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Separation of Racemic Chiral Drugs Using Immobilized CHIRALPAK IA: Methodology for Preparative Scale Development

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Abstract: Immobilized stationary phases represent a new perspective for analytical as well as preparative scale separation of chiral species. In this study, the basic data necessary for further development of the production-scale processes using CHIRALPAK IA columns were acquired.

The new immobilized chiral stationary phase CHIRALPAK IA has proven to be useful for the chiral separations of propranolol, metoprolol, guaifenesin, and α-Tetralol enantiomers. A broad range of standard and non-standard solvents used as components of mobile phases were tested and their influence on the separations was evaluated. A satisfactory separation considering further utilization of simulated moving bed chromatography was found for propranolol, guaifenesin, and α-tetralol using the following mobile phases n-Heptane/methanol/ethanolamine (05/95/0.1, v/v/v), n-Heptane/ethanol (85/15, v/v) and n-Heptane/DCM (85/15, v/v), respectively.

Inverse size exclusion chromatography was implemented to measure the porosity of the column using the set of polystyrene standards. The multicomponent adsorption equilibrium was characterized by linear + Langmuir model for α-Tetralol and linear model for propranolol and guaifenesin due to their limited solubility in selected mobile phases. The isotherm model was successfully validated by comparison of pulse profiles obtained by solving the mathematical model and those obtained experimentally.

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Keywords: Adsorption equilibrium, CHIRALPAK IA, chiral separation, enantiomers, preparative chromatography

INTRODUCTION

Chirality exists everywhere and plays an essential role in some aspects of nature lives including human beings. The consideration of chirality is very significant in some industries, particularly in the pharmaceutical, agrochemical, food and beverages, and petrochemical industries. In the pharmaceutical and drug industries, the existence of chirality became particularly important in the 1960s.

Separation of pure enantiomers is becoming one of the most challenging issues since each enantiomer can act in a different way, i.e., can be inactive or toxic (1). Many of the naturally occurring chiral drugs as well as their derivates can contain multiple chiral centers (2). Due to the differences observed in the pharmacokinetics properties of the enantiomers of chiral molecules, the drugs stereoisomerism is an issue having research as well as clinical and regulatory implications (3–6).

Chromatography using chiral stationary phases (CSPs) has proven to be the most useful technique in the field of chiral separation (6–8). Various CSPs have been developed to separate the different chiral compounds. Among the various polymers of polysaccharides, derivatives of cellulose and amylose are the most readily available and have been found to be suitable for chiral separations due to their wide ranges of applications at the analytical and preparative scale. Therefore, most chiral applications involving chromatography have been reported using these two polysaccharides (9,10). Moreover, both chiral phases have been proven, even though the separation performance was rather small, by employing simulated moving bed chromatography (11,12).

Generally, the selection of the mobile phase plays the main role in chiral resolution and therefore the mobile phase is selected according to compatibility with CSPs as well as solubility and structure of the racemic mixture (13–16).

In the case of coated CSPs, the linkage between a macroporous silica matrix and polysaccharide derivatives is achieved by psychical adsorption. These CSPs are widely used applying normal phase, polar organic, and reverse-phase conditions but the use of the mobile phase is limited to a few solvents and the solvents such as chlorinated solvents, tetrahydrofuran, ethyl acetate, which may dissolve or swell the polymeric selector, must have been avoided.

The restriction on the mobile phase causes severe problems in the separation of enantiomers at the preparative scale such as the solubility

of analytes in the mobile phase, which is essential to achieve high productivity. To overcome this problem and obtain more versatile CSPs, the polysaccharide derivatives have started being immobilized by covalent bonding on a silica matrix and enhancing the separation potential of the polysaccharide chiral stationary phase (7,17,18).

This procedure results in the development of the brand new CSPs, which are more robust and compatible in terms of the broad range of organic solvent systems. The use of a new generation of CSPs based on amylose and cellulose derivates (e.g. amylose tris (3,5-dimethylphenylcarbamate) and cellulose tris (3,5-dimethylphenylcarbamate) arises a number of advantages as a new selectivity profile, the enhancement of sample solubility in the mobile phase, and the possibility of automatic injections for samples dissolved in various synthetic media. A series of successful analytical applications on CHIRALPAK IA for a vast number of racemates have already been reported (19–26) but there is still a lack of separation of racemic mixtures at the preparative scale. In this study metoprolol, propranolol, guaifenesin, methocarbamol, and α-Tetralol were selected as model racemic mixtures in order to measure the adsorption equilibria data for the design of production-scale chromatographic processes, e.g. simulated moving bed technology.

Metoprolol belongs to the group of beta-adrenergic blocking agents reducing the heart rate, force of heart muscle contraction, as well as lowers blood pressure. Propranolol is one of the most important β-blocker drugs since a variety of analogues compounds have been developed based on it. It is mainly used in the treatment of hypertension and cardiac arrhythmias. Guaifenesin is an expectorant used to reduce chest congestion caused by the common cold, infections, or allergies and a component in many cough and cold treating products. Methocarbamol is a muscle relaxant and used in the h treatment of injuries and other painful muscular conditions. The last studied compound, α-Tetralol is a secondary chiral alcohol and its enantiomer R-α-Tetralol is used as a precursor for calcium antagonists commonly utilized in drugs for the treatment of hypertension and angina pectoris (8,27,28).

The objective of this work is to obtain the basic information required for the design of production-scale chromatographic processes using CHIRALPAK IA column, namely:

1. Choice of the mobile phase,
2. Characterization of the preparative scale column and
3. Adsorption equilibria isotherms for propranolol, metoprolol, guaifenesin, and α-tetralol.

For this purpose the preparative scale method development column packed with particle size of 20 μm was used. The mobile phase composition was selected based on the results obtained by the preliminary mobile phase screening covering a broad range of solvents (standard and non-standard). The composition of the mobile phases was kept as simple as possible, i.e., limited to a maximum of two or three solvents; the solubility of analytes in a mobile phase was also taken into account.

EXPERIMENTAL PART

Instrumentation

All solutions of tested compounds were prepared in the mobile phase, which was degassed and filtrated through a 0.2 μm, 50 mm in diameter NL 16-membrane filter (Schleicher & Schuell, Germany). The HPLC system consisted of Smartline 1000 LC pump, UV detector model Smartline 2500, LPG block, and degasser (Knauer, Germany). Different wavelengths of 245 and 270 nm were used depending on the analyte. The injection valve with a 10 μL sample loop was loaded manually using a syringe. The column was placed in a thermostat Jetstream 2 (WO Industrial Electornics, Austria). Clarity (DataApex, Ltd., 2004) software was used for data acquisition and HPLC control.

Materials and Reagents

The preparative scale method development columns CHIRALPAK IA (250 mm × 4.6 mm I.D.) with amylose tris (3,5 dimethylphenylcarbamate immobilized onto 20 μm silica-gel) and CHIRALPAK AD (250 mm × 4.6 mm I.D.) with amylose tris (3,5-dimethylphenylcarbamate coated onto 20 μm silica-gel were kindly gifted by Chiral Technologies Europe (France). The structural unit of the packing composition of the CSP is shown in Fig. 1.

The analytical column CHIRALPAK IB (250 mm × 4.6 mm I.D.) with cellulose tris (3,5 dimethylphenylcarbamate immobilized onto 5 μm silica-gel) used for the quantitative analysis of samples was supplied by Chiral Technologies Europe (France).

The external and total porosity of CHIRALPAK IA was determined by inverse size exclusion chromatography. The experiments were performed with a set of polystyrene standards with molecular weight from 580 to 2,100,000 g/mol (Polymer Laboratories LTD, UK) and toluene ($M = 92$ g/mol), 1,3,5-tri-tert-butylbenzene (TTB, $M = 246.44$ g/mol) and 2-propanol ($M = 60$ g/mol) as a non-retained components, all from

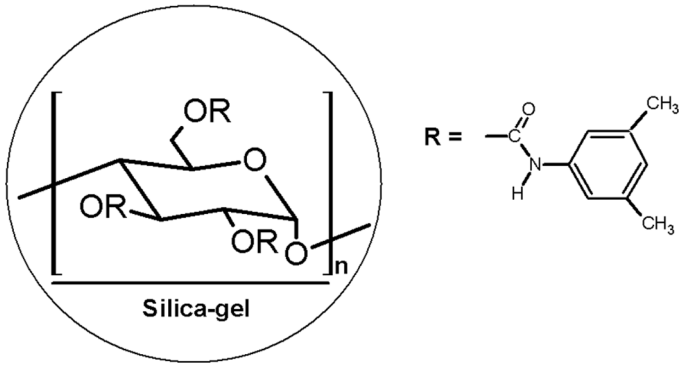

Figure 1. The structural unit of the chiral adsorbent CHIRALPAK IA.

Fluka Chemie (Switzerland). 1,4-Dioxane (99%, HPLC grade, Fluka Chemie, Switzerland) was used as mobile phase and solvent for polystyrene standards. The GC-grade organic solvents *n*-hexane, *n*-heptane, methanol (MeOH), ethanol (EtOH) 2-propanol (PrOH), dichloromethane (DCM), ethyl acetate (EtOAc), tetrahydrofuran (THF), methyl *tert*-butyl ether (MtBE) and ethanolamine (ENA) were purchased from Sigma-Aldrich Chemie, Germany. Analytes (±)-Metoprolol(+)-tartrate salt (A), (±)-Propranolol hydrochloride (B), Guaiacol glyceryl ether (Guaifenesine) (C), Guaiacol glyceryl ether carbamate (Methocarbamol) (D) and (S,R)-(±)-1,2,3,4-tetrahydro-1-naphthol (α-Tetralol) (E) were purchased from Sigma-Aldrich Chemie, Germany. The structural formulas are presented in Fig. 2.

Methodology

Inverse Size Exclusion Chromatography Experiment

The inverse size exclusion chromatography (ISEC) experiment with a set of polystyrene standards and non-retained tracers (toluene, heptane, and 1,3,5-tri-tert-butylbenzene) were carried out in order to determine the external and total porosity of CHIRALPAK IA column.

The ISEC is based on the physical sieving process excluding any chemical interactions with stationary phase. It is frequently used to separate mixtures of macromolecules (polymers), according to their molecular size. The following conditions must be granted a priori to determine the chromatographic column porosity:

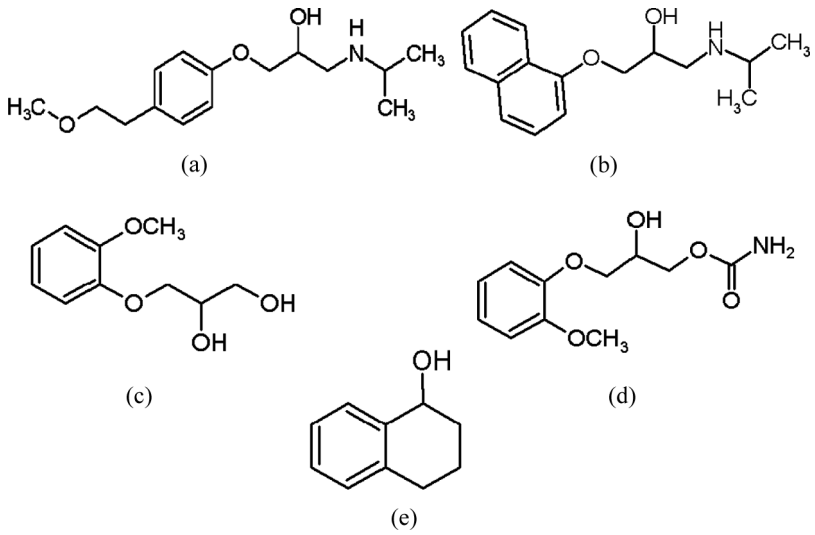

Figure 2. Structural formulas of the studied analytes: (±)-Metoprolol(+)-tartrate salt (a), (±)-Propranolol hydrochloride (b), Guaiacol glyceryl ether (Guaifenesine) (c), Guaiacol glyceryl ether carbamate (Methocarbamol) (d) and (S,R)-(±)-1,2,3,4-tetrahydro-1-naphthol (α-Tetralol) (e).

- The polymeric samples should be non-absorbable on the stationary phase;
- No agglomeration of the polymeric standard in the solvent used as mobile phase;
- The column efficiency under the experimental condition must be high;
- The elution peak profile must be close to Gaussian, with low asymmetry factor;
- The stationary phase must be rigid and the mobile phase flow-rate and temperature constant during whole experiment(29).

To assure the first two requirements 1.4-dioxane was selected as solvent for polystyrene standards.

The external porosity (ε_e) of the CHIRALPAK IA column was calculated as:

$$\varepsilon_e = \frac{V_e}{V_c} \tag{1}$$

where V_c is the volume of column ($V_c = 4.153$ mL). The elution volume of the excluded molecular mass (V_e) is obtained using the intersection point of the two straight lines when the logarithm of molecular weight (log M_w) for the various polystyrene standards is plotted versus the elution

volume. Elution volumes of the polystyrene standards were corrected for the dead volume of the HPLC system.

The total porosity was measured using species toluene, 2-propanol and 1,3,5-tri-tert-butylbenzene and different mobile phase compositions and calculated as follows

$$\varepsilon_T = \frac{V_0}{V_c} = t_0 \frac{Q}{V_c} \tag{2}$$

where V_0 is the retention volume of non-retained tracer (2-propanol), t_0 is the retention time of tracer and Q is the flow rate. The retention times of tracers were corrected for the dead time of the system.

The concentration of polystyrene standards and non-retained tracers was 5 g/L and an amount of 10 μL of each sample was injected into the column. The mobile phase (1,4-Dioxane) flow rates were 0.5, 1.0, and 1.5 mL/min. All measurements were acquired at a wavelength of 270 nm.

Elution Chromatography Experiments

The elution chromatography experiments were performed to determine the total porosity and adsorption constant of selected racemic mixtures on CHIRALPAK IA column.

The total porosity was evaluated by injecting the solution of a non-retained compound 1,3,5-Tri-tert-butylbenzene, toluene, and 2-propanol. The mobile phases used were methanol/n-Heptane/ENA (95/05/0.1, v/v/v) and n-Heptane/ethanol (85/15, v/v). The experiments were performed in the range of flow rates from 0.5 mL/min to 3.5 mL/min.

The elution experiments to determine the adsorption equilibrium constants under diluted conditions were conducted using pure enantiomers of R,S-(±)-α-Tetralol and (±)-Propranolol hydrochloride at concentration of 0.5 g/L for each enantiomer. The mobile phase used was n-Heptane/DCM (85/15, v/v) for R,S-(±)-α-Tetralol and n-Heptane/methanol/ENA (05/95/0.1,v/v/v) for (±)-Propranolol hydrochloride. The enantiomers were dissolved in the prepared mobile phase and an amount of 10 μL was injected into the column previously equilibrated with the corresponding mobile phase. The flow rates range was of 0.5 mL/min to 3.5 mL/min. All measurements were carried out at a constant temperature (25°C) and the chromatograms were recorded at a wavelength of 270 nm.

The Adsorption-Desorption Method

Among the various chromatographic methods available to determine multicomponent adsorption equilibrium isotherms, adsorption-desorption

analysis belongs to the most accurate(30–33). and was used for determination of the competitive adsorption equilibrium isotherm of R, S-(±)-α-Tetralol, (±)-Propranolol hydrochloride and Guaifenesin on the preparative scale method development column CHIRALPAK IA.

The adsorption-desorption method consists of two steps. In the first step the unloaded column is equilibrated with a feed concentration followed by the second step in which the solute is desorbed from the column using the pure mobile phase; the eluted volume resulting from the desorption step is collected and analysed. Subsequently, the whole system is re-equilibrated with pure mobile phase and the process repeated.

The adsorbed amount of each enantiomer was calculated as given:

$$V_d c_i^d = \varepsilon_T V_c c_i^f + (1 - \varepsilon_T) V_c q_i \tag{3}$$

where the concentration of each component retained in the adsorbent, q_i [g/L] in equilibrium with the feed concentration, c_i^f, [g/L], of each of the species in the mobile phase, c_i^d, [g/L], is the concentration of species "*i*" in the desorbed volume, V_d [L], V_c [L] is the column volume and ε_T is the total porosity. The total loading capacity for each concentration was calculated so that a point of the adsorption isotherm was obtained.

All measurements were carried out at a temperature of 25°C ± 0.1°C and at a flow rate of 1 mL/min. The mobile phases used were n-Heptane/DCM (85/15, v/v) for R,S-(±)-α-tetralol, n-Heptane/ethanol (85/15, v/v) for Guaifenesin and n-Heptane/methanol/ENA (05/95/0.1, v/v/v) for (±)-Propranolol. The racemic mixtures were prepared in pure mobile phase to avoid any concentration gradient during the measurement.

The equilibrium adsorption isotherm for the racemic mixture of α-tetralol was determined in the concentration range of 1 g/L to 24 g/L (a concentration range was divided evenly with 8 concentration points). Guaifenesin and propranolol showed limited solubility in the selected mobile phases and hence to avoid any precipitation inside the column the range of the racemate concentrations was of 1 g/L to 14 g/L (a concentration range was divided evenly with 7 concentration points).

The desorption and feed samples were analysed using analytical column CHIRALPAK IB. However, due to the different nature of the stationary phase of CHIRALPAK IB column a new separation condition leading to the baseline separation for each racemic mixture must have been found in order to quantitatively analyse each sample. The new separation conditions are n-Heptane/Ethanol/ENA (80/20/0.1, v/v/v) for (±)-Propranolol, n-Heptane/Ethanol (60/40,v/v) for Guaifenesin and n-Heptane/DCM/ethanol (78/20/2, v/v/v) for R,S-(±)-α-Tetralol.

Pulse Method

A large pulse of two minutes duration was carried out for propranolol, guaifenesin, α-Tetralol. All analytes were dissolved in the mobile phase. The total feed concentrations (racemate) were 2.6 and 12 g/L for α-Tetralol, 2.6 and 10 g/L for Guaifenesin, and 1.7 and 9 g/L for Propranolol.

A solution of the analyte was pumped to the column previously equilibrated with the pure mobile phase. After a period of 2 minutes, the feeding of the column with analyte was stopped and the pure mobile phase was started being pumped to the column. During the pulse elution, the samples were collected on the outlet of the column at certain times. The collected samples were analysed using analytical column CHIRALPAK IB under the same conditions as explained above.

MATHEMATICAL MODEL

The mathematical model used to describe the dynamic behavior of the fixed bed pulses considers the following assumptions:

- The axial dispersed plug flow model describes the fluid flow;
- External and internal mass-transfer for absorbable species are combined in a overall mass transfer coefficient;
- Isothermal process;
- Constant column length and packing porosity

The mass balance for species "*i*" in the bulk fluid and in the adsorbent particle are defined by Eq 1 and 2, respectively:

$$\frac{\partial C_i}{\partial t} + \nu_z \frac{\partial C_i}{\partial z} = D_L \frac{\partial^2 C_i}{\partial z^2} - \frac{1-\varepsilon}{\varepsilon} k_{\text{int}} \frac{3}{r_p} \left(C_i - C_{pi}\right) \tag{4}$$

$$\varepsilon_p \frac{\partial C_{pi}}{\partial t} + \left(1-\varepsilon_p\right) \frac{\partial q_i}{\partial t} = k_{\text{int}} \frac{3}{r_p} \left(C_i - C_{pi}\right) \tag{5}$$

where C_i is the concentration in the bulk fluid, C_{pi} is the average concentration in the particle pores, and q_i is the adsorbed phase concentration in equilibrium with C_{pi}, ν_z is the interstitial velocity, D_L is the axial dispersion coefficient, ε and ε_p are the bed and particle porosity, respectively, k_{int} is the internal mass transfer coefficient, and r_p is the radius of the adsorbent particle.

Multicomponent adsorption equilibrium isotherm:

$$q_i = f(C_{pi}) \tag{6}$$

The initial condition is:

$$t = 0 \;\; C_i = C_{pi} = 0 \tag{7}$$

The Danckwerts boundary conditions at the column inlet ($z = 0$) and column exit ($z = L$) for $t > 0$ are:

$$z = 0, \;\; D_L \frac{\partial C}{\partial z} = \nu_z (C_0 - C_i) \tag{8}$$

$$z = L, \;\; \frac{\partial C}{\partial z} = 0 \tag{9}$$

The mass transfer was calculated using an LDF (linear driving force) approximation suggested by Glueckauf (34) given by

$$k_{\text{int}} = \frac{5 \varepsilon_p D_m}{\tau r_p} \tag{10}$$

where ε_p is the particle porosity, D_m the free molecular diffusivity and τ is the tortuosity factor, and r_p is the particle radius. The tortuosity factor was estimated by $\tau = (2 - \varepsilon_p)^2 / \varepsilon_p$. The molecular diffusivities of the adsorbates (±)-Propranolol hydrochloride, R,S-(±)-α-Tetralol and Guaifenesin were calculated by the Wilke-Chang equation (35) and extended to mixed solvents by Perkins and Geankoplis (36)

$$D_m = 7.4 \times 10^{-8} \frac{T \sqrt{\phi M}}{\eta V_m^{0.6}} \tag{11}$$

where T is the absolute temperature (K), η the mobile phase viscosity (cP), calculated according to the Teja and Rice method for liquid mixture (37). The molecular diffusivities of all tested compounds are summarized in Table 3.

Numerical Solution of Model Equations

Model equations (Eqs. 1–6) were solved using the gPROMS software package (38). The mathematical model, involves a system of partial differential and algebraic equations. The system was solved by orthogonal

collocation in finite elements (OCFEM); the axial domain was discretized in 80 finite elements with two interior collocation points. An absolute and relative tolerance of 10^{-5} was used.

RESULTS AND DISCUSSION

Preliminary Mobile Phase Screening

It is well known that the performance of each CSP can be fully exploited if the appropriate mobile phase systems are used. The nature of the mobile phase can affect enantioselectvity, retention factors, and resolution degree and other parameters, i.e., pressure drop. Two groups of solvents can be distinguished with regard to the nature of the CSPs, namely the standard and non-standard solvents. The solvents in the first group including non-polar solvents such as hexane, heptane, and polar solvents like alcohols and acetonitrile can be safely applied on the coated CSPs. Whereas the solvents in the second group denoted as "non-standard" can only by used with the immobilized CSPs column.

Non-standard solvents are further classified in two groups based on enantioselectivity:

1. group with higher enantioselectivity: alcohols, THF, MtBE, CH_2Cl_2, and
2. group with lower enantioselectivity: ethylacetate, acetonitrile, toluene, $CHCl_3$, toluene, 1,4-dioxane, acetone.

The adsorption performance of the column CHIRALPAK IA was studied in order to determine the separation conditions for Metoprolol, Propranolol, Guaifenesine, Methocarbamol, and α-Tetralol. In the screening for the mobile phase, a broad range of solvents and their combinations were covered, however, for the sake of simplicity, only the best separation conditions for each racemic mixture are reported.

Metoprolol and Propranolol belong to the same group of drugs so called β-blockers. There are already known separation conditions using CHIRALPAK AD (5 μm) the stationary phase reported by various researchers (9). The authors have suggested polar mobile phase (methanol) with a trace of a basic additive, for instance ethanolamine at a typical concentration not exceeding 0.1%. The role of ethanolamine or other additives in the mobile phase is believed to suppress the undesirable effect of residual free silanols groups on the support surface and help elute the basic solutes in a reasonable time with improved peak symmetry, resolution, and enantioselectivity (39).

Encouraged by the results regarding the separation of metoprolol and propranolol using CHIRALPAK AD ($d_p = 20\,\mu m$) (see Fig. 3), the same chromatographic separation conditions were applied to CHIRALPAK IA ($d_p = 20\,\mu m$) column. However, no indication of enantiomers separation was found for metoprolol while in the case of propranolol a slight separation was observed. The chromatograms obtained for metoprolol and propranolol using CHIRALPAK AD and CHIRALPAK IA are shown in Fig. 3.

The separation factors for propranolol enantiomers on columns CHIRALPAK AD and CHIRALPAK IA under the same conditions were 3.52 and 1.34, respectively. Although the chiral selector in both

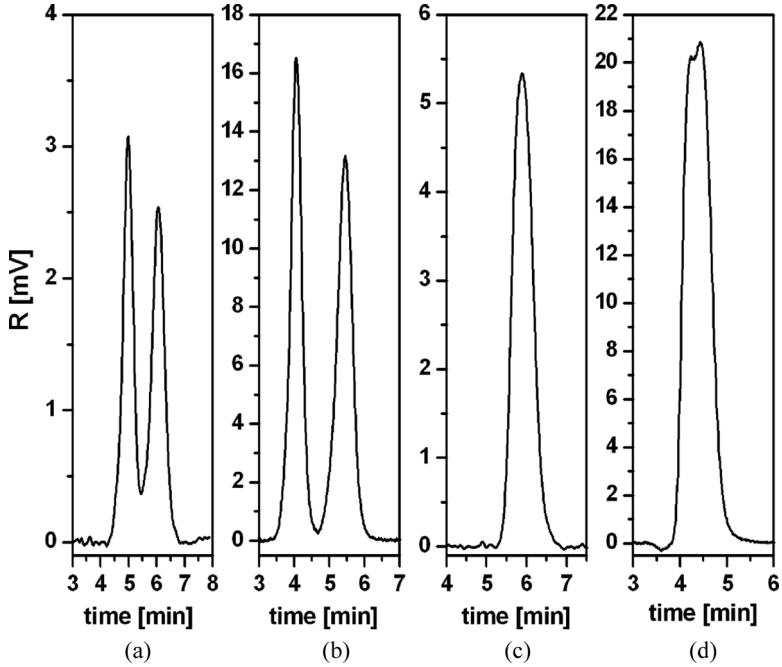

Figure 3. Chromatogram of propranolol and metoprolol; Separation conditions: (a). Metoprolol, CHIRALPAK AD, mobile phase: methanol/ethanolamine (100:0.1, v/v). Calculated retention factor: $k_2 = 0.73$ and separation factor: $\alpha = 1.72$; (b). (±)-Propranolol, CHIRALPAK AD, mobile phase: methanol/ethanolamine (100:0.1, v/v). Calculated retention factor: $k_2 = 0.55$ and separation factor:$\alpha = 3.52$; (c). Metoprolol, CHIRALPAK IA, mobile phase: methanol/ethanolamine (100:0.1, v/v). (d). Propranolol, CHIRALPAK IA, mobile phase: methanol/ethanolamine (100:0.1, v/v). Calculated retention factor: $k_2 = 0.28$ and separation factor: $\alpha = 1.34$.

CSPs is of the same nature, results suggest that immobilization of the amylase tris-(3,5 dimethylphenylcarbamate) on the silica matrix affects the chiral recognition ability comparing to coated CHIRALPAK AD as reported by Ghanem et al (9). The most possible explanation of the decrease in the enantioselectivity can be related to the alternation of the higher order structure of the polysaccharide derivate through the chemical bonding on the silica support (40).

Nevertheless, the separation using CHIRAKPAK IA was improved for both analytes by increasing the amount of non-polar solvent n-Hexane or n-Heptane in the mobile phase. The increase of enantioselectivity on CHIRALPAK IA for metoprolol was achieved using methanol/n-Hexane/ENA (80/20/0.1, v/v/v) with $k_2 = 0.41$ and $\alpha = 1.27$; for propranolol with methanol/n-Heptane/ENA, (95/05/0.1, v/v/v) with $k_2 = 0.34$ and $\alpha = 1.26$. Both chromatograms are depicted in Fig. 4 a, d. It is clear that adding a non-polar component to the mobile phase (Methanol/ENA) doubled the retention of the enantiomers though the separation factor α, remains constant.

The separation of β-blockers metoprolol and propranolol was also attempted using non-standard solvents namely MtBE, DCM, THF and ethylacetate. However, no improvement in separation was observed. Moreover, the solubility of β-blockers in the mentioned solvents was limited due to the fact that metoprolol and propranolol are available in a salt form. Therefore, their solubility is higher in polar solvents such as water and alcohols comparing to the solubility in heptane and THF and both are practically insoluble in MtBE. Table 1 summarizes all organic solvents and their combinations used as the mobile phases in the separation of all tested enantiomers.

Guaifenesin and methocarbamol were other compounds separated on CHIRALPAK IA. Both analytes have a good solubility in standard as well as in non-standard solvents. A reasonable separation of guaifenesin was achieved using n-Heptane and ethanol as the mobile phase. The increase of the alcohol content increases the mobile phase polarity, which results in the decrease of the retention time of the enantiomers. The highest separation factor was observed when the alcohol content was kept at a minimum, on the other hand, retention times as well as retention factors of the enantiomers increase. The chromatogram of guaifenesin using mobile phase n-heptane/ethanol (85/15, v/v/) is shown in Fig. 4b.

Among all tested mobile phases (Table 1) the only, but very poor, separation of methocarbamol enantiomers was observed using heptane/ethylacetate as the mobile phase. The use of other standard or non-standard solvents did not lead to a reasonable separation of methocarbamol using immobilized CHIRALPAK IA.

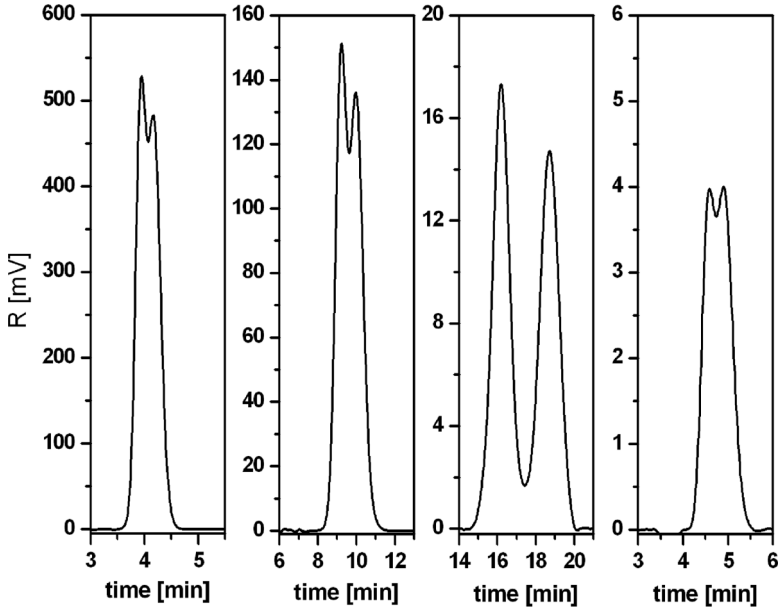

Figure 4. Chromatograms of Propranolol (a), Guaifenesin (b), α-tetralol (c), Metoprolol (d). Separation conditions: CHIRALPAK IA, mobile phase: (a) methanol/n-Heptane/ENA (95/05/0.1, v/v/v). Calculated retention factor: $k_2 = 0.34$ and separation factor: $\alpha = 1.26$. (b): mobile phase: n-Heptane/ethanol (85/15, v/v). Calculated retention factor: $k_2 = 2.32$ and separation factor: $\alpha = 1.12$. (c): mobile phase: n-Heptane/DCM (85/15,v/v). Calculated retention factor: $k_2 = 5.24$ and separation factor: $\alpha = 1.2$. (d): mobile phase: n-Hexane/methanol/ENA (20/80/0.1, v/v/v). Calculated retention factor: $k_2 = 0.41$ and separation factor: $\alpha = 1.27$.

Another compound of interest was α-tetralol for which there are already known separating conditions on CHIRALPAK AD (20 μm), (41,42) Therefore, our focus was to find the separation conditions for α-tetralol on CHIRALPAK IA using non-standard solvents. The conditions under which α-tetralol exhibited sufficient separation were found using n-Hexane or n-Heptane mixed with 2-propanol, THF and DCM.

A reasonable separation was achieved using n-heptane mixed with small amount (10%) of THF. However, the separation was extremely sensitive to change of THF concentration. A content of THF less than 10% caused a significant decrease of mobile phase elution strength and infinitive retention (no peak was detected within 40 minutes at flow rate

Table 1 All solvents and their mixture used as mobile phases in screening experiment

Solvent(S)	MtBE				n-heptane			MeOH/ EtOH/ PrOH
Co-eluent (CoE)	EtOH	MeOH	MeOH	EtOAc	PrOH	DCM	EtOAc	n-hexane/ n-heptane
Screening range (S/CoE)	50/50–100/0	80/20–100/0	80/20–100/0	90/10 80/20	100/0–80/20	90/10–0/100	95/05–20/80	100/0–05/95
Basic modifier (ENA)			0.1%					0.1%

Solvent(S)	DCM	n-heptane	MtBE
Co-eluent (CoE)	EtOH	THF	THF
Screening range (S/CoE)	30/70–70/30	95/05–30/70	99/01–30/70
Basic modifier (ENA)			

of 1 ml/min); alternatively, the increase of THF content (more than 15%) in the mobile phase causes fast elution with no separation.

The difference in separation performance using n-Hexane or n-Heptane was compared and insignificant selectivity differences could be noticed using either n-hexane or n-heptane.

Afterwards, the most suitable separation conditions for α-tetralol were found using a mixture of n-Heptane/DCM as a mobile phase. The chromatogram of α-tetralol with mobile phase n-heptane/DMC (85/15, v/v) is shown in Fig. 4 c. DCM was added to n-heptane in a proportional range of 15 to 60%. In this studied range, the separation factor (α) was almost independent of DCM concentration but the retention factor was strongly influenced by the increase of DCM content.

Preparative Scale Method Development

Determination of Porosities of the Column

The plot of the logarithm of molecular mass of the polystyrene standards versus their elution volumes is presented in Fig. 5.

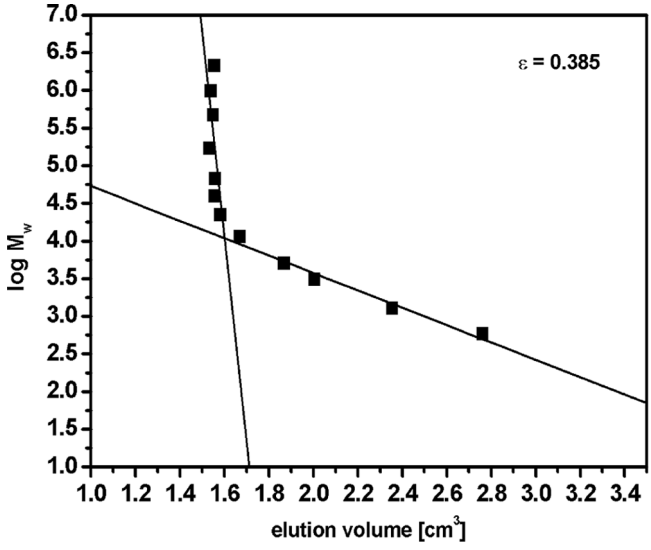

Figure 5. The ISEC plot of the logarithm of the molecular masses of the polystyrene standards versus their elution volume (flow rate 1 mL/min).

There are two lines which define two specific regions in the pore size distribution:

1. the internal pore zone describing the pore distribution in particles, and
2. the external pore zone corresponding to the pores between particles(43, 44).

The external porosity obtained for CHIRALPAK IA column is 0.385 which is a reasonable value for a well packed column.

The total porosity was measured using toluene, 2-propanol, and 1,3,5-tri-tert-butylbenzene and different mobile phase compositions methanol/n-Heptane/ENA (95/05/0.1, v/v/v) and n-Heptane/ethanol (85/15, v/v). It was found that the less absorbable compound from all three tested tracers was 2-propanol, and hence the total porosity of the column was calculated from its retention time.

The value of the total porosity measured in methanol/n-Heptane/ENA (95/05/0.1, v/v/v) mobile phase was 0.72 while the total porosity using mobile phases n-Heptane/ethanol (85/15, v/v) and n-Heptane/DCM (85/15, v/v) was 0.62. The possible explanation could be that ethanolamine is bonded on silanols groups and thus the hydrogen bonds between free silica centers could be broken down. The process results in the widening of the particles pores and the increase of the internal porosity.

The Multicomponent Adsorption Isotherm of Racemates

The CSPs used for chiral separation are usually heterogeneous. There are selective sites of the adsorbent where each of the enantiomers interacts differently and nonselective sites retain the enantiomers identically. Several multicomponent isotherm models were investigated (31) to predict the adsorption on heterogeneous surfaces. However, most of these multicomponent adsorption isotherm models consider the competition between species for the available selective sites but generally fail when the selectivity is concentration dependent. Concentration dependent selectivity can be simply described by the linear + Langmuir isotherm model, which has been previously used to characterize the adsorption isotherm on chiral stationary phases(11,45,46). The adsorption equilibrium isotherm equation for the species i and j is given by

$$q_i = H_i c_i + \frac{q_s b_i c_i}{1 + b_i c_i + b_j c_j} \tag{12}$$

where H_i [−] is the equilibrium constant for the adsorption of enantiomer "i" on the nonselective sites, b_i, b_j [L/g] are the equilibrium constants for

the adsorption of enantiomers "*i* " (S-enantiomer) and "*j*" (R-enantiomer) on the enantioselective sites and q_s [g/L] is the saturation capacity of the enantioselective sites.

The adsorption constant at infinitive dilution, K_i, for each enantiomer is as follows

$$K_i = H_i + q_s b_i \tag{13}$$

To obtain the parameters of the isotherm model thermodynamically consistent the saturation capacity, q_s of the enantioselective sites was assumed to be equal for both enantiomers as well as the equilibrium constants, H_i, for the adsorption on the nonselective sites.

The objective function used to minimize the overall error, δ, includes the sum of the weighted relative error as follows (30)

$$\delta = \sum_{i=1}^{N} \sqrt{\sum_{j=1}^{p} \frac{1}{n_e - p} \left(\frac{q_{\exp,j} - q_{theor,j}}{q_{\exp,j}} \right)^2} \tag{14}$$

where $q_{\exp,\,j}$ is the experimental value of the adsorbed amount, $q_{theor,\,j}$ is the theoretical value of the adsorbed amount calculated based on the isotherm equation, n_e is the number of experimental points, p is the number of parameters in isotherm model (for our case the number of parameters was four), N is number of compounds.

The parameters of the isotherm model in Eq. 12 for α-tetralol were determinate satisfying Eq. 13; the adsorption constant, K_i was obtained from the small pulse of pure enantiomers at different flow rates of the mobile phase except for guaifenesin because of lack of pure enantiomers.

The best fitted equilibrium isotherm parameters for α-tetralol are listed in Table 2 and the comparison of the isotherm model and experimental points is depicted in Fig. 6. Due to limited solubility of propranolol and guaifenesin in the given mobile phases, the linear model described the adsorption equilibrium ($q_i = KC_i$) was used. The adsorption constant for guaifenesin and propranolol are reported in Table 2 and experimental equilibrium points are shown in Fig. 7 and Fig. 8, respectively.

Table 2. Adsorption equilibrium parameters for the isotherm models

Compound	K_R	K_S	H [−]	q_S [g/L]	b_S [L/g]	b_R [L/g]
Propranolol	0.82	0.99	–	–	–	–
Guaifenesin	4.18	4.69	–	–	–	–
α-tetralol	12.83	11.13	3.308	61.853	0.127	0.154

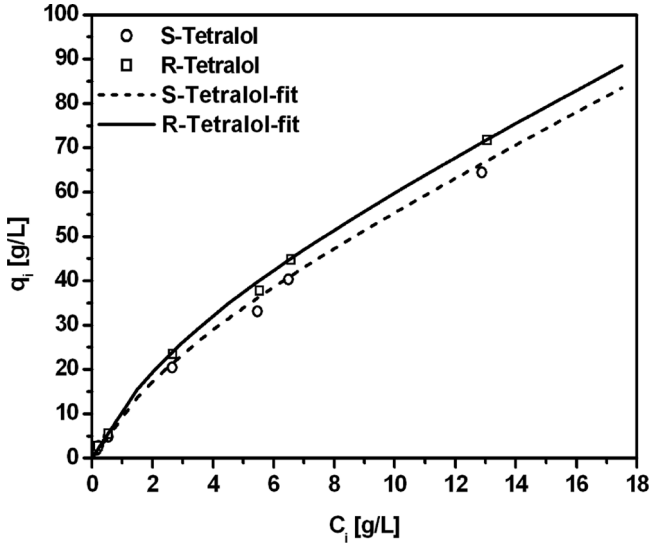

Figure 6. Adsorption competitive isotherms of α-tetralol enantiomers using Linear-Langmuir model.

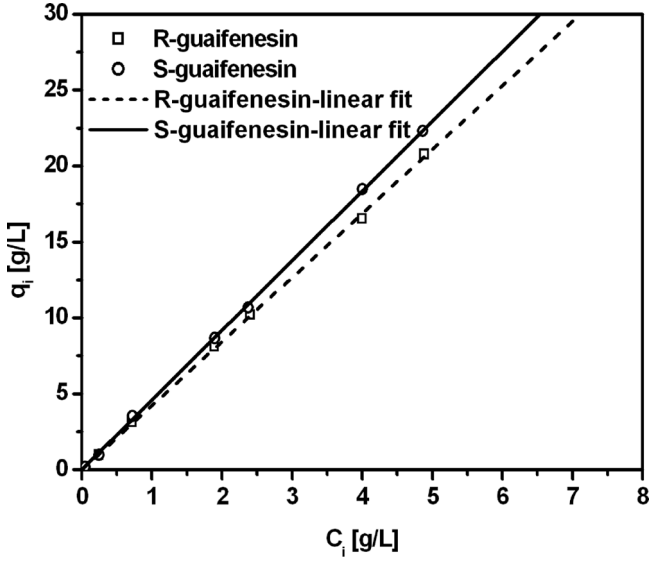

Figure 7. Adsorption isotherms of Guaifenesin enantiomers using linear model.

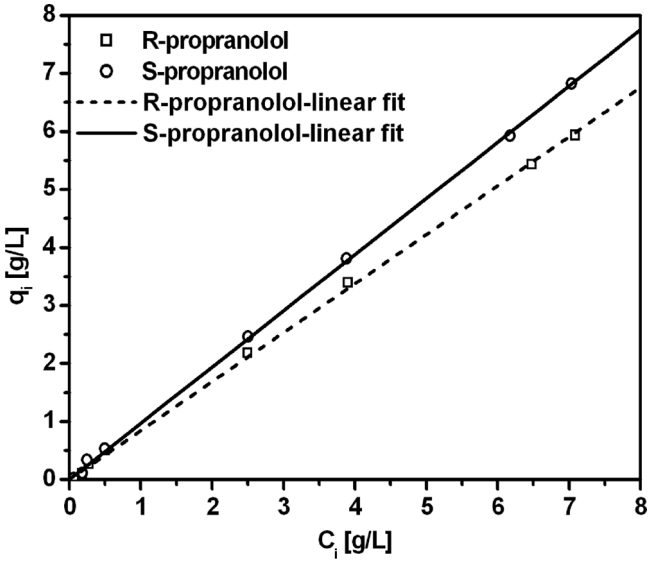

Figure 8. Adsorption isotherms of Propranolol enantiomers using linear model.

Validation of the Mathematical Model

In order to validate the adsorption equilibrium isotherm models the band profiles at different feed concentrations and the same pulse duration were carried out for all tested compounds and compared with the mathematical model.

The intraparticle pore diffusion was considered as the only resistance to mass transfer since it was approximately one order of magnitude smaller than external mass transfer, and therefore the rate-limiting step and the external mass transfer resistance was omitted.

Pulse experiments were carried out at flow rate 1.0 mL/min with pulse duration of 2 min and at an ambient temperature.

The parameters of mathematical model were calculated and are summarized in Table 3.

Experimental and simulated pulse responses for α-tetralol at total feed concentration 2.6 g/L and 12 g/L with pulse duration 2 minute column CHIRALPAK IA are shown in Figs. 9 and 10. Experimental and simulated pulse profiles of α-tetralol enantiomers are in good agreement.

The comparison between the experimental band profiles (points) and profiles calculated by the mathematical model (solid line) for guaifenesin and propranolol are depicted in Figs. 11–14.

Table 3. Parameters used in the mathematical model

Parameters	Column CHIRALPAK IA		
d_p [cm]	20×10^{-4}		
$L_c \times d_c$ [cm]	25×0.46		
Mobile phase	Propranolol n-Heptane/Methanol/ ENA (5/95/0.1)	Guaifenesin n-Heptane/Ethanol (85/15)	α-Tetralol n-Heptane/DCM (85/15)
η [cP]	0.511	0.512	0.255
ρ [g/L]	0.786	0.702	0.844
ε		0.38	
ε_T	0.72	0.62	0.62
D_L [cm^2/s]		1.0×10^{-3}	
D_m [cm^2/s]	9.92×10^{-6}	1.68×10^{-5}	3.83×10^{-5}
k_{int} [cm/s]	2.77×10^{-3}	4.68×10^{-3}	1.07×10^{-2}

A fair agreement between the experimental (symbols) and simulated (solid line) pulse profiles of guaifenesin and propranolol enantiomers can be observed as well. Furthermore, it is worth noting that experimental elution times of each enantiomer of guaifenesin and propranolol at both

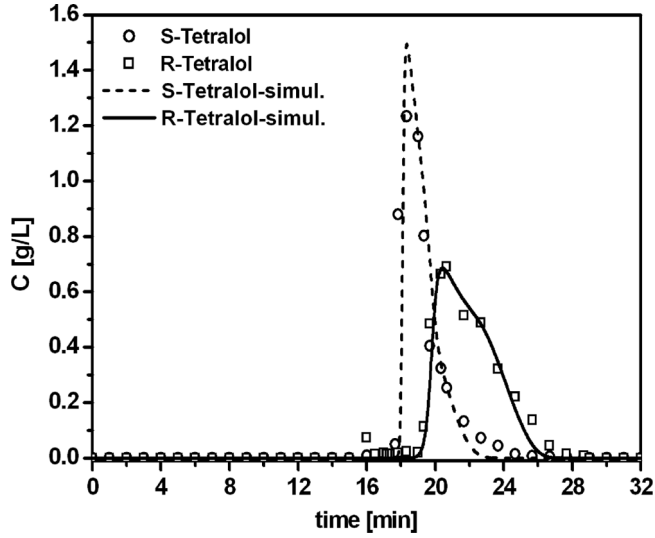

Figure 9. Pulse of α-tetralol with concentration 2.6 g/L and time period 2 min.

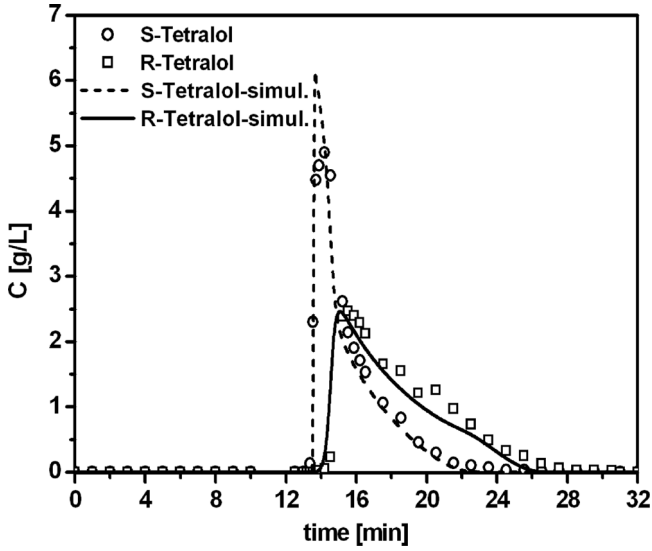

Figure 10. Pulse of α-tetralol with concentration 12 g/L and time period 2 min.

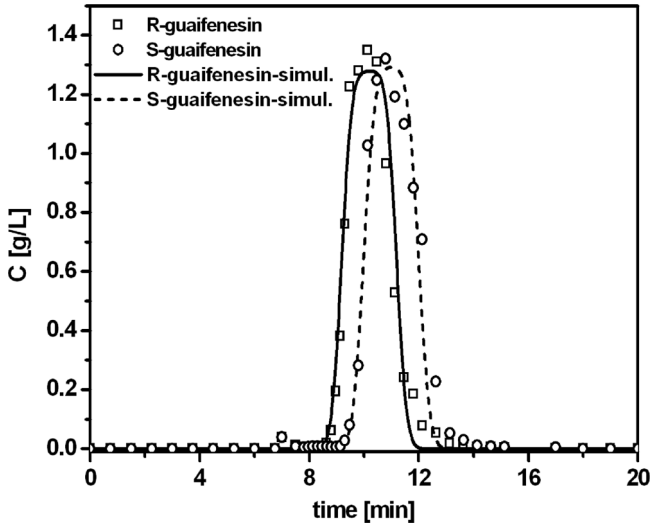

Figure 11. Pulse of Guaifenesin with concentration 2.6 g/L and time period 2 min.

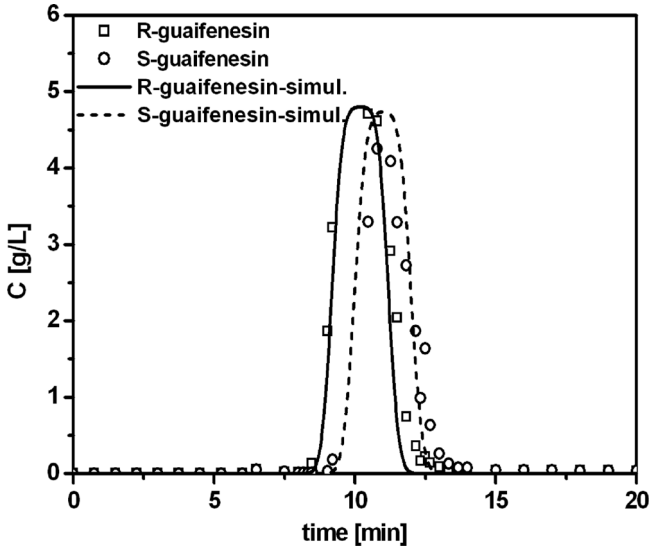

Figure 12. Pulse of Guaifenesin with concentration 10 g/L and time period 2 min.

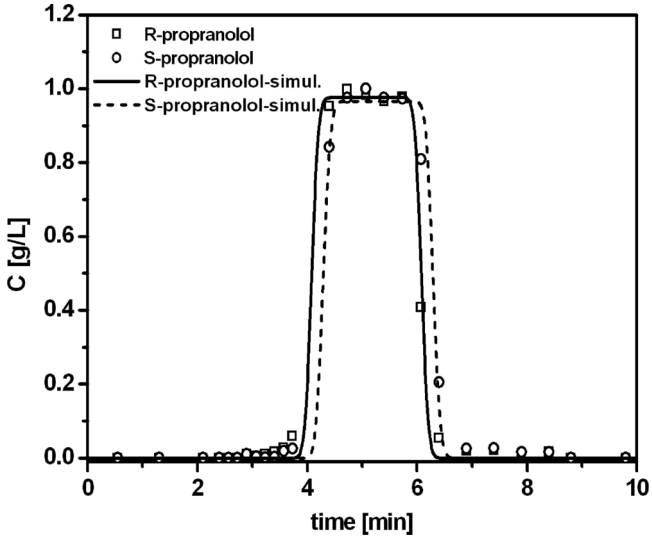

Figure 13. Pulse of propranolol with concentration 1.7 g/L and time period 2 min.

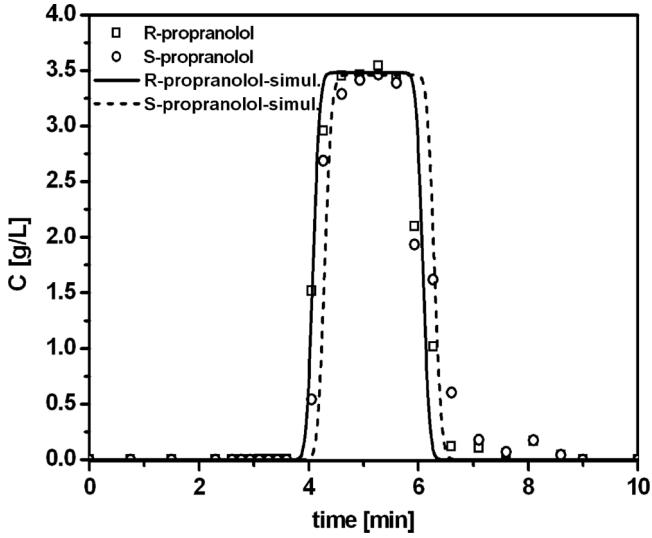

Figure 14. Pulse of propranolol with concentration 9 g/L and time period 2 min.

feed concentrations are very similar which clearly corresponds with linearity of isotherm.

Comparing both pulses of propranolol at feed concentration of 1.7 g/l and 9.0 g/L, an extensive tailing at feed concentration (Fig. 14) is noticeable. This could be caused by the base nature of the analyte and the presence of interactions between the free silanols groups even if ethanolamine is present in the mobile phase.

CONCLUSION

In view of a further design of production-scale chromatographic processes the selection of a proper chromatographic system, determination of the basic characteristics of the preparative scale column, as well as the measurement of multicomponent adsorption equilibria of propranolol, guaifenesin, and α-Tetralol on CHIRALPAK IA column were reported.

A suitable chromatographic separation conditions (mobile phase composition) for the β-blockers (metoprolol, propranolol), antitussive (guaifenesin), muscle relaxant (methocarbamol) and intermediate (α-Tetralol) on CHIRALPAK IA column ($d_p = 20\,\mu m$) using standard and non-standard solvents was selected. From the foregoing preliminary screening the reasonable separation conditions, if one intends using the simulated moving bed chromatography, were determined for propranolol,

guaifenesin, and α-tetralol using mobile phases n-heptane/methanol/ENA (5/95/0.1), n-heptane/ethanol (85/15) and n-heptane/DCM (85/15), respectively and obtained separation factors, α are 1.26 for propranolol, 1.12 for guaifenesin and 1.27 for α-tetralol. However, considerable differences between the separation factors of propranolol and metoprolol enantiomers were observed using CHIRALPAK AD and CHIRALPAK IA under the same experimental conditions and a fact that both columns possessing the same structural unit in the stationary phase.

Immobilization of the chiral stationary phase on the silica carrier allows the measurement of the external porosity by ISEC method using a set of polystyrene standards and 1,4 - dioxane as a mobile phase. The external porosity found was of 0.385, which is in accord with the external porosity of a well-packed column. The total porosity was obtained from the retention time of the solvent peak (2-propanol) with a value of 0.72 for the propranolol system and 0.62 for α-Tetralol and guaifenesin system.

The experimentally obtained adsorption equilibrium isotherms were mathematically described applying a competitive linear-Langmuir model for α-Tetralol and the linear model for guaifenesin and propranolol in the concentration range considered.

The mathematical model including intraparticle mass transfer resistance and adsorption equilibrium was used to predict the band profiles of pulses with duration of 2 minutes at different feed concentrations for all tested compounds. Good agreement between the experimentally measured and predicted pulses behaviors was found.

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NOMENCLATURE

b	equilibrium constant for the adsorption of enantiomers (m^3/kg)
C	bulk liquid phase concentration (kg/m^3)
C_0	initial (feed) concentration (kg/m^3)
C_p	liquid concentration (kg/m^3)

D_L axial dispersion coefficient (m^2/s)
d_c diameter of the column (m)
d_p diameter of particle (m)
D_m molecular diffusion (m^2/s)
H adsorption equilibrium constants of non-selective site (−)
K adsorption equilibrium constants (−)
k retention coefficient (−)
k_{int} internal mass transfer coefficient (1/s)
L length of the column (m)
q adsorbed phase concentration (kg/m^3)
q_s adsorbed phase saturation concentration of component (kg/m^3)
Q flow rate (m^3/s)
r_p particle radius (cm)
T temperature (K)
t time variable (s)
t_0 retention time of nontracer (s)
t_R retention time (s)
ν_z interstitial velocity (m/s)
V_0 retention volume of nontracer (cm^3)
V_c volume of the column (m^3)
V_e retention volume of excluded molecular mass (cm^3)
V_d elution volume (m^3)
V_m molar volume of the adsorbate at its normal boiling temperature (m^3/mol)
z axial variable (m)

Greek Symbols

α separation factor (−)
ε_e external porosity (−)
ε_p internal porosity (−)
ε_T total porosity (−)
ϕ association factor (−)
η viscosity (Pa.s)
ρ fluid density (kg/m^3)
τ tortuosity (−)

Subscripts and superscripts

i, j species in binary system
d desorption
f feed
R R-enantiomer
S S-enantiomer

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