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CHROMATOGRAPHIC SEPARATION OF VERAPAMIL RACEMATE USING A VARICOL CONTINUOUS MULTICOLUMN PROCESS

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Abstract - Verapamil is a chiral drug that is marketed in its racemic form, but because of the pharmacological effects due to molecule's chirality, one of the enantiomers is more potent, and the other exhibits different activities of therapeutic interest. The preparative separation of the verapamil enantiomers was performed using a continuous Varicol unit operated on a scale of 1 g/day. Amylose tris(3,5-dimethylphenylcarbamate) functioned as the stationary phase, and *n*-hexane/isopropanol/ethanol mixtures were used as the mobile phase. Diethylamine was used as the additive. The enantiomeric purities were 93.0% for *S*-(-)-verapamil and 92.0% for *R*-(+)-verapamil in the raffinate and extract streams, respectively. The unit provided productivities of 0.18 kg of raffinate per day per kg of adsorbent and 0.20 kg of extract per day per kg of adsorbent when using a feed concentration of 12.5 g L^{-1}.
Keywords: Continuous chromatography; Enantiomers; Varicol; Verapamil.

INTRODUCTION

Varicol is a continuous preparative multicolumn chromatographic system that, in general, operates with a smaller number of columns than the conventional simulated moving bed (SMB) process (Ludemann-Hombourger *et al*., 2000; Ludemann-Hombourger *et al*., 2002; Gomes and Rodrigues, 2012; Schulte *et al*., 2005). The underlying principle of the Varicol process is the asynchronous shift of the inlet and outlet ports during an overall switching time. In the Varicol process, the overall switching time is divided into several subintervals. The local switching of the ports is permitted in each sub-interval. Given the total number of the columns employed in the Varicol process, the number of columns in each zone varies with time during an overall switching time (Zhang *et al*., 2007). As a result, the Varicol process can have several column configurations, resulting in greater flexibility than the conventional SMB process. The more strongly adsorbed component is the primary compound in the extract stream, whereas the less strongly adsorbed component predominates in the raffinate stream (Ludemann-Hombourger *et al*., 2000; Adam *et al*., 2002). The Varicol process has been proven to be superior to the SMB process in terms of product purity and productivity in several studies (Ludemann-Hombourger *et al*., 2002; Wongso *et al*., 2004; Wongso *et al*., 2005; Zhang *et al*., 2002).

Multicolumn chromatographic processes, such as simulated moving bed (SMB) processes, have been applied especially for the separation of binary mix-

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tures (Ludemann-Hombourger *et al*., 2000; Ludemann-Hombourger *et al*., 2002; Pais and Rodrigues, 2003; Gomes *et al*., 2010; Gomes and Rodrigues, 2012; Gong *et al*., 2014). Several theoretical and experimental results reported in the literature indicate that, due to the new degree of freedom, the Varicol process is a more favorable chromatographic technique than SMB, especially in terms of productivity and solvent consumption. The first general modification is the use of a small number of columns, thus reducing the cost of the stationary phase. There is a tendency to construct multicolumn units with a maximum of six columns as an alternative to the conventional eight-column setup.

Verapamil (VER), 2-(3,4-dimethoxyphenyl)-5-[2-(3,4-dimethoxyphenyl)ethyl-methyl-amino]-2-propan-2-yl-pentanenitrile, is a calcium channel-blocking drug and is an effective agent for the treatment of hypertension, supraventricular arrhythmias and angina pectoris (Shibukawa and Wainer, 1992; Lankford and Bai, 1995; Sawicki, 2001; Yoshida *et al*., 2010). VER is a chiral drug that is administered as a racemic mixture of *S*-(-)-VER and *R*-(+)-VER. These enantiomers have different pharmacodynamic and pharmacokinetic properties (Lankford and Bai, 1995; Mateus *et al*., 2007; Pagel *et al*., 1998; Bhatti and Foster, 1997; Robinson and Mehvar, 1996). *S*-(-)-VER has negative chronotropic and inotropic effects, delays atrioventricular node conduction and causes systemic and coronary artery vasodilatation. In contrast, a similar concentration of *R*-(+)-VER results in vasodilatation of the arterial and coronary vasculature, but is relatively devoid of direct cardiac effects (Mateus *et al*., 2007; Pagel *et al*., 1998). *S*-(-)-VER is 10-20 times more potent than *R*-(+)-VER in slowing the cardiac A-V conduction velocity in humans, dogs and rabbits (Lankford and Bai, 1995; Mateus *et al*., 2007). Although *R*-(+)-VER has been shown to have lower antiarrhythmic potency, studies have found that this enantiomer has anti-tumor activity (Uehara *et al*., 1993; Warmann *et al*., 2002).

This study reports the semi-preparative chromatographic separation of verapamil enantiomers. This separation was performed with both dilute and concentrated solutions with chiral columns and a Varicol laboratory unit on the scale of 1 g/day to purify verapamil enantiomers from racemic mixtures of this chiral compound. The enriched enantiomer streams were obtained using amylose tris(3,5-dimethylphenylcarbamate) as the stationary phase, hexane-isopropanol-ethanol mixtures as the mobile phase and diethylamine as the additive. This work presents the first continuous preparative chromatographic separation data to be published for a racemic mixture of verapamil.

MATERIAL AND METHODS

Racemic Mixture, Stationary Phase and Mobile Phase

The racemic verapamil separated in this work was kindly donated by EMS® Pharmaceutical Industries (Hortolândia, São Paulo, Brazil). The structures of the verapamil enantiomers are presented in Figure 1.

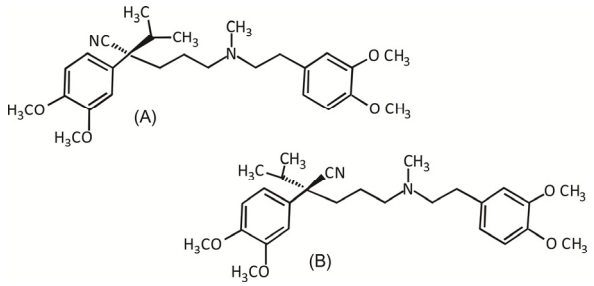

Figure 1: Chemical structures of verapamil enantiomers: (A) *S*-(-)-verapamil, and (B) *R*-(+)-verapamil.

The racemic mixture of verapamil was separated using chiral chromatographic columns packed with amylose tris(3,5-dimethylphenylcarbamate) supported on a matrix of silica. This stationary phase was developed primarily by the Okamoto group (Okamoto and Kaida, 1990; Okamoto and Kaida, 1994; Okamoto and Ikai, 2008) and has been successfully used to separate several chiral molecules (Silva Jr. *et al*., 2012; Francotte, 1994; Francotte, 2001; Lourenço *et al*., 2012). The chemical structure of the chiral selector is depicted in Figure 2.

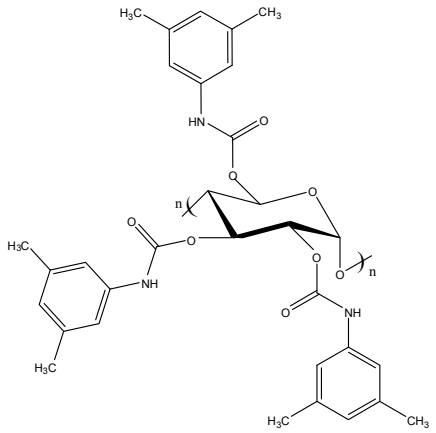

Figure 2: Chemical structure of the chiral stationary phase amylose tris(3,5-dimethylphenylcarbamate).

Six semi-preparative columns (10 cm x 1.0 cm I.D., 20 μm) packed with amylose tris(3,5-dimethylphenylcarbamate), commercially known as Chiralpak® AD

columns, for use in the Varicol system were purchased from Chiral Technologies Europe. The average mass of the chiral stationary phase in each column was 6.0 grams. The eluent used as the mobile phase, purchased from TEDIA (Brazil), was a mixture of *n*-hexane/isopropanol/ethanol (90:5:5 %, by volume), all HPLC grade, and diethylamine (0.1%, by volume) was used as an additive.

Chromatographic Parameters

Before running the Varicol unit, it was necessary to evaluate the interactions between the verapamil enantiomers and the chiral stationary phase, i.e., the chromatographic parameters had to be determined. This information was obtained by the injection of a dilute solution of the racemate into one of the six columns of the Varicol unit, and the capacity factor, selectivity and resolution were determined for different flow rates.

Experimental chromatographic parameters (retention factor, selectivity and resolution) were obtained from the peaks of chromatograms for the enantiomers S(-)-VER and R(+)-VER, separated in diluted phase on an analytical Chiralpak AD® column and calculated according to Collins *et al*. (2006).

The retention factor (k) is the ratio between the number of molecules of the solute in the stationary phase, and the number of solute molecules in the mobile phase (Collins *et al*., 2006). It was calculated according to Equation (1) in which t_R is the retention time of each enantiomer and t_m is the dead time of the column, which was determined by injection of 1,3,5-tri-tert-butylbenzene (TTBB).

$$k = \frac{(t_R - t_M)}{t_M} \tag{1}$$

The selectivity (α) measures the separation between two adjacent bands (Collins *et al*., 2006). It was calculated according to Equation (2).

$$\alpha = \frac{k_{R(+)VER}}{k_{S(-)VER}} \tag{2}$$

The resolution (R_s) is a quantitative measure of the separation. It was calculated by Equation (3), in which $t_{R\,R(+)VER}$ and $t_{R\,S(-)VER}$ are the enantiomer retention times, while w_{h1} and w_{h2} are the enantiomer peak widths at half height.

$$R_S = 1{,}177\ \frac{(t_{R\,R(+)VER} - t_{R\,S(-)VER})}{w_{R(+)VER} + w_{S(-)VER}} \tag{3}$$

The experiments were performed using the following chromatographic conditions: racemic mixture concentration, 0.5 g L^{-1}; Chiralpak® AD column (10 cm x 1.0 cm I.D., 20 μm); mobile phase, *n*-hexane/isopropanol/ethanol/diethylamine (90:5:5:0.1 %, by volume); flow rate, 1.0, 1.5, 2.0 and 2.5 mL min^{-1}; wavelength (λ), 270 nm; injection volume, 20 μL; and temperature, 25 ºC.

The Varicol Chromatographic Unit

The Varicol process introduces the operation of a continuous unit in a mode in which the feed and output streams are asynchronous. During the separation process, the column length and configuration are not constant. One cycle corresponds to the time required for each inlet/outlet stream to return to its initial position (Ludemann-Hombourger *et al*., 2000).

Figure 3 shows the operation mode and an example of a chromatogram for the six-column Varicol process. In this multi-column continuous chromatographic process, the columns are distributed among four sections (zones), each with a given role in the separation process. The section between the eluent stream and the extract stream is referred to as Section I, and its role is to desorb the more strongly retained component. Section II, between the extract stream and the feed stream, serves the purpose of desorbing the less strongly retained component. The role of Section III, between the feed stream and the raffinate stream, is to adsorb the more strongly retained component, and the role of Section IV, between the raffinate stream and the eluent stream, is to adsorb the less strongly retained component (Ludemann-Hombourguer *et al*., 2002).

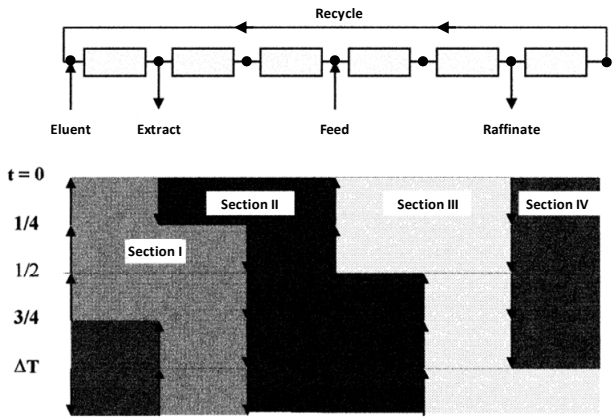

Figure 3: Operation mode and chronogram example in a six column Varicol system: switching of the streams over a cycle. Adapted from Ludemann-Hombourger *et al*., 2000.

The laboratory-scale Varicol-Micro system used in this work was obtained from NOVASEP (Pompey, France); this company owns the patent for this process (Adam *et al*., 2002). The unit has a processing capacity from 1 to 50 grams of the racemic mixture. The capacity depends primarily on the feed concentration and the size and number of the columns used. The experimental runs on the Varicol unit were performed with six stainless-steel columns (10 cm x 1.0 cm I.D., 20 μm) connected in series containing a total mass of 36 grams of chiral stationary phase. The number of columns chosen was based on the simulation results obtained with the HELP® 10.3 software from NOVASEP. This software was used to simulate runs with multiple columns from the one-column experimental chromatographic data and includes criteria for optimizing the pressure drop in the chromatographic system and the mass of the stationary phase.

Overload Experiments and Software Simulation

A stock solution of racemic verapamil (250 mg) was prepared by dissolving the compound in 25 mL of the same mobile phase used in the Varicol process. Volumes of 20, 50, 100, 200, 500, 600 and 800 μL of this solution were injected into the column. The overload experiments were performed using the following chromatographic conditions: feed concentration, 10 g L^{-1}; Chiralpak® AD (10 cm x 1.0 cm I.D., 20 μm) column; mobile phase, *n*-hexane/isopropanol/ethanol/diethylamine (90:5:5:0.1 %, by volume); flow rate, 1.0 mL min^{-1}; wavelength (λ), 260 nm; and temperature, 25 ºC.

The values for the inflection points of the chromatographic bands in the overload chromatograms were determined using a first-derivative plot produced with Origin® and were then used to determine the process parameters.

The process parameters for Varicol (feed, eluent, raffinate, extract and recycling flow rates) were calculated by HELP 10.3 software from NOVASEP®, which is based on a competitive Langmuir model modified to include a correction term for solution concentration (James *et al*., 1999).

In this software, a mathematical model is used to predict the separation performance of verapamil enantiomers by VARICOL processes, where axial dispersion and mass transfer resistances in the columns and chiral stationary phases are considered; the solid-film linear driving force model is used to describe the intraparticle mass transfer rate.

According to Guiochon *et al*. (1994) and Gong *et al*. (2014), for this mathematical model, the mass balance over a volume element of the packed column z and the mass balance in the chiral stationary phase are described by Equations (4) and (5), respectively.

$$\frac{\partial c_{i,z}}{\partial t} + \frac{u_z}{\varepsilon_T}\frac{\partial c_{i,z}}{\partial x} + \frac{(1-\varepsilon_T)}{\varepsilon_T}\frac{\partial q_{i,z}}{\partial t} = D_{ax,i,z}\frac{\partial^2 c_{i,z}}{\partial x^2} \quad (4)$$

$$\frac{\partial q_{i,z}}{\partial t} = k_{L,i,z}\frac{3}{r_p}(q_{eq,i,z} - q_{i,z}) \quad (5)$$

where i are the S(-)-VER and R(+)-VER enantiomers in the racemic mixture, $c_{i,z}$ (mg mL^{-1}) is the concentration of enantiomer i in the column z in the mobile phase, $q_{i,z}$ (mg mL^{-1}) is the average adsorbed phase concentration in the adsorbent, $q_{eq,i,z}$ (mg mL^{-1}) is the adsorbed phase concentration in equilibrium with the mobile phase concentration, ε_T is the total porosity in the column, u_z (cm s^{-1}) is the superficial velocity, x (cm) is the axial distance from the column entrance, t (s) is the time, $D_{ax,i,z}$ (cm^2 s^{-1}) is the axial dispersion coefficient of enantiomers i in the column z, and $k_{L,i,z}$ (cm s^{-1}) the mass transfer coefficient of enantiomers i in the column z.

To solve Equations (4) and (5), the initial and boundary conditions in each packed column z were:

$$t = 0 \quad c_{i,z} = 0 \quad (6)$$

$$x = 0 \quad D_{ax,i,z}\frac{\partial c_{i,z}}{\partial x} = u_z(c_{i,z} - c_{i,z}^{inlet}) \quad (7)$$

$$x = L_z \qquad \frac{\partial c_{i,z}(t, x = L_z)}{\partial x} = 0 \quad (8)$$

where L_z (cm) is the length of the column z, and $c_{i,z}^{inlet}$ (mg mL^{-1}) is the inlet concentration of enantiomers in the column z.

Once the concentrations of the enantiomers are calculated by Equations (4) and (5), together with the adsorption isotherm equation (competitive Langmuir model adapted), the mass balance was applied to each node to calculate the flow rates Q_1 (mL min^{-1}), Q_2 (mL min^{-1}), Q_3 (mL min^{-1}) and Q_4 (mL min^{-1}) in sections 1, 2, 3 and 4, respectively (Gong *et al*., 2014):

At the eluent node:

$$c_{i,z+1}^{inlet} = \frac{Q_4\ c_{i,z}(t, x = L_z)}{Q_1} \quad (9)$$

At the feed node:

$$c_{i,z+1}^{inlet} = \frac{Q_F\ c_i^F + Q_2\ c_{i,z}(t, x = L_z)}{Q_3} \quad (10)$$

where c_i^F (mg mL^{-1}) is the concentration of enantiomer *i* in the feed solution.

At the extract node:

$$c_{i,z+1}^{inlet} = c_{i,z}(t, x = L_z) \quad (11)$$

At the raffinate node:

$$c_{i,z+1}^{inlet} = c_{i,j}(t, x = L_z) \quad (12)$$

At the node between the other columns:

$$c_{i,z+1}^{inlet} = c_{i,z}(t, x = L_z) \quad (13)$$

Finally, the raffinate flow rate (Q_R) (mL min^{-1}), extract flow rate (Q_E) (mL min^{-1}), feed flow rate (Q_F) (mL min^{-1}) and eluent flow rate (Q_D) (mL min^{-1}) were calculated by global mass balances (Gong *et al*., 2014):

$$Q_1 = Q_D + Q_4 \quad (14)$$

$$Q_2 = Q_1 - Q_E \quad (15)$$

$$Q_3 = Q_2 + Q_F \quad (16)$$

$$Q_4 = Q_3 - Q_R \quad (17)$$

Analytical Performance of the Varicol Unit

The efficiency of the separation can be monitored by continuously measuring the purity of the raffinate and extract streams. To evaluate the success of the separation, the following criteria were used: the raffinate should have the highest possible purity for the less strongly adsorbed component, *S*-(-)-verapamil, whereas the extract should have the highest purity of *R*-(+)-verapamil, the more strongly adsorbed component. The purity of the enantiomer R(+)-verapamil in the extract stream is represented by $P_{R(+)VER}$, and the purity of the enantiomer S(-)-verapamil in the raffinate stream is represented by $P_{S(-)VER}$. Therefore, the purities (%) of more adsorbed enantiomer [*R*(+)-verapamil] in the extract stream and less adsorbed enantiomer [*S*(-)-verapamil] in the raffinate stream at steady state are defined as:

$$P_{R(+)VER} = \left(\frac{C_{Extract}^{R(+)VER}}{C_{Extract}^{R(+)VER} + C_{Extract}^{S(-)VER}} \right).100 \quad (18)$$

$$P_{S(-)VER} = \left(\frac{C_{Raffinate}^{S(-)VER}}{C_{Raffinate}^{S(-)VER} + C_{Raffinate}^{R(+)VER}} \right).100 \quad (19)$$

where $C_{Extract}^{R(+)VER}$, $C_{Extract}^{S(-)VER}$ are the average concentrations of more adsorbed enantiomer R(+)VER and less adsorbed enantiomer S(-)VER in the extract stream over the overall switching time. $C_{Raffinate}^{R(+)VER}$, $C_{Raffinate}^{S(-)VER}$ are the average concentrations of less adsorbed enantiomers R(+)VER and more adsorbed enantiomer S(-)VER in the raffinate stream over the overall switching time.

The total enantiomer concentration of the analyte in the raffinate and extract streams was determined with a UV/VIS detector (Smartline UV-detector 2500, Knauer, Germany) equipped with deuterium and halogen lamps and a flow cell. The wavelengths ranged between 190 and 710 nm with a standard deviation of ± 2 nm.

The difference between the enantiomer concentrations was evaluated with a polarimetric detector (IBZ Messtechnik, Germany) using tungsten and halogen lamps over a wavelength range of 380 to 680 nm with a precision greater than 0.05%. The polarimetric detector was also equipped with a flow cell.

In addition, the unit contained a sampling valve connected to one of the columns in series to permit the collection of samples for the experimental determination of the internal concentration profile of the verapamil enantiomers. This profile reflects the dynamics of the separation process within the columns.

Internal Concentration Profile

The internal concentration profile was obtained by quantifying the raffinate and extract samples at different positions of the Varicol cycle. To prepare the calibration standards, a stock solution of 0.5 g of verapamil in 25 mL of mobile phase, i.e., a mixture of *n*-hexane/isopropanol/ethanol (90:5:5 %, by volume), was prepared. Calibration samples with the following concentrations were then prepared: 0.20, 0.40, 0.60, 0.80, 1.00, 2.00, 4.00, 6.00, 8.00 and 10.00 mg mL^{-1}. Calibration curves were constructed by plotting the peak area against the concentration of each enantiomer. Raffinate and extract samples were collected at 1.26, 1.96, 2.66, 4.00, 4.70, 5.40, 6.69, 7.39, 8.09, 9.35, 11.31 and 15.03 min during cycle 30 of the Varicol process.

Determination of the Enantiomeric Purity

To ensure the purity of the extract and raffinate streams, samples were collected during the experiments for subsequent analysis by HPLC. This procedure provided purity values averaged over the time interval of collection.

The purity of each enantiomer was determined using an analytical HPLC system consisting of a Shimadzu LC-10AD pump (Kyoto, Japan), a SPD-10A variable wavelength UV/VIS detector and a Rheodyne valve with a 20 µL loop. This system was connected to a CMB-10A controller, and LabSolutions 1.23 from Shimadzu was used for data acquisition. The analytical column (250 mm x 46 cm I.D., 10 µm) contained the same stationary phase as that used in the Varicol column and was purchased from Chiral Technologies Europe®. The verapamil enantiomers were eluted with the same mobile phase as that used in the multi-column separation process.

RESULTS AND DISCUSSION

Elution Profile and Chromatographic Parameters for the Racemic Mixture

The versatility of polysaccharide stationary phases for the analytical-scale separation of racemic verapamil is well documented in the literature, with amylose-based chiral stationary phases being the most common (Shibukawa and Wainer, 1992; Mateus *et al*., 2007; Fieger and Blaschke, 1992). The Chiralpak® AD (10 cm x 1.0 cm I.D., 20 µm) column efficiently separated the verapamil enantiomers. This efficiency of the separation was analyzed by injecting dilute solutions (0.5 g L^{-1}) of the verapamil racemate into one of the six columns used in the continuous unit. Linear chromatographic data for dilute solutions are very useful for obtaining initial insight into the experimental conditions needed to determine the quantitative parameters of the interaction between the enantiomers of a racemic mixture and a chiral stationary phase.

The retention time of each enantiomer was measured, allowing the determination of the elution profile for the dilute racemic mixture of verapamil, as well as the retention time for the inert compound 1,3,5-tri-*tert*-butylbenzene (TTBB). The elution profiles of the inert compound and the racemic mixture (Figure 4) when a 0.5 g L^{-1} solution of the racemic mixture was injected into semi-preparative columns (10 cm x 1.0 cm I.D., 20 µm) packed with amylose tris(3,5-dimethylphenylcarbamate) supported on a matrix of silica are shown in Figure. 2. The capacity factor (k_i), selectivity (α) and resolution (R_s) of the chromatographic separation of the verapamil enantiomers were calculated (Table 1).

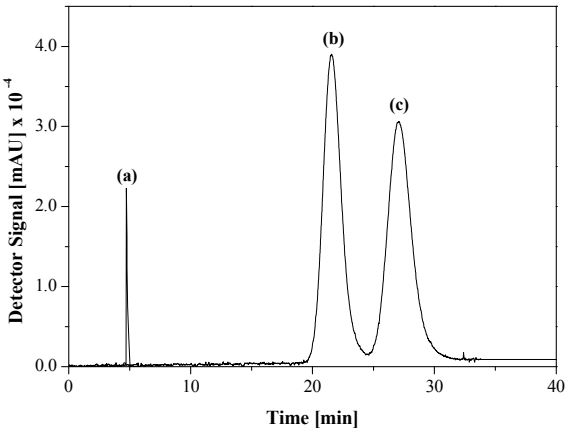

Figure 4: Elution profile for the diluted racemic mixture of verapamil and 1,3,5-tri-*tert*-butylbenzene. (a) 1,3,5-tri-*tert*-butylbenzene; (b) *S*-(-)-verapamil; (c) *R*-(+)-verapamil. Racemate concentration, 0.5 g L^{-1}; mobile phase, *n*-hexane: isopropanol: ethanol: diethylamine 90:5:5:0.1 % (by volume); stationary phase, amylose tris(3,5-dimethylphenylcarbamate); flow rate, 1.0 mL min^{-1}; detection, 270 nm; temperature, 25 ºC; injection volume, 20 µL.

Table 1: Capacity factors (k_i), selectivity (α), and resolution (R_s) for the verapamil enantiomers at different flow rates. Experimental conditions: Mobile phase, *n*-hexane: isopropanol: ethanol: diethylamine 90:5:5:0.1 % (by volume); stationary phase, amylose tris(3,5-dimethylphenylcarbamate); detection, 270 nm; temperature, 25 ºC; injection volume, 20 µL.

Flow rate (mL min^{-1})	Capacity factor		Selectivity	Resolution
	$k_{S(-)\text{-verapamil}}$	$k_{R(+)\text{-verapamil}}$	α	R_s
1.00	3.051	4.097	1.343	1.70
1.50	3.043	4.082	1.342	1.45
2.00	3.031	4.068	1.342	1.29

As shown in Table 1, the capacity factors for *S*-(-)-verapamil and *R*-(+)-verapamil were close to 3.0 and 4.1, respectively, for different flow rates. According to Collins *et al*. (2006), these values are within the optimal range for enantiomeric separation, which is $1.0 \leq k_i \leq 10.0$. The selectivity was close to 1.35 for three flow rates tested, which, according to Okamoto and Kaida (1994), represents complete separation of the enantiomers when using a polysaccharide as the chiral stationary phase whose minimum value for selectivity is 1.2.

Lastly, the resolution reached a maximum value (R_s = 1.70) for the flow rate of 1.0 mL min^{-1}. A sig-

nificant decrease in resolution was observed with increases in the flow rate. The observed decrease in resolution was associated with a reduction in interaction forces between the stationary phase and the molecules of the chiral drug, which in turn reduced the retention time. At low flow rates, the retention times are higher due to longer interaction times between the stationary phase and the individual molecules (Silva Junior *et al*., 2007).

Operating Conditions for the Varicol System

The optimization of the enantiomeric separation using the Varicol system was performed with modeling and simulation software after analytical chromatography experiments had been performed.

The proper simulation of a chromatographic separation requires reliable information about the equilibrium of the distribution of the enantiomers between the mobile and stationary phases, especially in cases with nonlinear conditions. Different volumes of the racemic mixture with the same concentration (10 g L^{-1}), representing different masses of drug, were injected into one of the semi-preparative Chiralpak® AD columns used in the Varicol system. The injection of different volumes provided overload chromatograms (Figure 5) that allowed the evaluation of the interactions between the enantiomers and the chiral stationary phase.

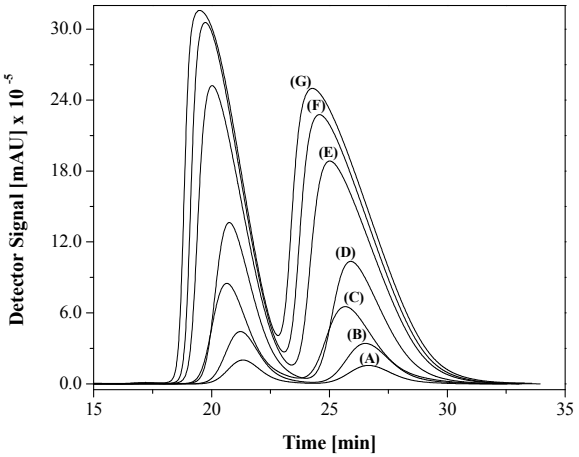

Figure 5: Elution profiles of overload enantiomeric mixtures of verapamil with different injection volume: (A) 20 µL (0.2 mg); (B) 50 µL (0.5 mg); (C) 100 µL (1.0 mg); (D) 200 µL (2.0 mg); (E) 500 µL (5.0 mg); (F) 600 µL (6.0 mg); (G) 800 µL (8.0 mg). Mobile phase, *n*-hexane: isopropanol: ethanol: diethylamine 90:5:5:0.1 % (by volume); stationary phase, amilose tris(3,5-dimethylphenylcarbamate); flow rate, 1.0 mL min^{-1}; detection, 260 nm; temperature, 25 ºC; racemic concentration, 10 g L^{-1}.

After determining the chromatographic parameters and the overloaded elution profiles for the enantiomeric mixtures of verapamil, it was possible to calculate the operating parameters (feed, eluent, raffinate, extract and recycle stream flow rates), column distribution and overall switching time of the streams used in the Varicol system using the program HELP® 10.3, which is based on a competitive Langmuir model modified to include a correction term for the solution concentration (Seidel-Morgenstern, 2004; James *et al*., 1999). The operating parameters are shown in Table 2.

Table 2: Process parameters for verapamil separation in the Varicol system.

Process parameters	Description and experimental values
Configuration (columns/section)	0.98/2.66/1.40/0.96
Stationary phase	Amylose tris(3,5-dimethyphenylcarbamate)
Mobile phase	*n*-hexane: isopropanol: ethanol: diethylamine 90:5:5:0.1 % (by volume)
Column number	6 column with 6 g/ column stationary phase
Column length	10 cm
Column diameter	1 cm
Feed concentration	12.5 g L^{-1}
Feed flow rate	0.61 mL min^{-1}
Eluent flow rate	3.87 mL min^{-1}
Recycle flow rate	10.53 mL min^{-1}
Extract flow rate	3.04 mL min^{-1}
Raffinate flow rate	1.44 mL min^{-1}
Overall switching time	2.8 min
Cycle time	6.8 min
Temperature	25 ºC
Pressure	30 bar

The feed concentration was set to 12.5 g L^{-1}, which corresponds to a concentration seven times less than the maximum solubility of the racemate in the mobile phase (90 g L^{-1}) at 25 ºC. When the appropriate operating parameters were applied, the system achieved steady state after twelve cycles. The total cycle time was 16.8 min, and the system was operated in the steady state for 5.30 h.

Results for Continuous Runs in the Varicol System

To evaluate the distribution of the enantiomers in the extract and the raffinate throughout one cycle, the experimental internal concentration profile was obtained by quantifying the enantiomers during cycle 30. Twelve samples were collected at specific times during the selected cycle, and then the enantiomeric purities were evaluated independently.

The experimental internal profiles presented in Figure 6 indicate that a good point for withdrawing the raffinate stream is located between columns 4 and 5, whereas a good point for withdrawing the extract stream is located between columns 1 and 2, with the feed stream being introduced between columns 3 and 4. This analysis also revealed a slight contamination of the raffinate and extract streams, which decreased the enantiomeric purity during the operation of the system. However, the experimental results are close to the internal concentration profile simulated by HELP® 10.3.

To evaluate the enantiomeric purities of the extract and raffinate streams during the separation process, samples were collected at the beginning of cycles 13 to 32. The enantiomeric purities averaged over the time interval of collection under the steady-state conditions of the continuous unit are depicted in Figures 7 and 8 as a function of the cycle number of the experimental run in the Varicol unit, working with a configuration of 0.98/2.66/1.40/0.96.

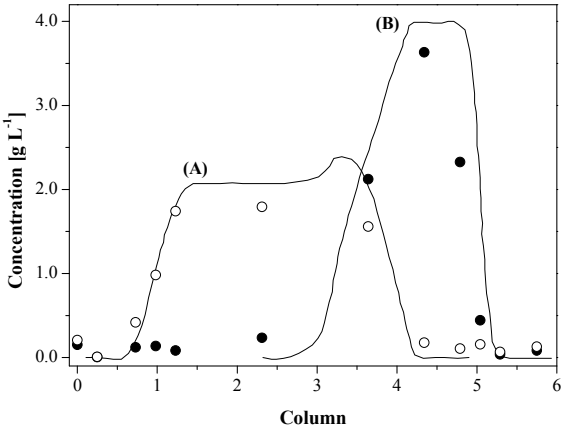

Figure 6: Experimental and simulated internal concentration profiles for the separation of the verapamil racemic mixture in the Varicol system (cycle 30). Feed concentration, 12.5 g L^{-1}. Experimental data (*S*-(-)-verapamil (●) and *R*-(+)-verapamil (○)) were taken in the steady-state regime. Simulated data for both enantiomers were obtained from HELP® 10.3 software developed by NOVASEP®.

Chromatograms obtained for the analysis of the purities of the raffinate and extract, also averaged over the time interval of collection under the steady-state conditions of the continuous unit, are displayed in Figures 9 and 10, respectively. The raffinate stream was rich in *S*-(-)-verapamil but was contaminated with a small amount of the *R*-(+)-verapamil enantiomer. In contrast, the extract stream was rich in *R*-(+)-verapamil, but was contaminated with the *S*-(-)-verapamil enantiomer.

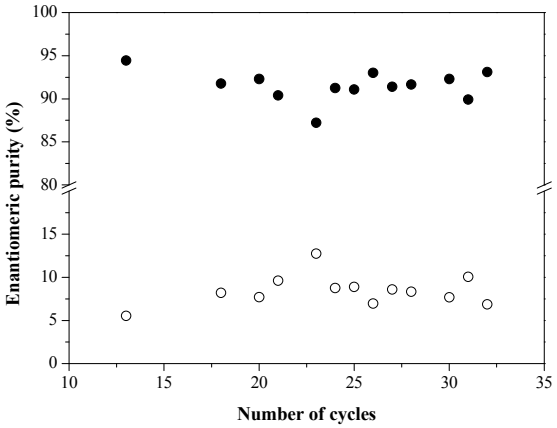

Figure 7: Enantiomeric purity obtained for the number of cycles in the Varicol system for the raffinate stream. (●) *S*-(-)-verapamil; (○) *R*-(+)-verapamil.

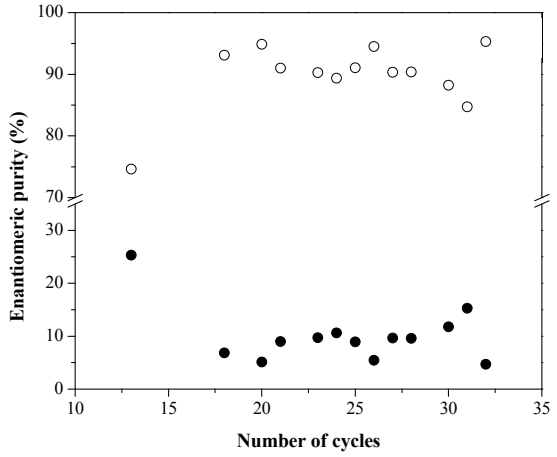

Figure 8: Enantiomeric purity obtained for the number of cycles in the Varicol system for the extract stream. (●) *S*-(-)-verapamil; (○) *R*-(+)-verapamil.

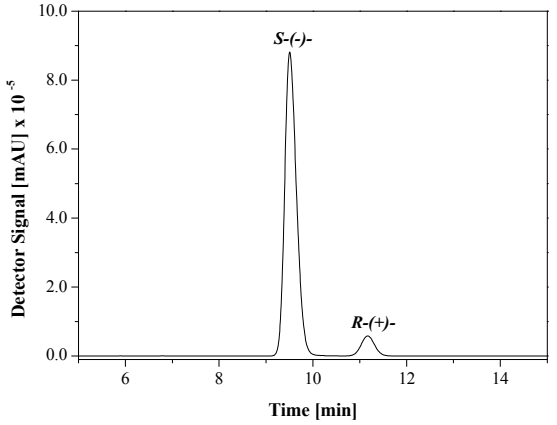

Figure 9: Chromatogram obtained for analysis of purity of the raffinate stream. Mobile phase, *n*-hexane: isopropanol: ethanol: diethylamine 90:5:5:0.1 % (by volume); stationary phase, amylose tris(3,5-dimethylphenylcarbamate); flow rate, 1.0 mL min^{-1}; detector, 270 nm (UV); temperature, 25 ºC; injection volume, 20 µL.

The $[\alpha]_D$ at 25 ºC was determined for both enantiomers, and the values obtained were close to those previously reported (Bhushan and Gupta, 2005). The extract was identified as (+)-verapamil, $[\alpha]_D$ = (+) 8.1, and the raffinate as (-)-verapamil, $[\alpha]_D$ = (-) 8.2. Note that these values are different because the enantiomers were not completely pure (Figure 9 and 10).

From the above results, the performance parameters can be calculated. A summary of these parameters for the enantiomeric separation of verapamil using the Varicol system is provided in Table 3.

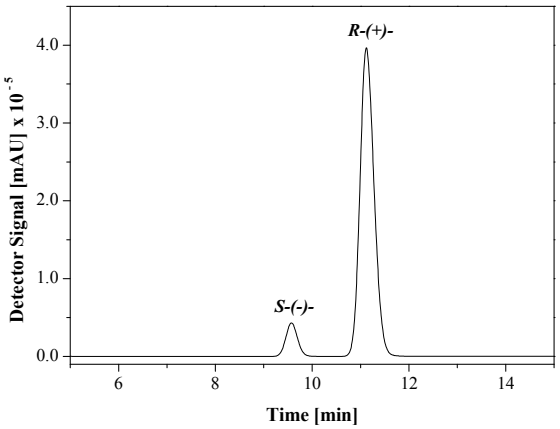

Figure 10: Chromatogram obtained for analysis of purity of the extract stream. Mobile phase, *n*-hexane: isopropanol: ethanol: diethylamine 90:5:5:0.1 % (by volume); stationary phase, amylose tris(3,5-dimethylphenylcarbamate); flow rate, 1.0 mL min^{-1}; detector, 270 nm (UV); temperature, 25 ºC; injection volume, 20 µL.

Table 3: Performance parameters for verapamil separation in the Varicol system.

Performance parameters	Experimental values
Enantiomeric purity *S*-(-)-verapamil	93.0%
Enantiomeric purity *R*-(+)-verapamil	92.0%
Productivity *S*-(-)-verapamil	0.18 kkd[(a)]
Productivity *R*-(+)-verapamil	0.20 kkd[(a)]
Recovery *S*-(-)- verapamil	81.0%
Recovery *R*-(+)-verapamil	84.0%
Solvent consumption	1.46 L/g enantiomer

[(a)]kkd = kilograms of purified enantiomer per kg of stationary phase per day.

The productivity index for the performance of preparative chromatography is typically expressed in units of kilograms of purified enantiomer per kilogram of stationary phase per day (kkd). The processing productivities of 0.18 kkd for *S*-(-)-verapamil and 0.20 kkd for *R*-(+)-verapamil indicate that the experimental procedure and the performance of the Varicol unit were satisfactory for separating the racemic mixture of verapamil, giving a purity of 93.0% for *S*-(-)-verapamil and 92.0% for *R*-(+)-verapamil. A good level of solvent consumption (1.46 L/g enantiomer) was observed for this process.

CONCLUSION

A chromatographic system using amylose tris(3,5-dimethylphenylcarbamate) as the stationary phase in six columns operating in Varicol mode achieved a good level of purification for racemic verapamil. The enantiomeric purities obtained were 93.0% for *S*-(–)-verapamil and 92.0% for *R*-(+)-verapamil in the raffinate and extract streams, respectively. The mobile phase was a mixture of *n*-hexane/ isopropanol/ethanol (90:5:5 %, by volume), and diethylamine (0.1%, by volume) was used as an additive.

The process parameters simulated by HELP® and the column overload data were suitable for determining the operating conditions that provide good separation over the range of feed concentrations examined herein. The unit provided processing productivities of 0.18 kkd for *S*-(-)-verapamil and 0.20 kkd for *R*-(+)-verapamil, which are considered satisfactory for preparative enantioselective chromatography.

To the best of our knowledge, this work provides the first continuous preparative chromatographic separation data described for the racemic mixture of verapamil.

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NOMENCLATURE

$C^{R(+)VER}_{Extract}$ concentration of the *R*-(+)-VER enantiomer in the extract stream [kg m^{-3}]

$C^{R(+)VER}_{Raffinate}$ concentration of the *R*-(+)-VER enantiomer in the raffinate stream [kg m^{-3}]

$C^{S(-)VER}_{Extract}$ concentration of the *R*-(+)-VER enantiomer in the extract stream [kg m^{-3}]

$C^{S(-)VER}_{Raffinate}$ concentration of the *S*-(+)-VER enantiomer in the extract stream [kg m^{-3}]

$P_{R(+)VER}$ purity of R(+)-verapamil enantiomer in the extract stream [-]

$P_{S(-)VER}$ purity of S(-)-verapamil enantiomer in the raffinate stream [-]
k_i capacity factor [-]
R_S chromatographic resolution [-]

Greek Letters

α selectivity [-]
α_D optical rotation [-]
λ wavelength [m]

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