{III} = 6.1489$ | $PR = 0.78\ gl^{-1}h^{-1}$ |
| | $Q_{IV}^* = 13.36$ | $Q_R = 1.86$ | $\gamma_{IV} = 5.2732$ | $SC = 3.10\ lg^{-1}$ |
| | $t^* = 5.327$ min | $Q_{REC} = 13.36$ | | |
| **XSelect** | $Q_I^* = 20.00$ | $Q_E = 7.24$ | $\gamma_I = 11.2160$ | $PUX = 99.3\%$ |
| | $Q_{II}^* = 14.15$ | $Q_F = 0.30$ | $\gamma_{II} = 7.6454$ | $PUR = 100.0\%$ |
| | $Q_{III}^* = 14.45$ | $Q_X = 5.85$ | $\gamma_{III} = 7.8272$ | $PR = 1.05\ gl^{-1}h^{-1}$ |
| | $Q_{IV}^* = 12.76$ | $Q_R = 1.69$ | $\gamma_{IV} = 6.7921$ | $SC = 2.53\ lg^{-1}$ |
| | $t^* = 6.927$ min | $Q_{REC} = 12.76$ | | |
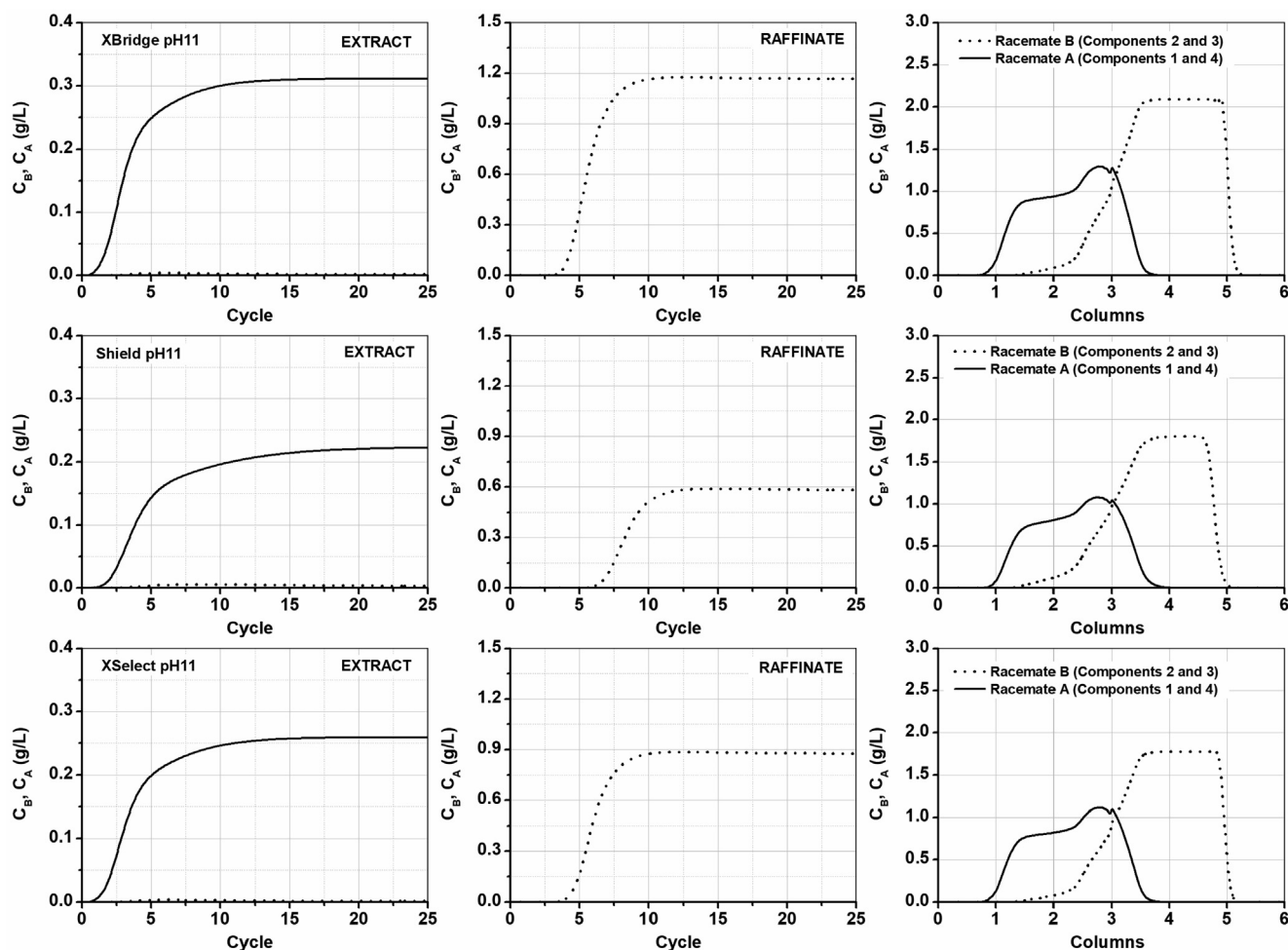
**Fig. 11.** SMB predicted transient evolution of the nadolol racemates' average concentrations in the extract (left) and raffinate (middle) streams for the three C18 adsorbents (XBridge, Shield and XSelect) and an ethanol:water:diethylamine (30:70:0.005) solvent composition (pH = 11). SMB predicted internal concentration profiles at cyclic steady-state (right; cycle 25). Dashed line for the less-retained racemate B (stereoisomers 2 and 3) and solid line for the more-retained racemate A (stereoisomers 1 and 4). Nadolol feed concentration of 10 g/L; model parameters of $\varepsilon$ = 0.4 (bed porosity), Pe = 5000 (Peclet number), and k = 5 s$^{-1}$ (mass transfer coefficient); linear + Langmuir (LLG) isotherm model parameters as in Table 3.

way, the results raised in this work introduce original and innovative solutions for the real separation of multicomponent chiral mixtures which represent an important step forward for the pharmaceutical and fine-chemistry industries.

## References

[1] K. Unger, R. Skudas, M. Schulte, Particle packed columns and monolithic columns in high-performance liquid chromatography-comparison and critical appraisal, J. Chromatogr. A 1184 (2008) 393–415.
[2] F. Gritti, G. Guiochon, The current revolution in column technology: How it began, where is it going? J. Chromatogr. A 1228 (2012) 2–19.
[3] D. Bell, Column watch - new chromatography columns and accessories for 2018, LCGC Europe 31 (2018) 202–211.
[4] J. Dang, Y. Cui, J. Pei, H. Yue, Z. Liu, W. Wang, L. Jiao, L. Mei, Q. Wang, Y. Tao, Y. Shao, Efficient separation of four antibacterial diterpenes from the roots of *Salvia Prattii* using non-aqueous hydrophilic solid-phase extraction followed by preparative high-performance liquid chromatography, Molecules 23 (2018) 623–635.
[5] W. Wang, Y. Tao, L. Jiao, M. Fan, Y. Shao, Q. Wang, L. Mei, J. Dang, Efficient separation of high-purity compounds from *Oxytropis falcata* using two-dimensional preparative chromatography, J. Sep. Sci. 40 (2017) 3593–3601.
[6] L. Jiao, Y. Tao, W. Wang, Y. Shao, L. Mei, Q. Wang, J. Dang, Preparative isolation of flavonoid glycosides from *Sphaerophysa salsula* using hydrophilic interaction solid-phase extraction coupled with two-dimensional preparative liquid chromatography, J. Sep. Sci. 40 (2017) 3808–3816.
[7] Y. Zhang, J. Jiao, C. Liu, X. Wu, Ying Zang, Isolation and purification of four flavone C-glycosides from antioxidant of bamboo leaves by macroporous resin column chromatography and preparative high-performance liquid chromatography, Food Chem. 107 (2008) 1326–1336.
[8] J. Aniceto, I. Azenha, F. Domingues, A. Mendes, C. Silva, Design and optimization of a simulated moving bed unit for the separation of betulinic, oleanolic and ursolic acids mixtures: Experimental and modeling studies, Sep. Pur. Technol. 192 (2018) 401–411.
[9] S. Wang, Y. Liang, S. Zheng, Separation of epigallocatechin gallate from tea polyphenol by simulated moving bed chromatography, J. Chromatogr. A 1265 (2012) 46–51.
[10] XBridge BEH OBD HPLC Columns Catalog, Waters Corporation (USA), 2015.
[11] XSelect OBD HSS HPLC Columns Catalog, Waters Corporation (USA), 2012.
[12] S. Atapattu, C. Pole, M. Praseuth, System maps for retention of small neutral compounds on a superficially porous ethyl-bridged, octadecylsiloxane-bonded silica stationary phase in reversed-phase liquid chromatography, Chromatographia 80 (2017) 1279–1286.
[13] G. Chen, F. Jirjees, A. Bawab, J. McElnay, Quantification of amlodipine in dried blood spot samples by high performance liquid chromatography tandem mass

spectrometry, J. Chromatogr. B 1072 (2018) 252–258.

[14] S. Dong, H. Niu, Y. Wu, J. Jiang, Y. Li, K. Jiang, X. Wang, M. Zhang, M. Han, S. Meng, Plasma pharmacokinetic determination of canagliflozin and its metabolites in a type 2 diabetic rat model by UPLC-MS/MS, Molecules 23 (2018) 1229–1244.

[15] D. McCalley, The challenges of the analysis of basic compounds by high performance liquid chromatography: Some possible approaches for improved separations, J. Chromatogr. A 1217 (2010) 858–880.

[16] N. Davies, M. Euerby, D. McCalley, Analysis of basic compounds by reversed-phase high-performance liquid chromatography using hybrid inorganic/organic phases at high pH, J. Chromatogr. A 1178 (2008) 71–78.

[17] W. Kiridena, C. Poole, S. Atapattu, J. Qian, W. Koziol, Comparison of the separation characteristics of the organic-inorganic hybrid octadecyl stationary phases XTerra MS C18 and XBridge C18 and shield RP18 in RPLC, Chromatographia 66 (2007) 453–460.

[18] L. Ferey, A. Raimbault, I. Rivals, K. Gaudin, UHPLC method for multiproduct pharmaceutical analysis by quality-by-design, J. Pharm. Biomed. Anal. 148 (2018) 361–368.

[19] L. Wang, T. Yan, L. Yang, K. Zhang, J. Jia, Monitoring the consistency quality and antioxidant activity of Da Hong Pao Teas by HPLC fingerprinting, J. Chrom. Sci. 55 (2017) 528–535.

[20] Y. Feng, C. Kunos, Y. Xu, Determination of triapine, a ribonucleotide reductase inhibitor, in human plasma by liquid chromatography tandem mass spectrometry, Biomed. Chromatogr. 29 (2015) 1380–1387.

[21] F. Wang, X. Liu, S. Lai, J. Fang, D. Semin, Chromatographic studies of unusual on-column degradations of aniline compounds on XBridge Shield RP18 column in high pH aqueous mobile phase, J. Chromatogr. A 1218 (2011) 3502–3510.

[22] K. Jayaraman, A. Alexander, Y. Hu, F. Tomasella, A stepwise strategy employing automated screening and DryLab modeling for the development of robust methods for challenging high performance liquid chromatography separations: A case study, Anal. Chem. Acta 696 (2011) 116–124.

[23] J. Zhou, X. Xue, F. Chen, J. Zhang, Y. Li, L. Wu, L. Chen, J. Zhao, Simultaneous determination of seven fluoroquinolones in royal jelly by ultrasonic-assisted extraction and liquid chromatography with fluorescence detection, J. Sep. Sci. 32 (2009) 955–964.

[24] E. Vanlaere, A. Baldwin, D. Gevers, D. Henry, E. Brandt, J. LiPuma, E. Mahenthiralingam, D. Speert, C. Dowson, P. Vandamme, K. Taxon, A complex within the *Burkholderia cepacia* complex, comprises at least two novel species, *Burkholderia contaminans* sp. nov. and *Burkholderia lata* sp. nov. Int. J. Syst. Evol. Microbiol. 59 (2009) 102–111.

[25] M. Douša, M. Slavíková, T. Kubelka, J. Černý, P. Gibala, J. Zezula, HPLC/UV/MS method application for the separation of obeticholic acid and its related compounds in development process and quality control, J. Pharm. Biomed. Anal. 149 (2018) 214–224.

[26] Z. Huang, Y. Hu, Y. Zha, R. Perrone, J. Ruan, W. Fish, Determination of polymeric impurities in asunaprevir drug substance and product using size exclusion effect of reversed-phase columns, J. Pharm. Biomed. Anal. 151 (2018) 200–208.

[27] M. Douša, J. Doubský, J. Srbek, Utilization of photochemically induced fluorescence detection for HPLC determination of genotoxic impurities in the vortioxetine manufacturing process, J. Chromatographic Sci. 54 (2016) 1625–1630.

[28] L. Bruton, J. Lazo, K. Parker (Eds.), Goodman & Gilmańs The Pharmacological Basis of Therapeutics, McGraw Hill, New York, 2001.

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

[30] J. Rekoske, Chiral separations, AIChE J. 47 (2001) 2–5.

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

[32] J. Sung, N. Nguyen, M. Park, S. Lee, Y. Lee, S. Choi, K. Kim, Preparation of 2R, 3S, 2'R-nadolol enantiomer using S-(-)-menthyl chloroformate as a chiral derivatizing reagent, Arch. Pharm. Res. 33 (2010) 1301–1306.

[33] X. Wang, C. Ching, Determination of the competitive adsorption isotherms of nadolol enantiomers by an improved h-root method, Ind. Eng. Chem. Res. 42 (2003) 6171–6180.

[34] X. Wang, C. Ching, Chiral separation and modelling of the three-chiral center-blocker drug nadolol by simulated moving bed chromatography, J. Chromatogr. A 1035 (2004) 167–176.

[35] X. Wang, C. Ching, Chiral separation of -blocker drug (nadolol) by five zone simulated moving bed chromatography, Chem. Eng. Sci. 60 (2005) 1337–1347.

[36] Y. Zhang, D. Wu, D. Wang, A. Tymiak, Enantioselective chromatography in drug discovery, Drug Discov. Today 10 (2005) 571–577.

[37] A. Ribeiro, A. Rodrigues, L. Pais, Separation of nadolol stereoisomers by chiral liquid chromatography at analytical and preparative scales, Chirality 25 (2013) 197–205.

[38] S. Jermann, M. Meijssen, M. Mazzotti, Three column intermittent simulated moving bed chromatography: 3. Cascade operation for center-cut separations, J. Chromatogr. A 1378 (2005) 37–49.

[39] R. Arafah, A. Ribeiro, A. Rodrigues, L. Pais, Separation of nadolol stereoisomers using chiralpak IA chiral stationary phase, Chirality 28 (2016) 399–408.

[40] 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.

[41] J. Kirkland, M. Straten, H. Claessens, High pH mobile phase effects on silica-based reversed-phase high-performance liquid chromatographic columns, J. Chromatogr. A 691 (1995) 3–19.

[42] M. Przybyclel, R. Majors, Column Watch – Phase Collapse in Reversed-phase LC, LCGC Europe, October (2002) 2-5.

[43] L. Edstrom, J. Samuelsson, T. Fornstedt, Deformations of overloaded bands under pH-stable conditions in reversed phase chromatography, J. Chromatogr. A 1218 (2011) 1966–1973.

[44] J. Kirkland, Development of some stationary phases for reversed-phase high-performance liquid chromatography, J. Chromatogr. A 1060 (2004) 9–21.

[45] Silicycle-ultrapure silica gels, New Generation of HPLC Silica-Based C18 Column for Both Highly Acidic and Basic pH Conditions: The SiliaChrom® SB C18 and SiliaChrom XT C18 Fidelity, Silicycle Inc., 2010.

[46] R. Majors, M. Przybyclel, Column Watch – Columns for Reversed-Phase LC Separations in Highly Aqueous Mobile Phases, LCGC Europe, December (2002) 2-7.

[47] YMC – Reversed Phase C18 columns (ODS), YMC General Catalog 2011.

[48] 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.

[49] 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.

[50] J. Aronson, The Henderson-Hasselbalch equation revisited, Biomed. Ed. 11 (1983) 168.

[51] L. Snyder, J. Kirkland, J. Glajch, Pratical HPLC Method Development, second ed., Wiley-Intersience, New York, 1997, pp. 292–349.

[52] K. Wyndham, T. Walter, P. Iraneta, U. Neue, P. McDonald, D. Morrison, M. Baynham, A review of waters hybrid particle technology. Part 2. Ethylene-Bridged (BEH technology) Hybrids and their use in liquid chromatography, Waters Corporation (2004).

[53] S. Fisher, G. Flaten, E. Hageæsther, G. Fricker, M. Brandl, In-vitro permeability of poorly water soluble drugs in the phospholipid vesicle-based permeation assay: the influence of nonionic surfactants, J. Pharm. Pharmacol. 63 (2011) 1022–1030.

[54] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, ISBN-13: 978-0471007036, second ed., John Wiley & Sons, Inc.,, 1997, pp. 21–58.

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

[56] 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.

[57] A. Ribeiro, N. Graça, L. Pais, A. Rodrigues, Optimization of the mobile phase composition for preparative chiral separation of flurbiprofen enantiomers, Sep. Purif. Technol. 8 (2009) 9–23.

[58] D. Ruthven, C. Ching, Counter-current and simulated counter-current adsorption separation processes, Chem. Eng. Sci. 44 (1989) 1011–1038.

[59] L. Pais, J. Loureiro, A. Rodrigues, Modeling strategies for enantiomers separation by SMB chromatography, AIChE J. 44 (1998) 561–569.

[60] S. Feist, Y. Hasabnis, B. Pynnonen, T. Frank, SMB chromatography design using profile advancement factors, miniplant data, and rate-based process simulation, AIChE J. 55 (2009) 2848–2860.

[61] F. Sincovec, K. Madsen, Algorithm 540, PDECOL, General collocation software for partial differential equation, ACTM Trans. Math. Softw. 5 (1979) 326–351.

[62] A. Ribeiro, P. Sá Gomes, L. Pais, A. Rodrigues, Chiral separation of ketoprofen enantiomers by preparative and simulated moving bed chromatography, Sep. Sci. Technol. 46 (2011) 1726–1739.

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

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

[65] L. Pais, J. Loureiro, A. Rodrigues, Modeling, simulation and operation of a simulated moving bed for continuous chromatographic separation of 1,1́-bi-2-naphthol enantiomers, J. Chromatogr. A 769 (1997) 25–35.

[66] C. Migliorini, A. Gentilini, M. Mazzotti, M. Morbidelli, Design of simulated moving bed units under nonideal conditions, Ind. Eng. Chem. Res. 38 (1999) 2400–2410.

[67] G. Biressi, O. Ludemann-Hombourger, M. Mazzotti, R.M. Nicoud, M. Morbidelli, Design and optimisation of a simulated moving bed unit: role of deviations from equilibrium theory, J. Chromatogr. A 876 (2000) 3–15.

[68] 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, ISBN: 978-0-12-802024-1 Butterworth-Heinemann, Elsevier, 2015, pp. 31–64.