INSTITUTO POLITÉCNICO
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Separation of Nadolol Stereoisomers by Liquid Chromatography using Chiralpak IA Chiral Stationary Phase

Rami Sami Arafah

Final dissertation report submitted to
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To obtain the Master Degree in

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To Senka

Abstract

The main objective of this work is to study the chiral separation of stereoisomers of nadolol by preparative liquid chromatography. In this report it is presented the state of the art and experimental results obtained for optimizing the methodology for preparative scale separation of nadolol stereoisomers using an immobilized chiral stationary phase (Chiralpak® IA).

The screening of the solvent composition is based on the best solvent or solvents mixture to perform the pseudo-binary separation (1+2+3/4) of the more retained stereoisomer using the simulated moving bed technology. The three least retained components (1, 2 and 3) co-elute in the raffinate outlet stream and the more retained component (4) will elute in the extract outlet stream.

After the solvent composition optimization, 100%methanol:0.1%diethylamine solvent composition was selected to perform the experimental SMB separation. Experimental results also include adsorption equilibrium isotherm and breakthroughs measurements followed by SMB experimental operation. Using a 2 g/L total feed concentration, the more retained stereoisomer was totally recovered at the extract outlet stream with 100% purity, obtaining a system productivity of 0.31 $g_{target\ product}/(L_{bed}.hr)$ and requiring a solvent consumption of 27.71 $L_{solvent}/g_{target\ product}$. Another SMB run was performed using a considerable higher feed concentration of 10 g/L and an improvement in the performance parameters were observed. For the more retained stereoisomer in the extract outlet stream, a purity of 99.5%, and a recovery of 97.6% was obtained, with a productivity of 1.98 $g_{target\ product}/(L_{bed}.hr)$ and a solvent consumption of 3.13 $L_{solvent}/g_{target\ product}$.

The extract and raffinate SMB outlet streams were collected and used to perform additional adsorption equilibrium isotherm and breakthroughs experiments. These results were used to evaluate the differences in the adsorption equilibrium dynamics for pure and racemic nadolol feed mixtures.

This work introduced new alternative results for the separation of nadolol stereoisomers and contributed for a future objective of the complete separation of the four nadolol stereoisomers by SMB technology using different modes of SMB operation, adsorbents and solvent compositions.

Keywords: Nadolol, Chiralpak® IA, preparative liquid chromatography, simulated moving bed (SMB)

Resumo

O principal objetivo deste trabalho consiste no estudo da separação quiral dos estereoisómeros do nadolol por cromatografia líquida preparativa. Neste relatório apresenta-se o estado da arte assim como alguns resultados obtidos da otimização da composição do solvente para a separação à escala preparativa utilizando uma fase estacionária quiral (Chiralpak® IA).

O estudo da composição do solvente é baseado na seleção do solvente ou mistura de solventes mais apropriada para realizar a separação pseudo-binária (1+2+3/4) dos estereoisómeros do nadolol utilizando a tecnologia de leito móvel simulado. Os três componentes menos retidos (1, 2 e 3) co-eluem na corrente de refinado e o componente mais retido é recuperado na corrente de extrato. Na fase inicial, realiza-se uma comparação dos resultados experimentais obtidos com a fase estacionária Chiralpak® IA com os resultados obtidos com a fase estacionária Chiralpak® AD. Para este estudo, empacotaram-se duas colunas com dimensões preparativas, com estas fases estacionárias e repetiram-se algumas análises cromatográficas com a fase estacionária Chiralpak®AD.

Após a otimização da composição do solvente, foi selecionada a mistura 100%methanol:0.1%diethylamine para realizar a separação preparativa utilizando a tecnologia de leito móvel simulado. Os resultados experimentais incluem a determinação experimental de dados de equilíbrio através da medição e modelização de isotérmicas de equilíbrio de adsorção assim como medições experimentais e a modelização de experiências de cromatografia frontal (*breakthroughs*). Finalmente, apersentam.se os resultados experimentais obtidos das experiências de separação pseudo-binária dos estereoisómeros de nadolol utilizando um sistema de leito móvel simulado (SMB). Utilizando uma concentração da solução de alimentação de 2 g/L, o estereoisómero mais retido foi totalmente recuperado na corrente de extrato com uma pureza de 100%, obtendo-se uma produtividade de 0.31 $g_{target\ product}/(L_{bed}.hr)$ sendo necessário um consumo de solvente de 27.71 $L_{solvent}/g_{target\ product}$. Uma outra experiência de SMB foi realizada utilizando uma concentração da corrente de alimentação de 10 g/L, tendo-se obtido um aumento da produtividade e uma diminuição do consumo de solvente. Para o estereoisómero mais retido, obteve-se na corrente de extrato, uma pureza de 99.5% e uma recuperação de 97.6%, uma produtividade de 1.98 $g_{target\ product}/(L_{bed}.hr)$ e um consumo de solvente de 3.13 $L_{solvent}/g_{target\ product}$.

As correntes de extrato e de refinado das operações de SMB foram recolhidas e utilizadas para realizar experiências adicionais de medidas de isotérmicas de equilíbrio de adsorção e experiências de *breakthrough* de forma a avaliar a diferença do comportamento de adsorção dos estereoisómeros quando presentes na mistura ou na forma "pura" (1+2+3 e 4).

Este trabalho introduz novas alternativas para a separação dos estereoisómeros do nadolol, contribuindo para o objetivo futuro que será a separação completa dos quatro estereoisómeros do nadolol, utilizando diferentes modos de operação SMB, adsorventes e composições de solvente.

Palavras-chave: Nadolol, Chiralpak® IA, Cromatografia líquida preparativa, Leito móvel simulado (LMS)

الخلاصة

إن الهدف الرئيسي من هذا العمل هو دراسة عملية الفصل الكيرالي للنظائر الفراغية لمركب النادولول بواسطة الكرومتغرافيا السائلة التحضيرية. نتطرق في هذا التقرير إلى بعض الدراسات السابقة إضافةً لعرض النتائج المُحصّلة بهدف التوصل إلى افضل منهجية لفصل النظائر الفراغية للنادولول ضمن النطاق التحضيري بإستخدام الطور الكيرالي الثابت (Chiralpak® IA).

يستند فحص تركيب المذيب على أفضل مذيب أو خليط من المذيبات التي تسمح بإجراء عملية الفصل الثنائية (4/3+2+1) للنظير الذي يمتلك زمن البقاء الأكبر وذلك بإستخدام تقنية محاكاة السرير المتحرك (SMB). المركبات الثلاثة التي تمتلك زمن بقاء أقل (1, 2 و 3) تمتزج مع المذيب لتخرج مع التيار الثانوي, أما المركب الأكثر بقاءً (4) يخرج مع تيار الإستخلاص.

بعد الإنتهاء مع عملية تحسين تركيب المذيب, 100% ميثانول: 0.1 % داي إيثيل أمين هو المذيب الذي تم إختياره لإجراء عملية الفصل بتقنية SMB. النتائج التجريبية تتضمن الإمتزاز التوازني عند درجة حرارة ثابتة إضافةً إلى تجارب الإختراقات أو ما يعرف بالكروموتغرافية الأمامية (breakthrough), في النهاية نعرض النتائج التجريبية لمحاكي السرير المتحرك SMB. بإستخدام 2 g/L كتركيز كلي للمحلول المغذي, تم الإسترجاع وبشكل كامل للنظير الفراغي الأكثر إحتفاظاً من تيار الإستخلاص وبنفاوة وصلت إلى 100%, بإنتاجية بلغت 0.31 $g_{target\ product}/(L_{bed}.hr)$ إضافة إلى إستهلاكية المذيب 27.71 $L_{solvent}/g_{target\ product}$. بإستخدام تركيز أعلى للمحلول المغذي 10 g/L, تمت عملية الفصل مرة آخرى بواسطة تقنية SMB حيث لوحظ تحسن في معايير الأداء. نقاوة النظير الأكثر بقاءً الخارج مع تيار الإستخلاص وصلت إلى 99.5% , وإسترجع بنسبة 97.6%, الإنتاجية وصلت إلى 1.98 $g_{target\ product}/(L_{bed}.hr)$ إضافة إلى إستهلاكية المذيب 3.13 $L_{solvent}/g_{target\ product}$.

تمّ جمع عينات من تيارات الإستخلاص والرشاحة الخارجة من SMB لغرض القيام بدراسات إضافية حول الإمتزاز عند درجة حرارة ثابتة وكذلك تجارب الغروماتوغرافيا الأمامية. لتقييم الفروقات في ديناميكية الإمتزاز التوازني لكلا الحالتين للنادولول سواء إن كان نفياً أو إن كان كمركب عنقودي.

هذا العمل قدم نتائج إضافية لفصل النظائر الفراغية لمركب النادولول وساهمت هذه النتائج في وضع الخطة المستقبلية لفصل نظائر النادولول بشكل كامل بإستخدام تقنية SMB بمتواليات مختلفة من طرق التشغيل, كذلك الممتزات وأخيراً تراكيب المذيب.

الكلمات المفتاحية: نادولول, Chiralpak® IA, الكرومتغرافيا السائلة التحضيرية, محاكي السرير المتحرك (SMB)

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List of Symbols

b_i	Model parameter for species *i* of the adsorption equilibrium isotherm (L/g)
c	Fluid phase, g/L
c_i^d	Concentration of component *i* in the eluted volume, g/L
C_i^F	Concentration of component *i* in the feed solution, g/L
d_p	Particle diameter, μm
k	Mass transfer coefficient, s^{-1}
L	Length of column, mm
N_c	Number of columns per section in the SMB system
Pe	Peclet number
PR	System productivity, grams/ day L
PUR	Raffinate Purity (%)
PUX	Extract Purity (%)
q_i^*	Concentration of component *i* in the particle, g/L
SC	Solvent consumption, L/g
t	Time variable, s
t*	Switch time interval, s
u_0	Superficial Velocity, cm/min
V_c	Column volume, cm^3
V^d	Eluted volume, cm^3
m	Numerical parameter of the adsorption equilibrium isotherm
M	Total number of experimental points for the adsorption equilibrium isotherm
N	Total number parameters to be estimated for the adsorption equilibrium isotherm model
Q:	Capacity factor of the adsorption equilibrium isotherm of the Langmuir term (g/L)

Greek Symbols

β	Safety margin (SMB operation)
ε	Bed porosity
γ	Ratio between fluid and solid interstitial velocities in the TMB operation
γ^*	Ratio between fluid and solid interstitial velocities in the SMB operation
μ_1	First moment of the peak
μ_k	Moment of order k
σ^2	Peak variance

Subscripts and Superscripts

A	Less retained component (binary mixture)
B	More retained component (binary mixture)
E	Eluent
F	Feed

i	Component index (*i= 1, 2, 3, 4)*
R	Raffinate
REC	Recycle
X	Extract

List of Abbreviations

ACN	Acetonitrile
C7	Heptane
CSP	Chiral Stationary Phase
DEA, D	Diethylamine, strong basic modifier
ESA	Ethanesulfonic acid
E	Ethanol
HAc	Acetic Acid
HETP	Height Equivalent to a Theoretical Plate
Hex	Hexane
HPLC	High-Performance Liquid Chromatography
IPA	Iso-propanol alcohol
LDF	Linear driving force
LLG	linear+Langmuir adsorption equilibrium isotherm model
MeOH, M	Methanol
RSD	Relative Standard Deviation
SMB	Simulated Moving Bed
THF	Tetrahydrofuran
TEAA	Triethylammonium acetate
TMB	True Moveng Bed
UV	Ultraviolet

Chapter 1

Motivation and Outline

1.1 Background and Motivation

Direct racemic resolution of enantiomers by means of liquid chromatography using chiral stationary phases is nowadays a very popular technique. This popularity is mainly due to development of new chiral stationary phases and also by exploring and developing new and more efficient modes of operation. The use of chiral liquid chromatography, from analytical scale, by means of the high performance liquid chromatography (HPLC), to preparative and industrial scale, through the simulated moving bed (SMB) technology has gained a renewed interest.

Nadolol is a nonselective beta-adrenergic receptor antagonist (β-blocker) pharmaceutical drug, widely used in the treatment of cardiovascular diseases, such as hypertension, ischemic heart disease (angina pectoris), congestive heart failure, and certain arrhythmias. Its chemical structure has three stereogenic centers which allows for eight possible stereoisomers. However, the two hydroxyl substituents on the cyclohexane ring are fixed in the cis-configuration, which precludes four stereoisomers. Nevertheless, the separation of nadolol stereoisomers is a considerable challenge, since this is a multicomponent separation of major interest at preparative and industrial scales.

The separation of nadolol stereoisomers on Chiralpak® AD at both analytical and preparative scales was recently reported [Ribeiro *et al*., 2013]. The Chiralpak® AD is, currently, the most used commercially available chiral stationary phase (CSP). It is an amylose-based CSP and is produced by physical coating of the chiral polymer on a matrix. However, due to their coated nature, this CSP can only be used with a limited range of solvents such as alcohols (as polar solvent) and alkanes (as non-polar solvent).

1.2 Objectives

In the present work the adsorption behaviour of nadolol stereoisomers using an immobilized chiral stationary phase (Chiralpak® IA) is studied. Immobilization of a polysaccharide-derivative on the support is an evolutionary strategy to make the CSP compatible with the whole range of organic solvents, which will consequently extend its

application scope. The Chiralpak® IA CSP has the amylose polymer immobilized onto silica gel, making the CSP compatible with all types of solvents. Additionally, the use of "non-traditional" solvents, such as acetonitrile, tetrahydrofuran or ethyl acetate, among others is now allowed, and possibly promoting better separation performances (at analytical or preparative scales).

The present study will be conducted using the following methodology: screening of mobile phase composition, measurements of adsorption equilibrium isotherms, measurement and simulation of fixed-bed adsorption behaviour, and prediction of the preparative separation process (SMB) performance, by estimation of process productivity and solvent consumption. Finally, after the solvent composition selection, the preparative separation of nadolol stereoisomers will be performed using a laboratory scale SMB unit.

1.3 Outline

In Chapter 2 is presented the case study: the preparative chiral separation of the four stereoisomers of nadolol. This chapter describes an actual review on the preparative liquid chromatography of nadolol stereoisomers. The main types of chiral stationary phases, the methodology used for preparative liquid chromatography of chiral compounds and a brief introduction to the simulated moving bed technology is also revised.

Chapter 3 presents the experimental procedures used for packing the preparative columns, and for the pulses experiments using both Chiralpak® AD and IA stationary phases using different mobile-phase compositions. In this chapter are also presented the experimental procedures, modelling and simulation tools used to describe the fixed-bed adsorption behaviour. Finally, are presented the tools used to estimate the initial SMB operation conditions as well the experimental operation of the FlexSMB unit.

In Chapter 4 are presented and discussed the experimental results obtained using the previous methodology presented in Chapter 3.

Finally, in Chapter 5 are presented the main conclusions of this work and proposed some suggestions for future work.

Chapter 2

Separation of Nadolol Stereoisomers by Analytical and Preparative Liquid Chromatography

2.1 Chirality and Nadolol Stereoisomers

Chirality can be simply described by the characteristic of molecules of being mirror images of each other. The structure of chiral molecules is usually a tetrahedral geometric configuration with a carbon as the central atom attached to four different groups. The pair of these two molecules looks like a two mirror-image, have the same chemical structure, and are called enantiomers [Pais, 1999]. To better understand enantiomers, we can take the stereochemical structure of butan-2-ol (Figure 2.1). The structure of S-butan-2-ol is similar to R-butan-2-ol but they are not superimposed images.

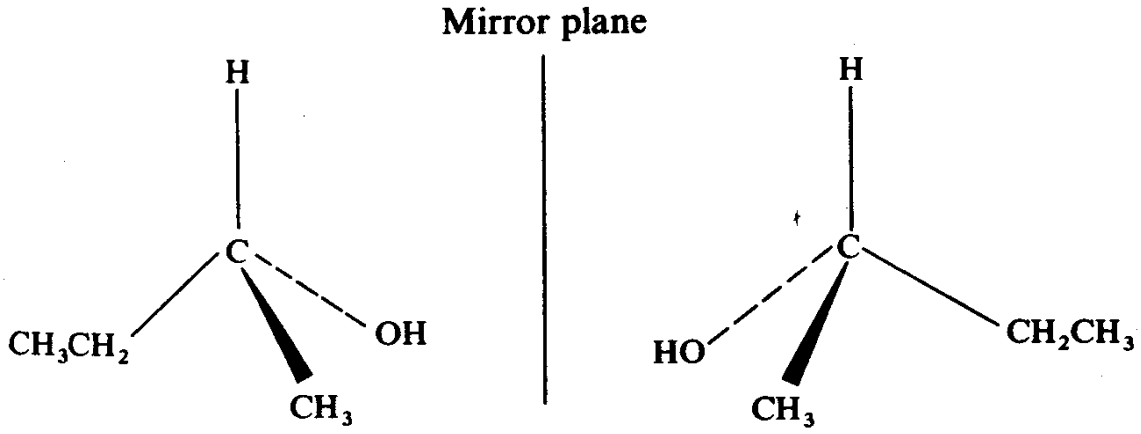

Figure 2.1. *Stereo-chemical structure of butan-2-ol. [Source: Subramanian, 1994].*

The chemical structure of nadolol has three stereo-genic centers which allows for eight possible stereoisomers. However, the two hydroxyl substituents on the cyclohexane ring are fixed in the *cis*-configuration, which precludes four stereoisomers [Bruton *et al.*, 2001].

Regardless of considerable evidence that it is important to characterize the stereo-chemical components when describing the pharmacodynamics and pharmokinetics of a racemic drug, the narrow international legislation concerning chiral drugs safety still allows nadolol commercialization in the form of a racemic mixture of four stereoisomers [McCarthy, 1994] (Figure 2.2).

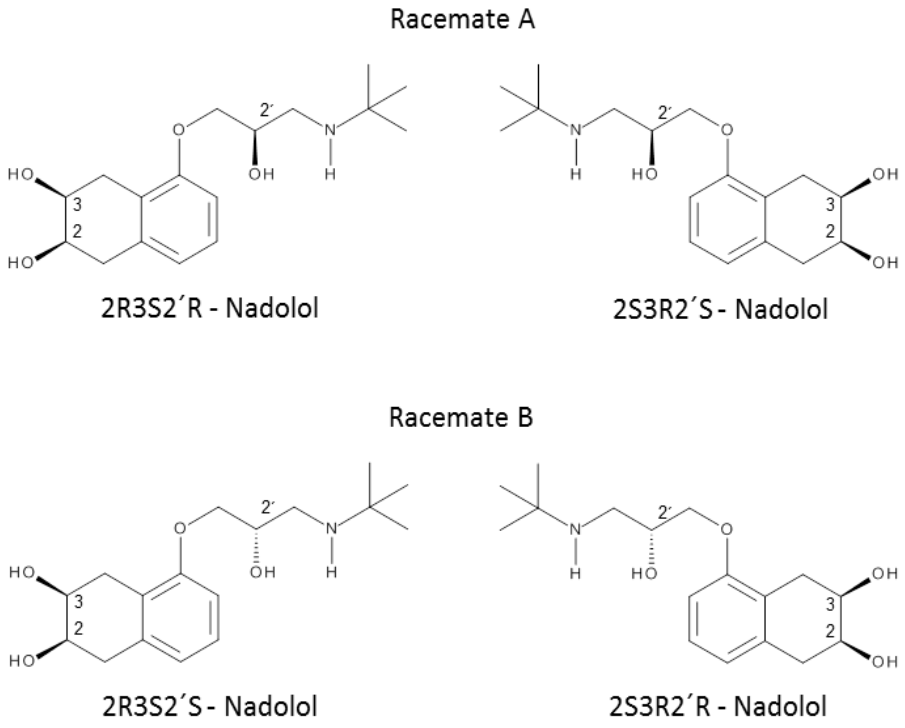

Figure 2.2. *Molecular structures of the four nadolol stereoisomers. [Source: Ribeiro et al., 2013]*

Nadolol is a nonselective beta-adrenergic receptor antagonist (β-blocker) containing more than one chiral centre and is marketed as a mixture of four stereoisomers. Nadolol is a pharmaceutical drug, widely used in the relief of high blood pressure, and in the treatment of cardiovascular diseases, such as hypertension, ischemic heart disease (angina pectoris), congestive heart failure, and certain arrhythmias [Ribeiro *et al.*, 2013]. Nadolol's therapeutic effects are attributed to its ability to competitively bind to β-adrenergic receptor sites on the heart and by doing so preventing the production of hormones that signal the heart to beat faster. Hence, nadolol reduces the demands on the heart by causing it to beat more slowly and with less force which in turn improves blood flow and decreases the amount of oxygen the heart needs to pump blood around the body [Hashem, 2012].

2.2 Chiral Stationary Phases

The first CSP was introduced by Pirkle *et al.*, 1981. Actually, there are commercially available more than eighty CSP for liquid chromatography.

Normally, CSPs are divided into five main groups, according to the type of interaction between enantiomers and the stationary phase. Table 2.1, presents the classification of the different CSPs types [Subramanian, 1994].

Table 2.1. Classification of chiral stationary phases according to the interaction between enantiomer and CSP molecules.

Classification	First reported	Type of Interactions
Type I	Pirkle and House, 1979	Attractive interaction, hydrogen bonding, π-π interactions and dipole interactions
Type II	Ichida *et al.*, 1984	Both attractive interactions and inclusion complex formation
Type III	Wainer, 1987	Retention by formation of inclusion complexes within chiral cavities
Type IV	Busker *et al.*, 1985	Ligand exchange mechanisms with metal complexes
Type V	Hermansson, 1983	Hydrophobic and polar interactions with bound protein phases

In present work, two different chiral phases were used: Chiralpak® IA and Chiralpak® AD. Both CSPs belongs to type II, and are considered as amylose derivatives. This class of CSP has a widespread utility for preparative applications in different industries, such as, in the pharmaceutical industry. Chiralpak® IA and Chiralpak® AD stationary phases have the same chiral selector nature based on 3,5-dimethylphenylcarbamate of amylose (Figure 2.3). However, Chiralpak® AD is manufactured by coating and Chiralpak® IA is obtained by immobilizing the chiral selector on the silica support. In fact, more than 90% of racemic compounds can be separated using Chiralpak® AD in analytical scale. However, it can be used with a limited range of solvents, such as alcohols and hydrocarbons.

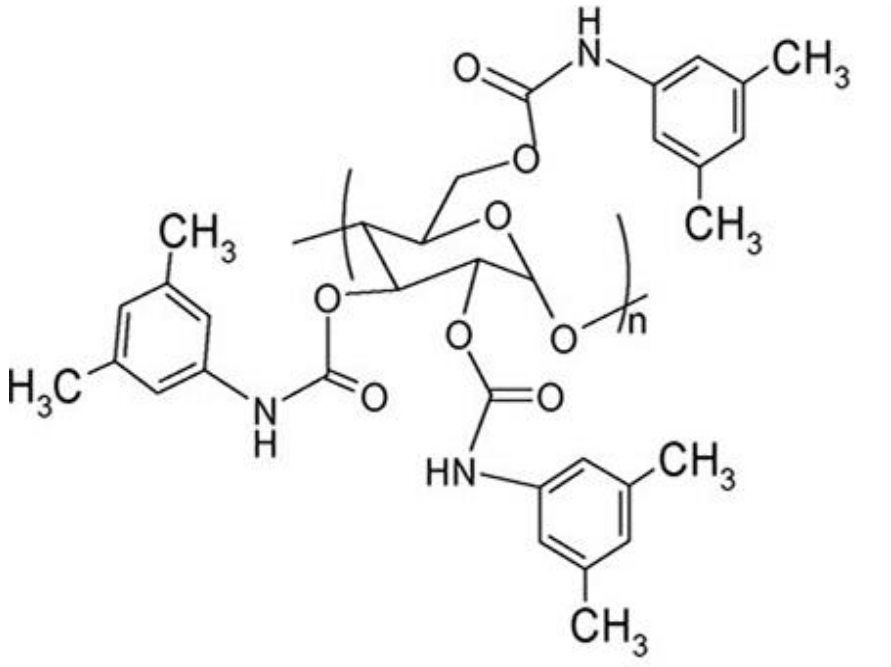

Figure 2.3. *Chemical structure of Amylose tris-3,5-dimethylphenylcarbamate.*
[Source: Zhang et al., 2008]

Daicel Chemical Industries Ltd, has introduced in the market several immobilized CSPs. Chiralpak® IA is able to improve the separation performance of enantiomers at both analytical and preparative scales. This CSP being immobilized in nature, presents an excellent solvent versatility. It allows the use of a wide range of mobile phases containing non-standard solvents, such as acetonitrile, tetrahydrofuran, ethyl acetate and dichloromethane and could be a step forward compared to coated CSPs such as the Chiralpak AD [Zhang *et al.*, 2005].

2.3 Preparative Chiral Separation Technologies

All separations techniques which allow the isolation of a certain amount of product can be qualified as being 'preparative'. Actually, not all preparative techniques are useful at the same scale; some preparative techniques are easily used for manipulation of large amounts of material, while others are suitable for isolation of few milligrams of materials which may be enough for the purposes.

There are several preparative techniques used in the resolution of enantiomers. In Table 2.2 are presented the most common used ones divided into two different strategies: Single enantiomer synthesis or racemic resolution approaches.

Table 2.2. Main preparative methods used to obtain single enantiomers.

Chiral Synthetic Approach	**Asymmetric Approach**	This method uses a chiral catalyst, auxiliary; by introducing a temporary stereocenter to bind the molecule in a way that influences the reaction by using steric hindrance [Sheldon, 1993].
	Biological Methods	This method uses enzymatic catalysis, in which an enzyme acting as chiral catalysts is combined with a racemic mixture [Rasor and Voss, 2001].
Racemic Approach	**Crystallization Techniques**	Traditional optical resolution techniques based on crystallization; Is the most widely employed method to obtain pure enantiomers in the pharmaceutical industries [Lorenz *et al.*, 2007].
	Chromatography Techniques	New resolution technique depends on the differential adsorption of enantiomers; or special selective columns that result in increased retention of one enantiomer over the other. The last method is by forming a diastereoisomeric derivative using standard CSPs [Bojarski *et al.*, 2005].
	Enantioselective Membranes	This technique constitutes nowadays well-established process methods for industrial treatments of fluids, this technique can be performed in continuous mode [Pirkle and Doherty, 1989].

In present work, it will be used the racemic approach to obtain single enantiomers, namely, the preparative liquid chromatography using the simulated moving bed technology.

2.4 Simulated Moving Bed Technology (SMB)

One of the most powerful techniques used for the preparative chromatography is the Simulated Moving Bed (SMB) technology. One of the most important advantages of this technology is that it allows the continuous injection and separation of binary mixtures and the mass-transfer driving force is maximized by the counter-current contact between the solid and liquid phases. The counter-current contact in SMB was originally achieved by means of a rotary valve that periodically shifted the feed, eluent, raffinate and extract streams at a selected time intervals in the direction of fluid flow phase [Pais, 1999].

The first appearance of the SMB technology in the chemical engineering field was in 1961, when the first patent by UOP (Universal Oil Products, Des Plaines, Illinois, USA) appeared. Then this technology was applied to the petroleum refining and petrochemicals, and become known as the Sorbex process, which was designed for *p*-xylene recovery from mixed xylenes.

In the pharmaceutical industry the SMB is a much useful tool particularly for small scale tests, where only a few grams of the chiral drug are needed. However, the use of SMB technology in the pharmaceutical industry is not limited to laboratory tests. Large scale chromatographic separations were in the past limited mainly due to the high cost of the adsorbent, the high dilution of products, and the large amounts of mobile phase needed. With the introduction of SMB technology, large scale separation can now be carried out under cost-effective conditions. A diagram of a Simulated Moving Bed is presented in Figure 2.4.

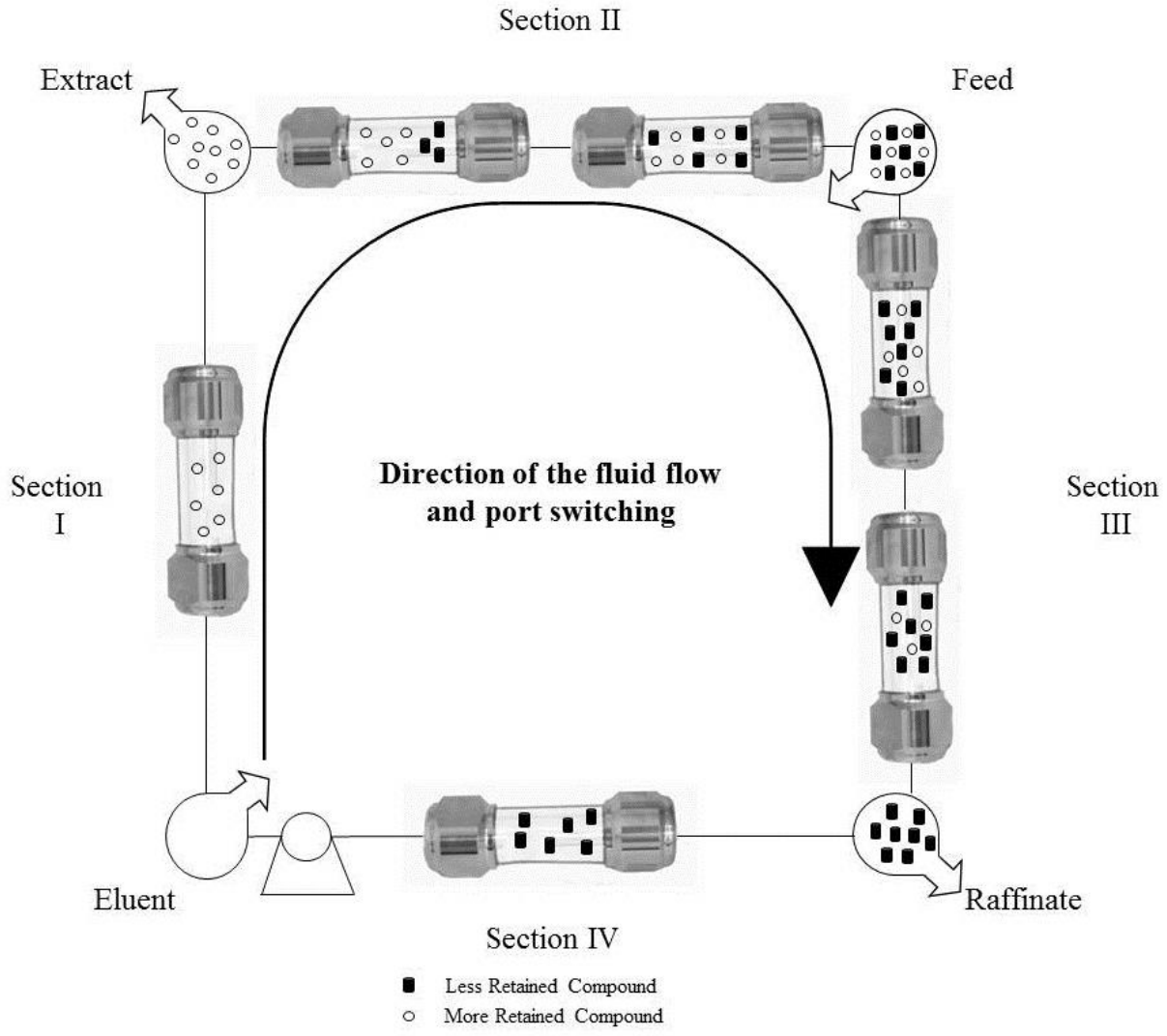

***Figure 2.4**. Diagram of a Simulated Moving Bed.*

To simplify the description of the operation of each section, let us consider a feed mixture containing only two components: component A, the less retained component, recovered in the raffinate outlet stream, and species B, the more retained component recovered in the extract outlet stream.

Section I is the zone of solid regeneration. In this section, both components must be desorbed in order to obtain a solid phase free from both components at the beginning of this zone. Since component B is the more retained species, the conditions for adsorption of this component will allow also, the desorption of the less retained component. In sections II and III the two components must move in opposite directions. The less retained component A must be desorbed and carried with the liquid phase, while the more retained species B must be adsorbed and carried with the solid phase. Considering that in these zones the objective is to prevent the contamination of the extract and raffinate streams by the undesired component, section II the zone of desorption of the less retained species A, while section III is the zone of the adsorption of the more retained component B. In section IV both components must be adsorbed in order to regenerate the eluent that will be recycled to the first zone. Since the component A is the less retained species, the conditions for adsorption of this component will allow also the adsorption of the more retained species [Pais, 1999].

2.5 State of the Art

The main published works on chiral separation of nadolol stereoisomers by preparative liquid chromatography, using different chiral stationary phases and different solvent compositions are presented in Table 2.3.

Table 2.3. Recent publications on nadolol stereoisomers chiral separation by liquid chromatography.

Author	Year	Column	Mobile Phase Composition	UV(nm)
McCarthy	1994	Analytical: Chiralpak® AD, 10 μm	EtOH/Hex/IPA (25/75/0) to (75/0/75) EtOH/Hex/DEA (20/80) Hex/EtOH/IPA (77/15/8) all with 0.3%DEA H_2O/EtOH (75/25) with 0.5%DEA	270
Aboul-Enein and Aboul-Basha	1996	Analytical: Chiralcel OD	EtOH/Hex/DEA (x/y/0.4) different x/y	254
Wang and Ching	2002	Analytical: β-cyclodextrin, 15 μm	H_2O/MeOH/TEAA (80/20/1) pH=5.5	280
Wang and Ching	2003	Analytical: β-cyclodextrin, 15 μm	H_2O/MeOH/TEAA (80/20/1) pH=5.5	280
Wang and Ching	2004	SMB (2-2-2-2): β-cyclodextrin, 15-25 μm Analytical: β-cyclodextrin, 5 μm	H_2O/MeOH/TEAA (80/20/1)	280
Wang and Ching	2005	SMB (2-2-2-2-2): β-cyclodextrin, 15-25 μm Analytical: β-cyclodextrin, 5 μm	H_2O/MeOH/TEAA (80/20/1)	280
Singh *et al.*	2006	Analytical: Chiralpak® OD, 10 μm	EtOH/Hex/DEA/HAc (15/87/0.2/0.2)	276
Wang and Ching	2007	Analytical: Chiralpak® AD-H, 5 μm	EtOH/Hex/DEA (20/80/0.3)	280
Van Arnum	2008	Analytical: Chiralpak® AD-H	0.1%ESA in Hex/EtOH	---
Sung *et al.*	2010	Preparative HPLC: JAIGEL-ODS-BP-L Analytical: Chiralpak® AD-H	MeOH/H_2O	230
Lee and Wankat	2010	SMB (2-2-2-2): β-cyclodextrin	Simulation	---
Hashem	2012	Data from Wang/Ching	Simulation	---
Ribeiro *et al.*	2013	SMB (1-2-2-1): Chiralpak® AD	80E/20C7/0.3DEA	230/270
Jermann *et al.*	2015	3C-ISMB: Chiralpak® AD	70E/30C7/0.3DEA	240

The chiral separation of all four stereoisomers at analytical scale was first reported by McCarthy in 1994. In that work, the analytical chiral separation was performed with both normal and reverse-phase methods, using a tris 3,5-dimethylphenyl carbamate (Chiralpak® AD, 10 μm) chiral stationary phase. Several mobile-phase compositions were tested using hydrocarbons, alcohols, and acetonitrile. The complete separation of all the four stereoisomers was reported under normal-phase mode, using a 20:80:0.3 %ethanol:%hexane:%diethylamine

mobile-phase composition. The last retained compound, referred as the most active stereoisomer (RSR-nadolol), elutes the chromatographic column after 32 min using a flow-rate of 1.2 mL/min.

Wang and Ching reported in 2004, for the first time, the separation of nadolol stereoisomers at preparative scale [Wang and Ching, 2004 and 2005]. However, the adsorbent used was based on perphenyl carbamoylated β-cyclodextrin (15–25 μm) that normally presents low saturation capacity (0.1–5 mg solute/g CSP) and, consequently, low preparative performance when compared with the polysaccharide-derivatives-based CSP (5–150 mg solute/g CSP) [Zhang *et al.*, 2005].

In 2007, Wang and Ching published another study reporting the analytical separation of nadolol stereoisomers using Chiralpak® AD column (5 μm), but no further work was reported considering the experimental preparative chiral separation using this chiral stationary phase [Wang and Ching 2007].

Another published study on preparative separation of racemic nadolol by four-section simulated moving bed (SMB) was reported by Hashem in 2012. That work present a theoretical study with the aim of the complete separation of the most therapeutic potent stereoisomer (RSR-nadolol) using a beta-cyclodextrin CSP.

Ribeiro *et al.* published a work in 2013 reporting the preparative pseudo-binary separation of the four nadolol stereoisomers of Chiralpak® AD by chiral liquid chromatography. That work shows how retention and resolution depend on the polarity of the solvent composition (ethanol-hexane and ethanol-heptane mixtures). The selected mobile phase composition was a 80:20:0.3 %ethanol:%heptane:%diethylamine mixture used to perform the preparative separation of the more retained stereoisomer of the nadolol (RSR-nadolol). The SMB separation was done using a 2 g/L feed concentration of the four nadolol stereoisomers. Both recovery and purity were 100%, with a solvent consumption of SC = 9.6 $L_{solvent}/g_{target\ product}$ and a system productivity of PR = 0.65 $g_{target\ product}/(L_{bed}.hr)$.

Recently, in another work reported by Jermann *et al.* in 2015, it was studied and applied the three columns intermittent simulated moving bed technology (3C-ISMB) for the separation of nadolol stereoisomers. The separation was carried out using the Chiralpak® AD 20 μm stationary phase using a 30:70:0.3 %heptane:%ethanol:%diethylamine solvent composition and a productivity of 2.10 $g_{target\ product}/(L_{bed}.hr)$ and solvent consumption of 12 $L_{solvent}/g_{target\ product}$ of product were obtained.

Chapter 3

Experimental Procedures and Methodology

3.1 Chemicals and Adsorbents

The mixture of the four nadolol stereoisomers and 1,3,5-tri-tertbutylbenzene, used as a non-retained compound, were both obtained from Sigma-Aldrich (Schnelldorf, Germany). The HPLC-grade solvents ethanol, methanol, hexane, heptane, acetonitrile, tetrahydrofuran and the basic modifier diethylamine (DEA) were obtained from Fluka (Bunchs, Switzerland). The chiral stationary phase used was the amylose tris(3,5-dimethylphenylcarbamate), commercially available as Chiralpak®IA and Chiralpak® AD and were both obtained from Daicel Company (Chiral Technologies Europe).

3.2 Equipment

The packing of the preparative columns was done using a Knauer Wellchrom Pneumatic pump K-1900 equipped with a 250 mL/min pump head (Figure 3.2). The packing solvent was pure iso-propanol.

The columns characterization, screening of the mobile-phase composition, adsorption isotherms and breakthroughs measurements were carried out using a ‘‘preparative’’ Knauer HPLC system equipped with a Smartline UV detector 2520 set at 270 nm wavelength, two Smartline 1050 pumps with 50 mL pump heads, a manual injection valve and two different loops (100 and 1000 μL) (Figure 3.3). A constant flow-rate of 5 mL/min was used. Two kinds of columns were used, seven preparative (SMB) columns (100mm L×21.2mm ID) were home-packed with Chiralpak® IA and one with Chiralpak® AD, both bulk materials have a particle size diameter of 20 μm.

The pseudo-binary SMB separation of nadolol stereoisomers was performed using a laboratory-scale SMB unit. This unit was completely built on the LSRE group, Faculty of Engineering, University of Porto, and is designated as “FlexSMB-LSRE®” (see Figure 3.4). The unit was operated using a [1-2-2-1] configuration. An analytical Kanuer HPLC system was used to measure the nadolol concentrations in the outlet extract and raffinate streams. This system was equipped with a Smartline UV detector 2520 set at 270 nm wavelength, one Smartline 1050 pump with 10 mL pump head, a manual injection valve and a loop of 20 μL.

A Chiralpak AD column with analytical dimensions (250 mm L×4.6 mm ID) and a particle size diameter of 5 μm was used (Figure 3.1).

Figure 3.1. *A-Preparative column; B-Analytical column.*

Figure 3.2. *Preparative column packing system.*

Figure 3.3. *Knauer HPLC system.*

Figure 3.4.*A detail of the laboratory scale SMB unit; FlexSMB-LSRE®*

3.3 Methodology for Chiral Separations by SMB

The optimization of a liquid chromatographic chiral separation process is based on a careful selection of the proper combination between the chiral stationary phase and the solvent composition to separate a specific chiral mixture.

In the present work, the use of Chiralpak® IA material as stationary phase for the separation of nadolol stereoisomers was studied. The global experimental methodology can be divided in five steps:

1st step: The solubility of the chiral mixture is measured using different solvents (pure solvents and different solvent mixtures). Then, using the most promising solvents, elution chromatographic pulses are carried out at low (analytical conditions) and high feed concentrations (preparative conditions) in order to collect retention and selectivity data. However, this step was done by the group before this master thesis;

2nd step: The screening of the mobile-phase composition is carried out by using a Knauer HPLC system and a column with preparative dimensions and bulk material with 20 µm particle size diameter. Different solvents mixtures were used in order to optimise the mobile-phase composition: ethanol, methanol, hexane, heptane, acetonitrile and tetrahydrofuran. The selectivity and retention data was collected and used to select a 100%methanol:0.1%DEA solvent composition to perform next steps;

3rd step: The adsorption equilibrium isotherm data is obtained experimentally and fitted to a model able to describe the fixed-bed adsorption behavior. This is a very critical step, since a week description of the adsorption equilibrium data will also lead to a week description of the adsorption process and to a wrong optimization of the final separation process at preparative scale;

4th step: The kinetic data is estimated by performing breakthrough experiments (Peclet number and mass transfer resistances). This step is also used to validate the adsorption equilibrium isotherm model previously selected;

5th step: In the final step, the SMB separation is performed at pilot scale unit. This step needs the estimation of the SMB operating conditions, using simulation and the concept of separation regions through the methodology validated by Ribeiro et al. (2011a, 2011b, and 2013). The system performance is evaluated through the performance parameters of purity, productivity and solvent consumption.

3.4 Column Packing and Characterization

3.4.1 Columns Packing Procedure

Eight preparative columns (i.e., preparative dimensions) were packed with different CSPs, seven columns were packed with Chiralpak® IA and one column packed with Chiralpak® AD.

A proper packing of the preparative column is one important step since good separation performances also depend on the adequate hydrodynamic behaviour of the chromatographic column. A methodology was developed for packing the preparative columns and is based on the following steps:

a) 21.5 g of the CSP bulk material is weighted and pure iso-propanol (used as the packing solvent) is added, obtaining a 90 mL of slurry mixture;
b) This mixture is let for 30 min and two different phases and obtained. After this time, around 25 mL of liquid phase and 65 mL of slurry are obtained. Then with a syringe the upper liquid was removed and the slurry was energetically mixed;
c) The column was completely filled with pure and cold iso-propanol (T=4ºC).
d) All amount of slurry was inserted in the upper packing reservoir. The reservoir was closed and connected to the pneumatic pump;
e) Then the packing system was operated using cold iso-propanol to compact the adsorbent bed, using the five stages of the procedure described in Table 3.1;
f) After the final packing 45-min stage, the pump was turned off, and the upper reservoir was opened and the remaining solvent removed. The reservoir was disconnected from the column and using a blade the extra slurry on the top of the column cut. Finally the column was closed;
g) The packed new column was inverted and cold iso-propanol was circulated for more 5 minutes using a liquid pressure of 50 bar.

Table 3.1. Packing procedure for preparative chromatographic columns.

Stage #	Liquid pressure (bar)	Air pressure (bar)	Time (min)
1	**50**	**0.7**	**0-5**
2	**100**	**1**	**5-10**
3	**150**	**1.7**	**10-15**
4	**200**	**2**	**15-25**
5	**250**	**2.6**	**25-45**

3.4.2 Columns Characterization

After packing the seven preparative columns using Chiralpak® IA the quality of all the columns packing was evaluated using elution chromatography. This evaluation was done by analyzing the dispersion and measuring variation of the retention time of both the non-retained compound (tracer) and nadolol racemic mixture. The efficiency of all SMB columns was also tested by measuring the influence of the flow-rate upon the height equivalent to a theoretical plate.

3.4.2.1 Tracer Experiments

These studies were performed using elution chromatography using a 100%methanol:0.1%diethylamine solvent composition. The analysis were carried out by injecting 2 g/L of *1,3,5*-tri-*tert.*-butylbenzene (tracer) and 2 g/L of nadolol solution using a loop of 100 µL and a constant flow-rate of 5 mL/min using the preparative HPLC Knauer system.

3.4.2.2 Columns Efficiency

The preparative column number 5, packed with Chiralpak® IA, was selected to measure the efficiency of the SMB columns that is characterized by the Height Equivalent to a Theoretical Plate (HETP). The efficiency is calculated for each compound from the experimental chromatographic peak as a function of superficial velocity by:

$$HETP = \sigma^2 L/\mu_1^2 \quad (3.1)$$

where σ^2 is the peak variance and $\mu_1$is the first moment of the peak. The moments of order k are given by:

$$\mu_k = \int_0^\infty t^k C(t)dt \qquad k = 0,1,2,3 \ldots \quad (3.2)$$

and the variance is:

$$\sigma^2 = \mu_2 - \mu_1^2 \quad (3.3)$$

Where *C(t)* is the concentration history obtained under experimental condition and *L* is the length of the bed.

3.5 Comparison between Chiralpak AD and IA Adsorption Behaviour

Elution chromatographic pulses of 2 g/L nadolol solution are carried out using the preparative Chiralpak® AD and Chiralpak® IA CSPs. The comparison of the pulses was done using a 20%ethanol:80%heptane:0.1%DEA composition. This mobile phase composition was the same used in a previous work with Chiralpak AD [Ribeiro *et al.*, 2013].

3.6 Screening of Mobile Phase Composition

In a liquid chromatographic chiral separation the choice of the solvent composition is a crucial step. This selection normally has a remarkable impact on both retention and selectivity.

Several pulses of a 2 g/L nadolol solution were carried out at preparative scale using the Chiralpak® IA selected column (column "number 5"). Different solvent compositions with increasing ethanol-methanol content were tested, namely, ethanol/methanol, ethanol-methanol/acetonitrile, and ethanol-methanol/tetrahydrofuran mixtures. All the mixtures prepared adding 0.1% diethylamine (a strong basic modifier).

Basically, the selection of the most promising solvent compositions for (1+2+3)/4 pseudo-binary separation is based on the selectivity and retention. The selected compositions are 100%ethanol, 100%methanol, 95%methanol:5%acetonitrile, 75%methanol:25%acetonitrile, 25%methanol:75%acetonitrile, 80%methanol:20% tetrahydrofuran, all with 0.1% DEA.

Additional experiments were carried out using a bigger injection volume and higher concentrations (2, 5, 10 g/L of nadolol stereoisomers), trying to approach the real conditions under preparative/production conditions (loading experiments).

To better understand how to prepare the eluent, let us give this example, to prepare 2 g/L of nadolol in 20%ethanol:80%heptane:0.1%DEA. For a final volume of 1000mL a 20:80:0.1 ethanol-heptane-diethylamine solvent mixture is prepared by adding 200 mL of ethanol to 800 ml of heptane and 1 mL of diethylamine. 2 g/L nadolol solution is prepared by dissolving 20 mg of nadolol in a 10 mL volumetric flask using the previous prepared mobile phase composition. All volumetric liquid measurements were done using volumetric flasks

and micropipettes and for mass measurements and analytical precision balance was used (±0.0002g).

3.7 Measurement and Modeling of Adsorption Equilibrium Isotherms

An accurate method is advised to experimentally measure the adsorption equilibrium isotherm, since equilibrium data has an utmost impact in the quality of the preparative separation process performance. In this work, we are focusing on the separation of nadolol stereoisomers at preparative scale. Due to that, it's advisable to obtain the competitive isotherm experimentally, instead of predicting those from single component isotherms.

3.7.1 Experimental Determination of Competitive Adsorption Isotherms

The experimental determination of the competitive adsorption isotherms was carried out using the adsorption–desorption method. According to this method, the preparative column is saturated with a large amount of the feed solution with known racemic nadolol concentration (2, 4, 6, 8 and 10 g/L), until equilibrium is achieved. The column is then completely regenerated with pure eluent. The eluted volume, resulting from this desorption step, is collected and analysed using the analytical Knauer HPLC system, in order to measure each enantiomer concentration (Figure 3.5).

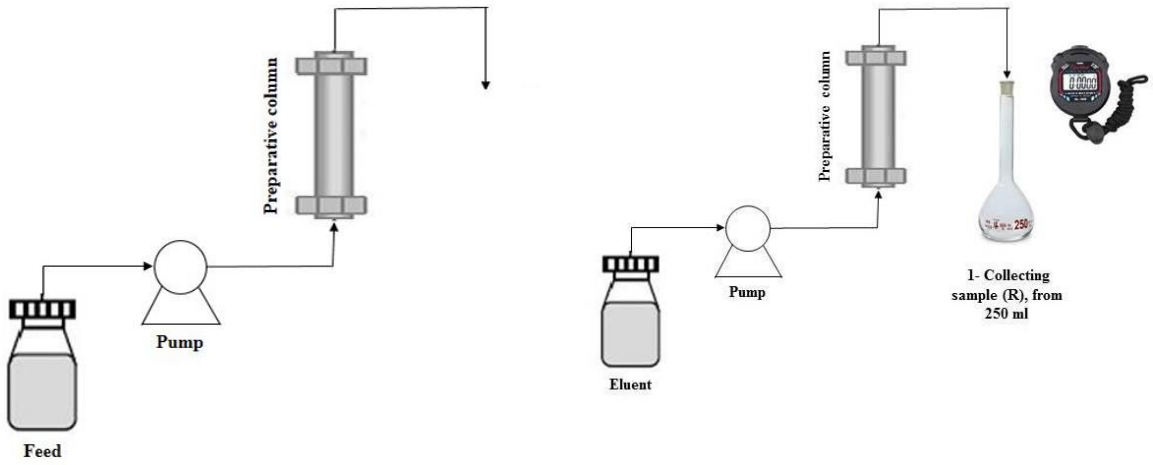

***Figure 3.5**. The adsorption–desorption method; (a) saturation step, (b) regeneration step.*

Under this saturation scenario, the concentration of component *i* in the solid phase, q_i^*, in equilibrium with the concentration of component *i* in the liquid phase (that is equal to feed concentration, C_i^F), can be calculated using the following mass balance:

$$V^d c_i^d = \varepsilon V_c c_i^F + (1-\varepsilon) V_c q_i^* \quad (3.4)$$

where:

V^d: Eluted volume [cm^3];

c_i^d: Concentration of each component in the eluted volume [g/L];

ε : The bed porosity, equal to *0.4;*

V_c : The column volume [cm^3];

c_i^F: The concentration of component *i* in the feed solution [g/L];

q_i^* : Concentration of each component retained in the particle [g/L]

In fact, q_i^* is considered as the total concentration retained in the adsorbent, considering the mass of each species retained in the adsorbent and the mass of each species retained inside the particle porous. This procedure is consistent with the simulation of the chromatographic process, based in a model that considers the particles as homogeneous.

The previous procedure produces one single point of the adsorption isotherm for each component and for a given feed nadolol solution concentration. Due to that, it's necessary to apply the mass balance equation for different feed solutions concentrations to obtain the complete adsorption isotherm.

3.7.2 Modeling Adsorption Isotherms

Irving Langmuir has presented in 1916, probably the most used mathematical equation to model the adsorption of species into simple surfaces. In the case of chiral mixtures, Langmuir model is the most used one. However, this model normally fails in the prediction of the preparative separation process. It is well known that, when leading with chiral mixtures under competitive conditions (high concentrations), the selectivity factor decrease with the increase of species concentration. The Langmuir model thus not considers this behavior since it predicts a constant value over the entire range of concentrations. A practical way to overcome this limitation is to introduce a linear term into the Langmuir model. This "linear+Langmuir" model (LLG6), can be expressed for a quaternary mixture as:

$$q_i^* = mC_i + \frac{Qb_iC_i}{1+\sum_{j=1}^{4} b_jC_j} \quad (3.5)$$

where:

$i = 1, 2, 3, 4$ $(number\ of\ species)$

m: Linear term of the adsorption equilibrium isotherm

C_i: Concentration of species *i* in the liquid phase (g/L)

Q: Capacity factor of the adsorption equilibrium isotherm of the Langmuir term (g/L)

b_i: Model parameter for species *i* of the adsorption equilibrium isotherm (L/g)

The adsorption isotherm parameters were estimated using the Levenberg-Marquardt algorithm written on FORTRAN language. For these simulations the objective function was defined to minimize the SD parameter, defined as,

$$SD = \sqrt{\frac{SQ}{M-N}} \quad (3.6)$$

with,

M: Total number of experimental points for the adsorption equilibrium isotherm

N: Total number parameters to be estimated for the adsorption equilibrium isotherm model

$$SQ = \sum_{j=1}^{M/4} \left[\left(q_{1j}^{*T} - q_{1j}^{*E}\right)^2 + \left(q_{2j}^{*T} - q_{2j}^{*E}\right)^2 + \left(q_{3j}^{*T} - q_{3j}^{*E}\right)^2 + \left(q_{4j}^{*T} - q_{4j}^{*E}\right)^2 \right] \quad (3.7)$$

were,

q_{ij}^{*T} and q_{ij}^{*E} are the equilibrium solid concentration of species *i* predicted from the model and experimentally obtained, respectively.

3.8 Breakthroughs Measurements and Simulation

3.8.1 Experimental Determination of Breakthroughs

Breakthrough measurements were done using the preparative Knauer HPLC system equipped with a preparative column (100mm L×21.2mm ID) home-packed with Chiralpak® IA, and using for mobile phase a solvent composition of 100%methanol:0.1%DEA. A 10 g/L of nadolol feed concentration was used to perform the saturation-regeneration steps. During these steps, several different samples were collected immediately at the end of the chromatographic column each 15 seconds, as represented in Figure 3.6.

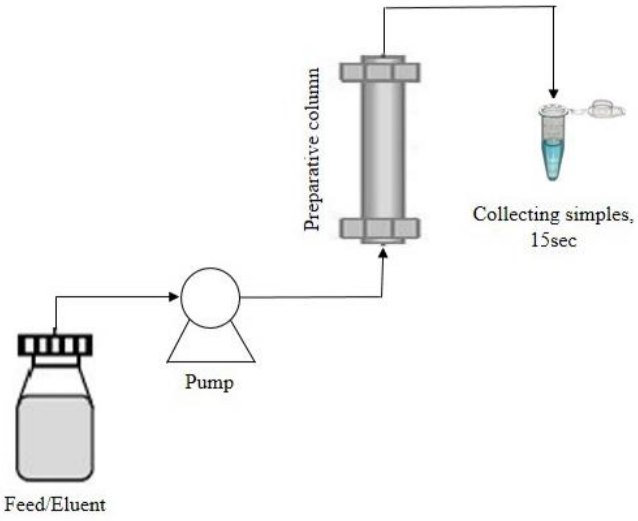

***Figure 3.6**. Experimental scheme for breakthroughs measurements.*

All samples, collected on the saturation and regeneration steps, were then analysed on the analytical Knauer HPLC system.

3.8.2 Modelling and Simulation of Breakthroughs

Generally, in preparative competitive liquid chromatography, the modelling of fixed-bed saturation and regeneration steps can be determined using the Linear Driving Force (LDF) model. This model assumes that the adsorbent particles are homogeneous, the flow is composed by the sum of a convective term and a dispersive term, and the mass transfer resistance is described by a linear driving force model. The model equations can be found in a previous published work (Ribeiro *et al.*, 2008). The modelling and simulation were performed using a PDECOL subroutine written in FORTRAN.

3.9 SMB Operation

As it was mentioned before, one of the main goals of this master thesis was to perform the SMB pseudo-binary separation of nadolol stereoisomers using the Chiralpak® IA stationary phase. After the screening of solvent composition, measurement of the adsorption isotherm and breakthroughs is necessary to predict the SMB operating conditions. In this task,

were used several modelling and simulation tools that are detailed described in other previous published works [Ribeiro *et al.*, 2011a].

3.9.1 Prediction of the SMB Initial Operating Conditions

The design of a SMB process consists in taking decisions at different levels, from the unit configuration to its operating conditions, in order to achieve and optimize the desired separation. This includes the selection of the system configuration (the number, dimension, and distribution of its columns), its operation mode, the choice of the separation system (stationary phase and mobile-phase composition), and the choice of the operating conditions, namely, the definition of the flow-rates in each section and the switching time interval.

In this work, the experimental operation of a SMB pilot unit was carried out with the objective of obtaining the more retained nadolol stereoisomer pure from a mixture containing an equal concentration of all its four stereoisomers. Considering this multicomponent mixture (components 1, 2, 3, and 4), some constraints have to be met if one wants to recover the more retained component 4 in the extract and the other three less retained components 1, 2, and 3 co-eluting in the raffinate. To ensure the complete separation of a binary or pseudo-binary mixture, the net-fluxes for each component in all four SMB sections must be properly set. Considering the case of the pseudo-binary separation where the more retained nadolol stereoisomer 4 is expected to be recovered pure in the extract, while the other three stereoisomers should co-elute in the raffinate. The net-flux constraints can be expressed in terms of the ratio between the fluid and the solid interstitial velocities in each section, γ_j, leading to the following conditions for the complete separation of the more retained stereoisomer 4 to be recovered pure in the extract stream, and considering linear isotherm:

$$\gamma_I > \frac{(1-\varepsilon)}{\varepsilon}(m + Qb_4) \tag{3.8}$$

$$\frac{(1-\varepsilon)}{\varepsilon}(m + Qb_3) < \gamma_{II} < \gamma_{III} < \frac{(1-\varepsilon)}{\varepsilon}(m + Qb_4) \tag{3.9}$$

$$\gamma_{IV} < \frac{(1-\varepsilon)}{\varepsilon}(m + Qb_1) \tag{3.10}$$

The previous inequalities for sections I and IV are frequently satisfied by introducing a safety margin β (β > 1). In this way, eqs. 3.8 and 3.10 can be rewritten as,

$$\gamma_I = \frac{(1-\varepsilon)}{\varepsilon}(m + Qb_4) \times \beta \tag{3.11}$$

$$\gamma_{IV} = \frac{(1-\varepsilon)}{\varepsilon}(m + Qb_1)/\beta \quad (3.12)$$

And the switching time interval, t*, can then be defined by

$$t^* = \frac{\varepsilon V_C}{Q_I^*}(\gamma_I + 1) \quad (3.13)$$

The flow-rate inside section I, Q_I^*, (the largest inside the SMB unit) is fixed taking into account the pressure limit imposed for the SMB operation. In this work, we used a safety margin of 50%, that is, β = 1.5, and imposed a maximum pressure drop of 40 bar. After fixing Q_I^* , γ_I, γ_{IV}, and t*, the region of SMB complete separation can be defined through the triangle theory methodology using the adaptation proposed for non-linear adsorption isotherms (linar+Langmuir model, complete separation 100%) [Mazzotti *et al.*, 1997]. A pair of (γ_{II},γ_{III}) values inside the triangle can be chosen and the corresponding γ_j values can be determined by using the correspondence between the simulated moving bed and the true moving bed operations,

$$\gamma_j^* = \gamma_I + 1 \quad (3.14)$$

Then, the internal SMB flow rates, Q_j^*, in sections II, III, and IV, can be defined using

$$Q_j^* = \frac{\varepsilon V_C}{t^*}\gamma_j^* \quad (3.15)$$

And the inlet, outlet, and recycle flow rates can finally be evaluated by

$$Q_E = Q_I^* - Q_{IV}^* \quad (3.16)$$

$$Q_X = Q_I^* - Q_{II}^* \quad (3.17)$$

$$Q_F = Q_{III}^* - Q_{II}^* \quad (3.18)$$

$$Q_R = Q_{III}^* - Q_{IV}^* \quad (3.19)$$

$$Q_{REC} = Q_{IV}^* \quad (3.20)$$

The SMB process performance is usually evaluated by means of four parameters: purity, recovery, solvent consumption, and system productivity. For the present objective of getting a pure form of the more retained stereoisomer (the target compound RSR-nadolol) from a mixture containing the four nadolol stereoisomers, it is assumed that the target compound is recovered in the extract while the other three components co-elute and are

recovered in the raffinate stream. In Table 3.2, are presented the expressions used to evaluate recovery, purity, productivity and solvent consumption.

Table 3.2. SMB performance parameters (the target product is the more retained stereoisomer, component 4, to be recovered in the extract stream).

Parameter	Equation	
Recovery	$RCX = \frac{Q_X \overline{C}_4^X}{Q_F C_4^F}$	(3.21)
Purity	$PUX = \frac{\overline{C}_4^X}{\overline{C}_1^X + \overline{C}_2^X + \overline{C}_3^X + \overline{C}_4^X}$	(3.22)
Productivity ($g_{target\ product}/(L_{bed}.hr)$)	$PR = \frac{Q_X \overline{C}_4^X}{V_T} = \frac{\varepsilon}{N_C t^*}(\gamma_1 - \gamma_2)\overline{C}_4^X$	(3.23)
Solvent Consumption ($L_{solvent}/g_{target\ product}$)	$SC = \frac{Q_E + Q_F}{Q_X \overline{C}_4^X} = \frac{1}{\overline{C}_4^X}\left[1 + \frac{\gamma_3 - \gamma_4}{\gamma_1 - \gamma_2}\right]$	(3.24)

3.9.2 SMB Experimental Operation

At the initial stage of the FlexSMB unit operation, feed and eluent pumps were purged, the mass of the eluent and feed flasks and the extract and raffinate flasks were measured. Then, the installation was started introducing the estimated values of solvent density, rotation time, and eluent, extract, raffinate, feed and recycle flow-rates. Using the "on-line" values obtained for the extract stream and the flow-rate inside section II and with the outlet streams samples collected at the end of one complete cycle (6t*) for measure their masses and determine the outlet flow rates, was performed a correction to the flow-rates, as needed.

The extract and raffinate outlet samples are, simultaneously, collected and analyzed using the analytical Knauer HPLC system. The obtained concentrations were used to monitor the concentration transient evolution of the four stereoisomers in the extract and raffinate outlet streams. The cycle steady-state was considered to be achieved when, after at least 5 complete cycles, the purities of both outlet streams had a minimum of 99.0%. In this moment, several samples were collected to monitor the internal profiles inside the six SMB columns, using a six-way valve at the end of section IV. These samples were collected at different times of the rotation time (25%, 50% 75% and 95% of t*). Finally, the system was operated

using pure methanol in order to cleaning the complete unit. The SMB operation has covered 3 different runs: 2 runs using a total nadolol feed concentration of 2 g/L and one single run using a feed concentration of 10 g/L.

3.9.3 Recovering, Distillation and Fixed-Bed Studies of the Outlet Streams

As soon as the cycle steady-state was achieved, both extract and raffinate outlet streams were collected in individual containers in order to perform further studies. We must state that after the SMB separation the extract outlet stream is a mixture of the three less retained stereoisomers and the raffinate contains only the most retained stereoisomer. Since three different SMB runs were performed, a total of six containers to collect the "pure" outlet streams were used, three for the extract and three for the raffinate. After the SMB separation runs performed in the University of Porto, the containers were stored and transported to LSRE-IPB in Bragança, to carry out further studies.

All the "pure" outlet streams were distillated under vacuum using a rotor-vapor system installed in the laboratory of analytical chemical (ESTiG). After distillation, the samples were used to perform adsorption equilibrium isotherm and breakthroughs measurements, using the same experimental methodology presented before for the quaternary mixture.

Chapter 4

Results and Discussion

4.1 Column Packing and Characterization

4.1.1 Tracer Experiments

To have good separation in simulation moving bed, it's very important to pack all columns with exact the same procedure to have the same adsorption behavior for all preparative columns. The procedure used to pack all seven preparative columns using Chiralpak® IA is mention in paragraph *(3.4).*

Figure 4.1 presents the elution chromatographic pulses of 2 g/L nadolol and tracer (*1,3,5*-tri-*tert.*-butylbenzene).

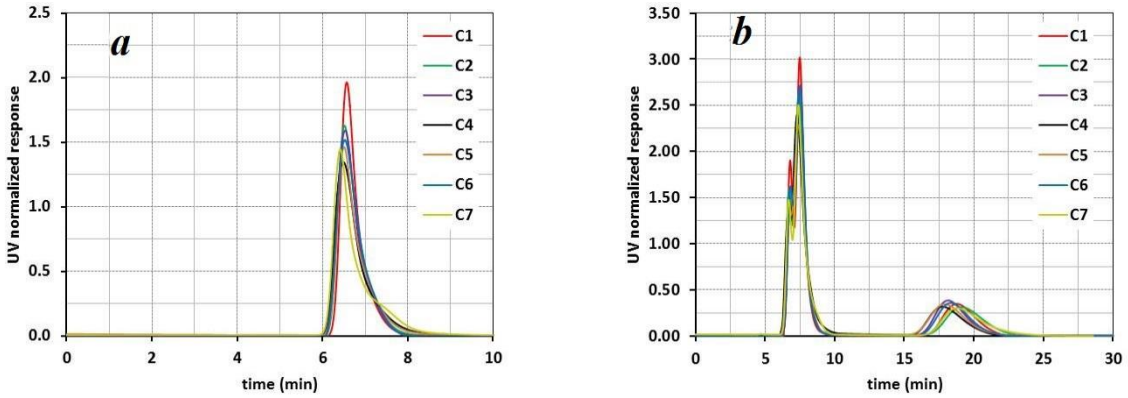

Figure 4.1. *UV normalized response to pulses experiments of tracer (a) and nadolol (b) on seven SMB columns packed with Chiralpak® IA, using 100%methanol:0.1%diethylamine solvent (C_F^T= 2.0 g/L; Q=5.0 mL/min; Injection volume=100 μL).*

Obtained results show that all columns have approximately the same adsorption behaviour. The retention time dispersion for nadolol and tracer pulses, were determined and are presented in Table 4.1.

Table 4.1. Quantification of dispersion in retention time measurements for nadolol and tracer pulses.

	Tracer	**Stereoisomer *I, II, III***	**Stereoisomer *IV***
Mean (min)	6.717	7.398	18.976
SD (min)	0.01033	0.02537	0.3099
RSD (%)	0.1538	0.3430	1.6331

4.1.2 HETP Measurements

After packing seven columns with the same procedure, one SMB column was selected (SMB column 5) to perform the HETP measurements. This study was carried out by injecting a non-retained compound (*1,3,5*-tri-*tert.*-butylbenzene) using three different flow-rates (5, 10 and 15 mL/min; Figure 4.2) in order to compare the HETP values of the non-retained compound in parallel with the HETP values of nadolol stereoisomers.

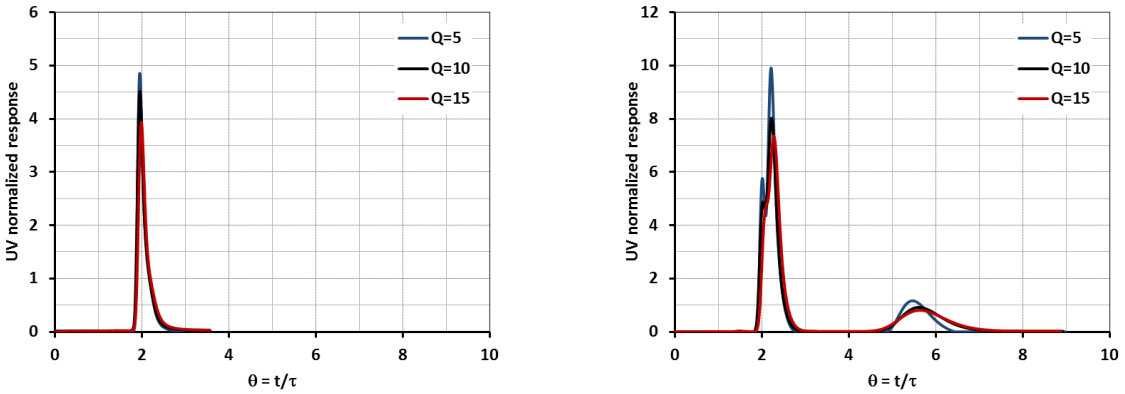

Figure 4.2. *UV normalized response to pulses experiments of tracer (left) and nadolol (right) on SMB column 5 packed with Chiralpak® IA, using 100%methanol:0.1%diethylamine solvent (C_F^T= 2.0 g/L; Q=5, 10 and 15 mL/min; Injection volume=100 µL).*

Figure 4.3 presents the linear dependency of HETP on the superficial velocity u_0.

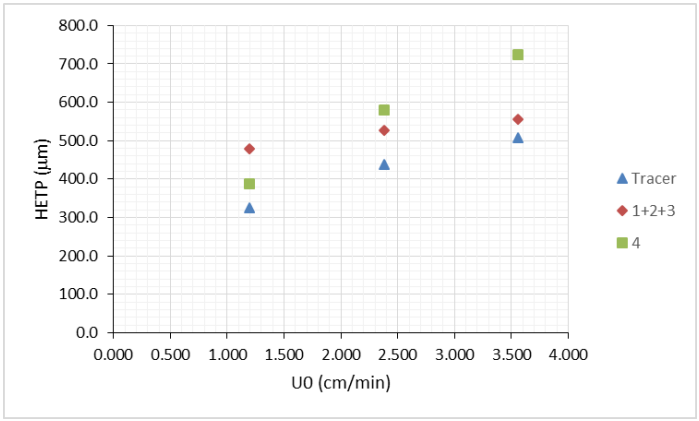

Figure 4.3*. HETP as a function of superficial velocity, u_0.*

The obtained results show that HETP values increase, as it was expected, with the increase of the superficial velocity. Using flow-rate between 5 and 15 mL/min, HETP in the range of 300 and 700 µm were obtained for tracer, the mixture 1+2+3, and stereoisomer 4.

4.2 Comparison between Chiralpak AD and IA Adsorption Behaviour

Elution chromatographic pulses of 2 g/L nadolol solution were carried out using the preparative Chiralpak® IA CSP. The Chiralpak® IA material allows a wider range of solvents compositions when compared with and Chiralpak® AD. The tested compositions were done by using 0.1% DEA (diethylamine, a strong basic modifier) in all mixtures.

Chiralpak® AD was also used for comparison purposes, since this CSP was validated for the nadolol separation by Ribeiro *et al.* (2013) using an 80:20:0.1 ethanol-heptane-diethylamine mobile phase.

Figure 4.3 presents the comparison of the pulses obtained with the two CSP (IA and AD) using the mobile phase selected by Ribeiro *et al.* (2013). We conclude that, using the 80:20:0.1 ethanol-heptane-diethylamine mobile phase, both the Chiralpak® AD and IA materials present the capacity of performing a good (1+2+3)/4 separation; that is, to separate the most valuable nadolol stereoisomer as the more retained component from the other three nadolol stereoisomers. Chiralpak® AD presents some higher selectivity between the first three species which is not an important advantage when the objective is to obtain the (1+2+3)/4 separation. On the other hand, Chiralpak® AD presents also higher selectivity between the fourth and the first three nadolol stereoisomers but together with higher retention time. These are two characteristics of opposite value at a preparative/production scale. Higher selectivity and short retention times are both welcome to the preparative separation process since both enhances the system productivity. In fact, this is the main trade-off to be achieved when choosing the best mobile phase composition for a certain solute and CSP: how to get higher selectivity with short retention at preparative conditions? To answer this question, these pulses only serve as preliminary results and for the identification of potential mobile phase compositions. The final answer will need the measurement of the adsorption isotherms and kinetic data at high feed concentrations, as to be used under preparative/production scale conditions. For now, Figure 4.4 shows that both Chiralpak® IA and AD materials, using the 80:20:0.1 ethanol-heptane-diethylamine mobile phase, can perform the (1+2+3)/4 separation at preparative scale.

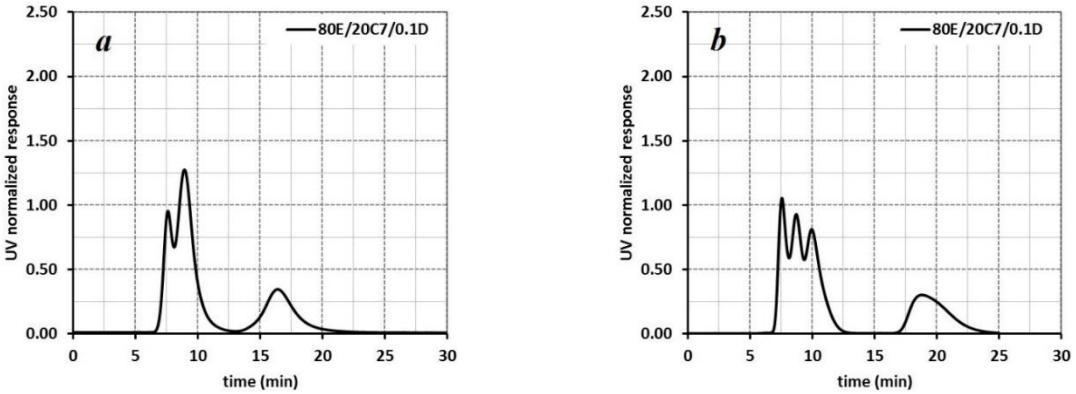

Figure 4.4. *Pulse experiments of nadolol using 80%ethanol:20%heptane:0.1%DEA mobile-phase composition for: (a) Chiralpak® IA and (b) Chiralpak® AD CSPs (C_T^F=2.0 g/L; injection volume = 100 μL; Q= 5.0 mL/min;). Legend: E-Ethanol; C7–Heptane; D-Diethylamine.*

4.3 Screening of Mobile Phase Composition

The presented results are obtained by using the traditional mobile phase compositions: alcohol/hydrocarbon mixtures with alcohol being methanol, ethanol, 1-propanol, 2-propanol and butanol, and hydrocarbon being hexane and heptane. Also, other organic solvents/mixtures were used such as, alcohol-acetonitrile, with alcohol being methanol, ethanol and 2-propanol, methanol-ethanol, dichloromethane-heptane, ethanol-dichloromethane, tetrahydrofuran-heptane, ethanol-tetrahydrofuran, ethyl acetate-heptane and ethanol-ethyl acetate mixtures. The obtained results show different possibilities to perform the separation of nadolol stereoisomers, depending on the target component or components to be obtained (Figures 4.5, 4.6, and 4.7).

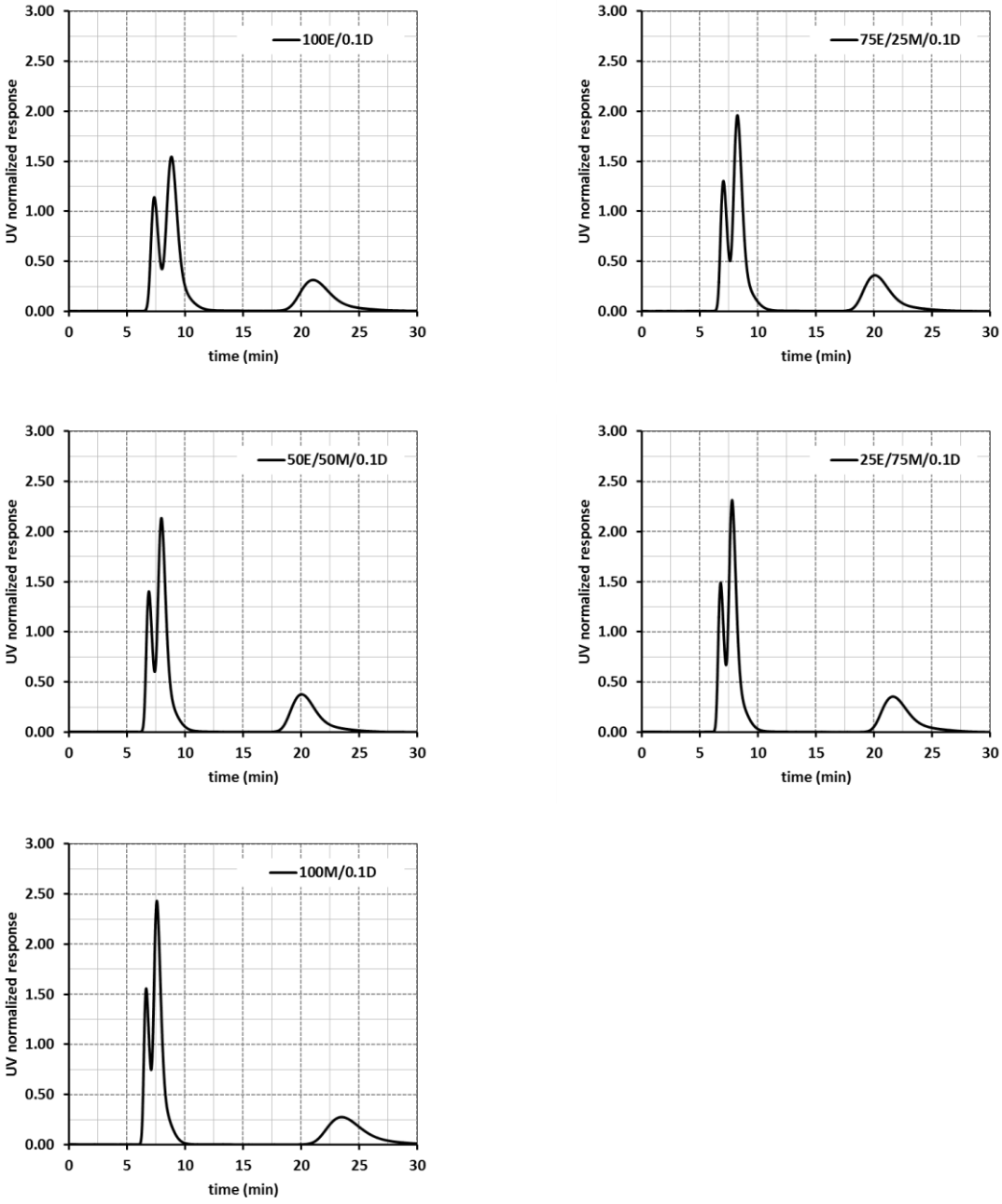

Figure 4.5 *UV normalized response to pulse experiments of nadolol solution on Chiralpak® IA, using different five ethanol(E)/methanol(M)/Diethylamine(D) solvent mixtures (C_F^T= 2.0 g/L; Q=5.0 mL/min; Injection volume=100 μL).*

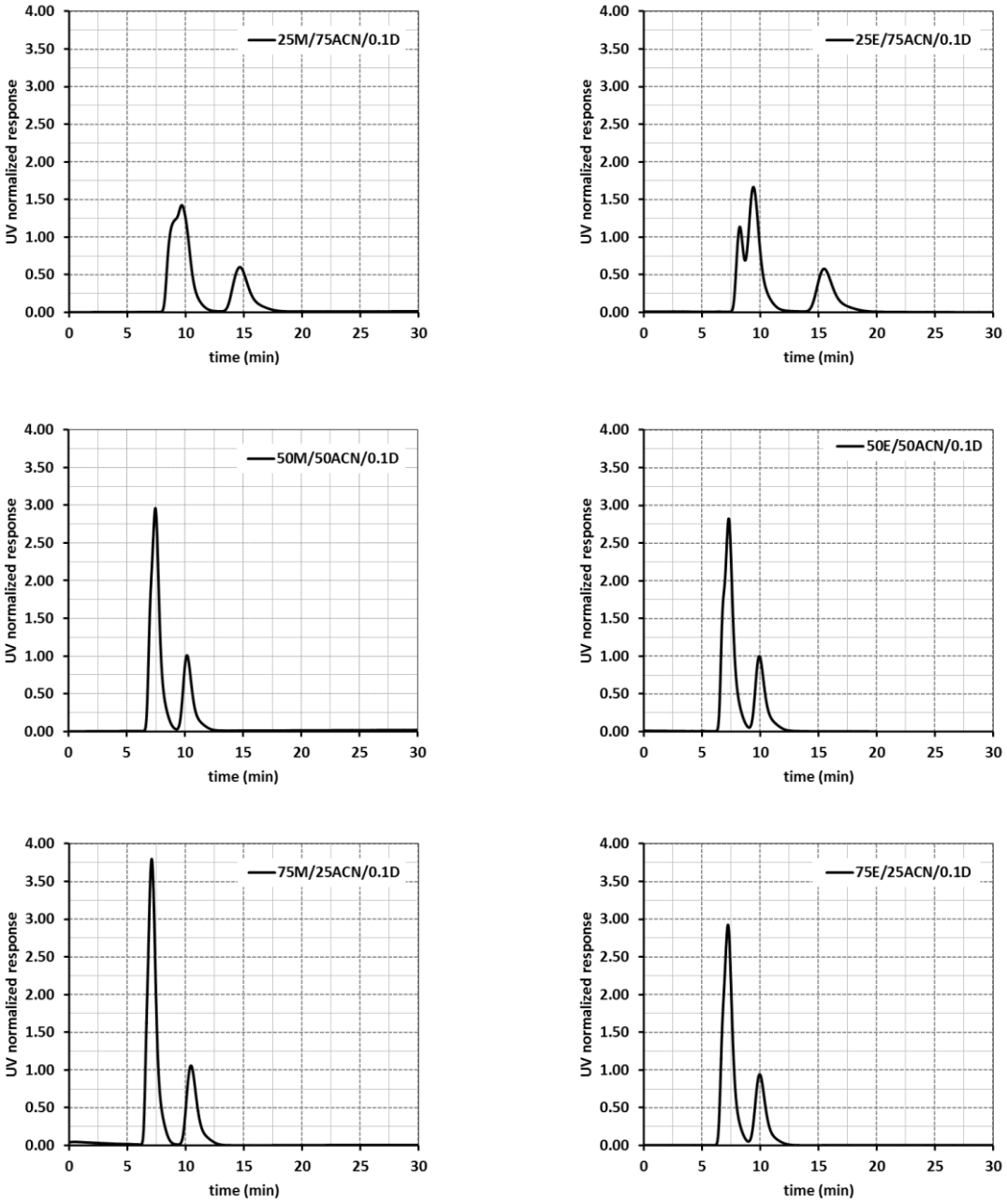

Figure 4.6. *UV normalized response to pulse experiments of nadolol solution on Chiralpak® IA, using different methanol/acetonitrile/diethylamine and ethanol/acetonitrile/diethylamine solvent mixtures (C_F^T= 2.0 g/L; Q=5.0 mL/min; Injection volume=100 μL).*

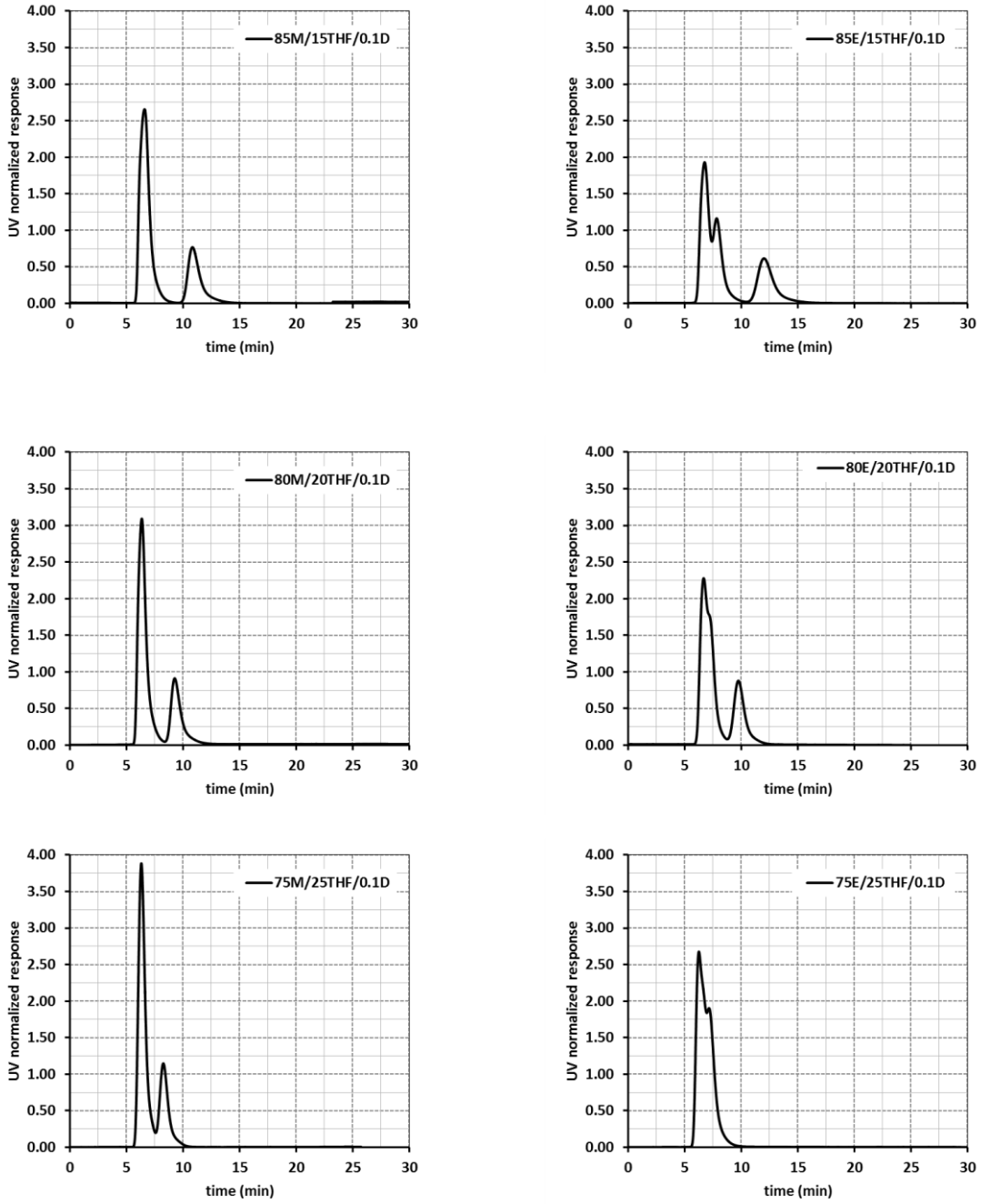

Figure 4.7. *UV normalized response to pulse experiments of nadolol solution on Chiralpak® IA, using different methanol/tetrahydrofuran/diethylamine and ethanol/tetrahydrofuran/diethylamine solvent mixtures (C_F^T= 2.0 g/L; Q=5.0 mL/min; Injection volume=100 μL).*

4.3.1 Most promising Solvent Compositions for Pseudo-Binary Separation

The main advantage of using Chiralpak® IA over Chiralpak® AD is the opportunity to use a wider range of solvents, such as acetonitrile, tetrahydrofuran, dichloromethane and ethyl acetate. Preliminary tests prove that the use of dichloromethane and ethyl acetate mixtures are not suitable to achieve the separation of nadolol stereoisomers. But, some very

interesting results were obtained when using acetonitrile and tetrahydrofuran. Also, it was tested the use of methanol instead of ethanol with some very potential results.

Figure 4.8 presents a summary of the most promising mobile phase compositions for the pseudo-binary (1+2+3)/4 separation using Chiralpak® IA under different mobile phase mixtures and compositions. For all the results presented, the first three nadolol stereoisomers co-elute together (at similar retention times), being the more retained stereoisomer completely resolved from the other species.

As for the previously presented ethanol/heptane results, these new mobile phase mixtures and compositions for Chiralpak® IA show two opposite advantages to be optimized at preparative level (at high concentrations): some are very interesting because of the very high selectivity (between the last and the first three nadolol stereoisomers) but with high retention times for the more retained component; other overcome this last drawback but with a considerable loss in selectivity.

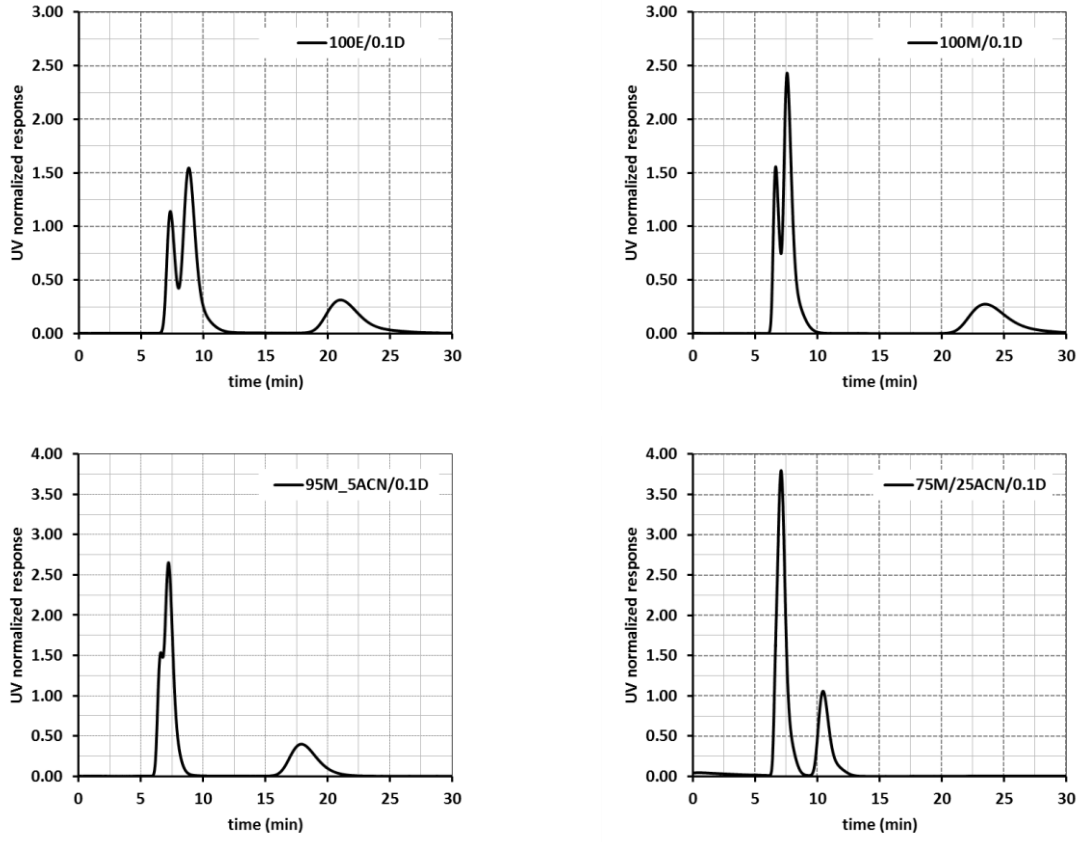

Figure 4.8. *Pulse experiments of nadolol using some potential mobile phase mixtures and compositions using Chiralpak® IA CSP (C_T^F=2.0 gL; injection volume = 100 μL; Q= 5.0 mL/min). Legend: M-Methanol; E-Ethanol; ACN – Acetonitrile; THF-Tetrahydrofuran; D-Diethylamine.*

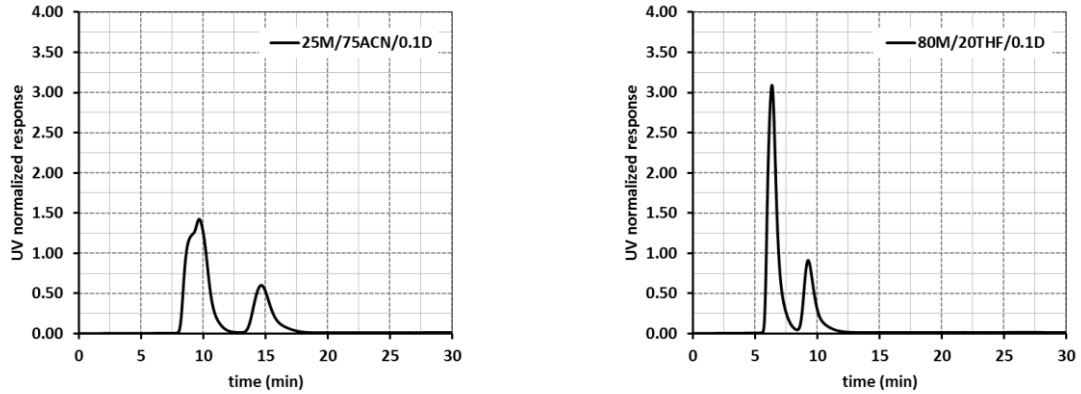

Figure 4.8 (Cont). *Pulse experiments of nadolol using some potential mobile phase mixtures and compositions using Chiralpak® IA CSP (C_T^F=2.0 g/L; injection volume = 100 μL; Q= 5.0 mL/min). Legend: M-Methanol; E-Ethanol; ACN – Acetonitrile; THF-Tetrahydrofuran; D-Diethylamine.*

4.3.2 Loading Experiments

Additional experiments were carried out using a bigger injection volume and higher feed concentrations, trying to approach the real conditions under preparative/production conditions. Several pulses experiments were performed on two different SMB columns packed with Chiralpak® IA and Chiralpak® AD. These experiment were done using different nadolol concentrations (2, 5 and 10 g/L) and a 1000 μL injection volume. The 80%ethanol:20%heptane mobile phase composition was selected to be the same as the used by Ribeiro *et al.* (2013) in order to compare the chromatographic separation on both chiral stationary phases. The experimental results obtained in this study are present in Figure 4.9. Other six different solvent compositions were studied: 100%ethanol, 100%methanol, 95%methanol:5%acetonitrile, 75%methanol:25%acetonitrile, 25%methanol:75%acetonitrile and 80%methanol:20%tetrahydrofuran, (Figure 4.10).

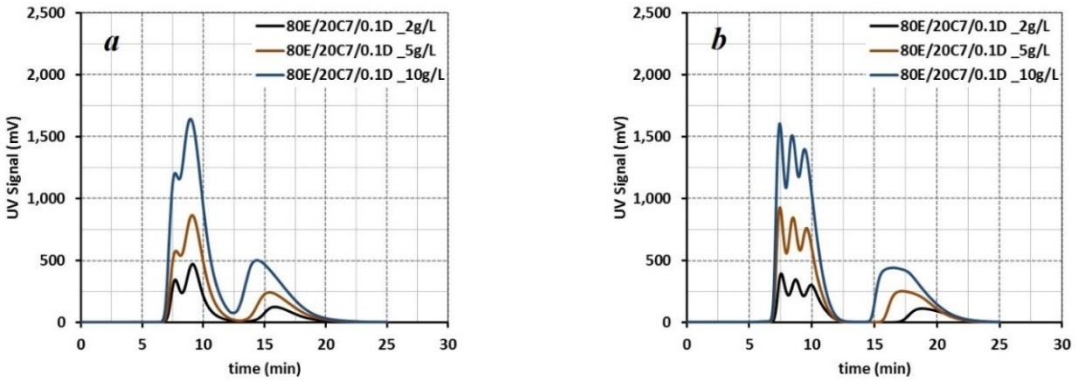

Figure 4.9. *Pulse experiments of nadolol using 80%ethanol:20%heptane:0.1%DEA mobile-phase composition for: (a) Chiralpak® IA and (b) Chiralpak® AD CSPs (C_T^F=2, 5 and 10 g/L; injection volume = 1000 μL; Q= 5.0 mL/min;). Legend: E-Ethanol; C7–Heptane; D-Diethylamine.*

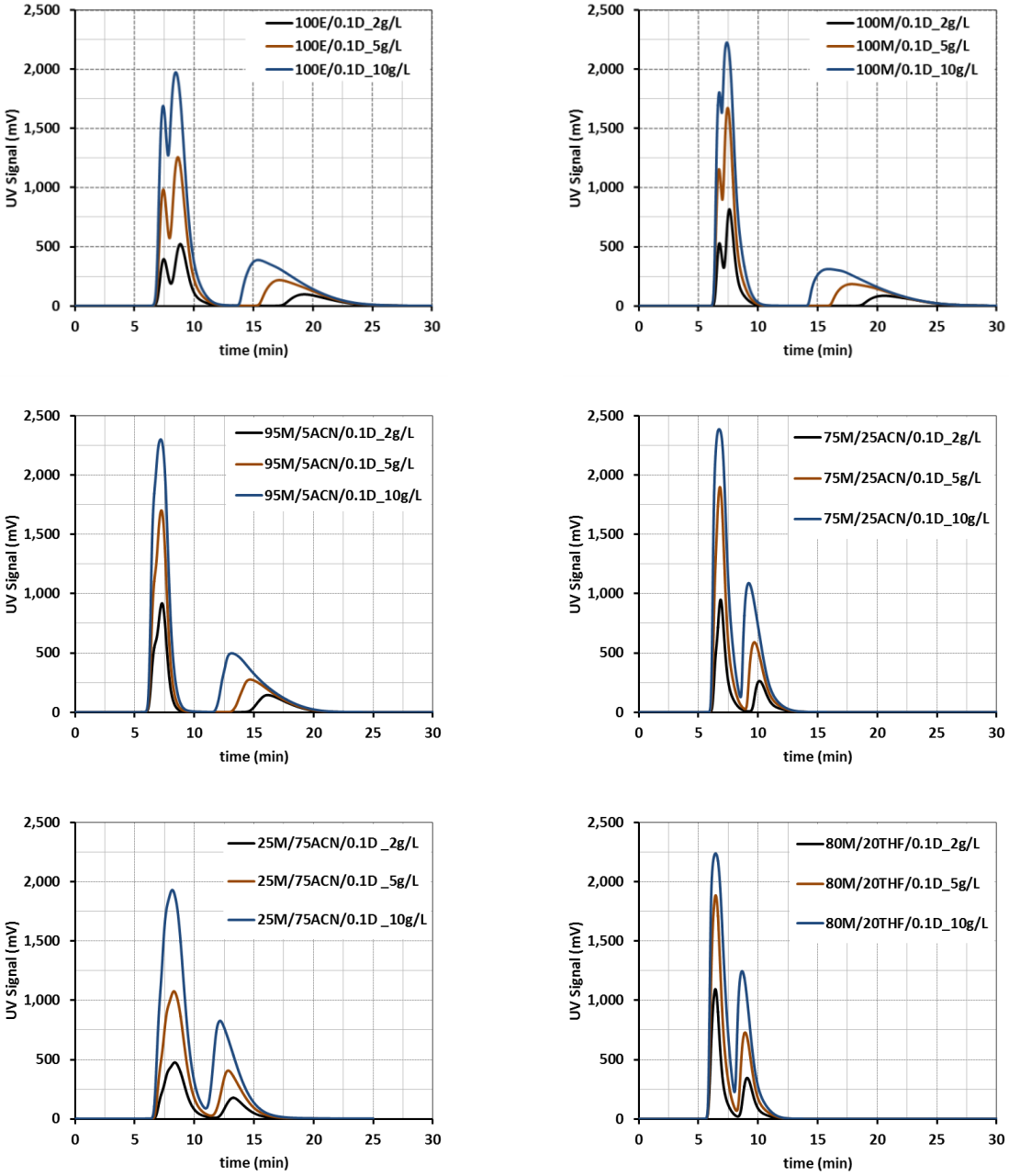

Figure 4.10. *Pulse experiments of nadolol using some promising mobile phase mixtures and compositions using Chiralpak® IA CSP (C_T^F=2, 5 and 10 g/L; injection volume = 1000 µL; Q= 5.0 mL/min ;). Legend: M-Methanol; E-Ethanol; ACN – Acetonitrile; THF-Tetrahydrofuran; D-Diethylamine.*

The use of pure methanol and pure ethanol presents good selectivity between the component trio (1+2+3) and the more retained component 4. Nevertheless, its use introduces a strong non-linear behaviour for the more retained component which can be an important drawback at a preparative scale separation process. The use of alcohol mixtures (particularly methanol) with acetonitrile and tetrahydrofuran (75-80% methanol : 25-20% acetonitrile or

tetrahydrofuran) introduces a significant diminishing on that non-linear adsorption behaviour but together with a decreasing on the retention of the more retained component and, consequently, a diminishing on the selectivity of the (1+2+3)/4 pseudo-binary separation. A compromise between more linear adsorption behaviour and good selectivity (both welcome for a preparative scale separation) can be obtained by using alcohol/acetonitrile mixtures with low (25% for instance) and very high (95%) methanol contents. All of these mixtures are potentially very interesting for a pseudo-binary (1+2+3)/4 preparative separation process. We stress out that these last results clearly show that the higher retention of the last nadolol stereoisomers is accompanied by a stronger non-linear adsorption behaviour which is, once again, an important drawback for the preparative separation process. Taking on account the results presented on Figure 4.10. We diced to continue the work with the mobile phase of 100%methanol:0.1%DEA, since it is present better solubility of nadolol and also a higher selectivity between third and fourth stereoisomers of nadolol. Although it is present some strong non-linear behaviour particularly for the most retuned component.

4.4 Measurement of Adsorption Equilibrium Isotherms

As mentioned before, the linear+Langmuir adsorption isotherm model (LLG6 model) with six parameters was used to describe the adsorption equilibrium isotherm experimental behavior. The fitting procedure of the model to the experimental points allowed obtain the following model parameters: m=0.6887; Q=2.365 g/L; b_1=1.096x10^{-7} g/L; b_2=2.573x10^{-2} g/L; b_3=5.633x10^{-2} g/L; b_4=4.379x10^{-1} g/L with a corrected standard deviation of SD=0.0418.

Experimental results and the obtained model are presented in Figure 4.11. Figure 4.12 shows the effect of the concentrations of the nadolol stereoisomers on the selectivity factor.

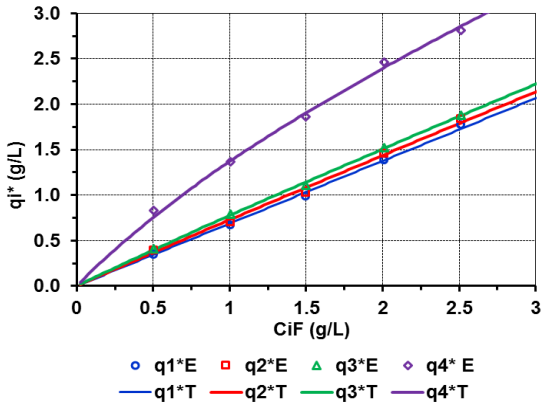

Figure 4.11*. Competitive adsorption isotherm. Comparison between experimental (points) and model (lines) using 100%MeOH:0.1%DEA mobile phase.*

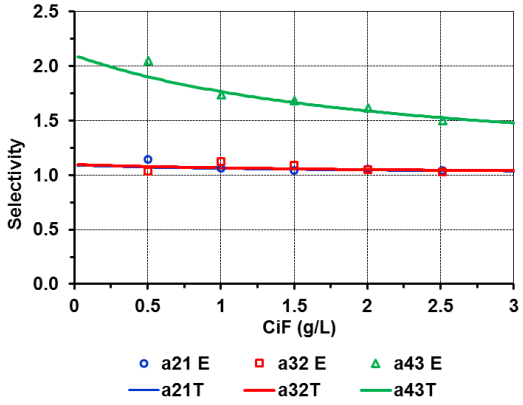

***Figure 4.12**. Effect of the stereoisomers concentration on the selectivity factor. Comparison between Experimental (points) and model (lines) using 100%MeOH:0.1%DEA mobile phase.*

There is a good agreement between the experimental points and the adsorption behaviour predicted by the obtained LLG6 model. For Fig 4.12, we can observe a decrease of selectivity with the increase of the nadolol stereoisomers concentration as it is expected for competitive chromatography systems. This behaviour is more important between stereoisomers 3 and 4.

4.5 Breakthrough Measurements and Simulation

The breakthrough measurements are very important to validate the adsorption equilibrium isotherm model. Previously, the prediction of the axial dispersion was estimated using a feed solution containing the non-retained compound (1,3,5-tri-tert-butylbenzene) leading to a Peclet number of 1000. After, a 10 g/L of nadolol feed solution was used to estimate the mass transfer resistance of 1.0 s^{-1}. Breakthroughs measurements were carried out using a 10 g/L feed concentration of nadolol stereoisomers and using a 100%methanol/0.1%DEA mobile phase composition and a constant flow rate of 5 mL/min. Figure 4.13 presents the experimental results (points) obtained from the saturation and regeneration steps of the preparative column, as well as the fixed-bed adsorption behavior (lines) predicted by the selected adsorption isotherm model.

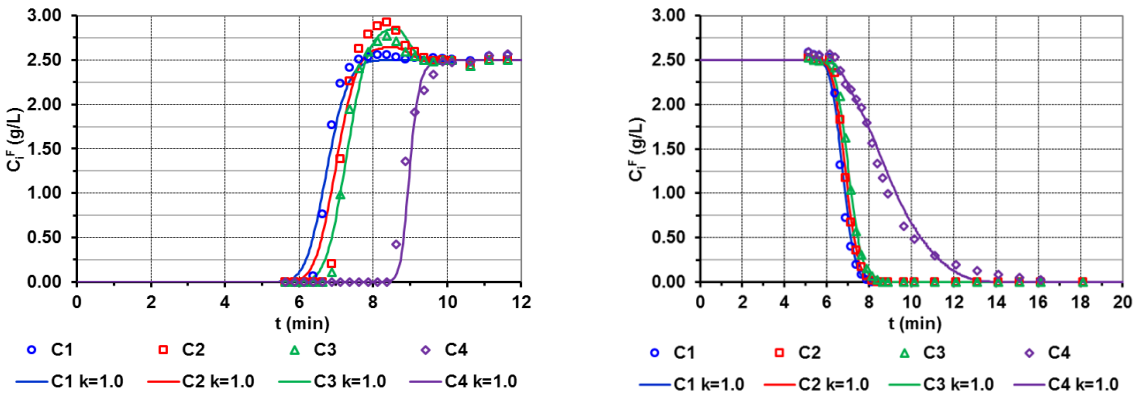

Figure 4.13. *Saturation (adsorption) and regeneration (desorption) curves for total feed concentration of 10 g/L of nadolol. Comparison between experimental (points) and simulation (lines) results. Mobile phase 100%metanol:0.1DEA; flow rate: 5 mL/min. Model parameters used: ε = 0.4, Pe = 1000; k = 1s^{-1}.*

These results shows a roll-up phenomenon for the less retained species, where the stereoisomers concentration reaches a maximum that is larger than its concentration in the feed concentration, due of its displacement by the more retained species and due to the competition of stereoisomers for adsorption under overload nonlinear conditions.

4.6 Nadolol Stereoisomers Pseudo-Binary Separation by SMB

The prediction of the initial SMB operation conditions were carried out using the data collected on previous sections. The SMB initial operation conditions were held using the equilibrium theory, adapted for non-linear systems, and calculating the triangle that defines a region of complete separation. Figure 4.14 shows two separation regions obtained for two feed solution concentrations of 2 and 10 g/L and the experimental measured points obtained for γ_{II} and γ_{III} for three different SMB runs: two runs using the same 2 g/L feed concentration and one run using a 10 g/L of nadolol feed solution concentration.

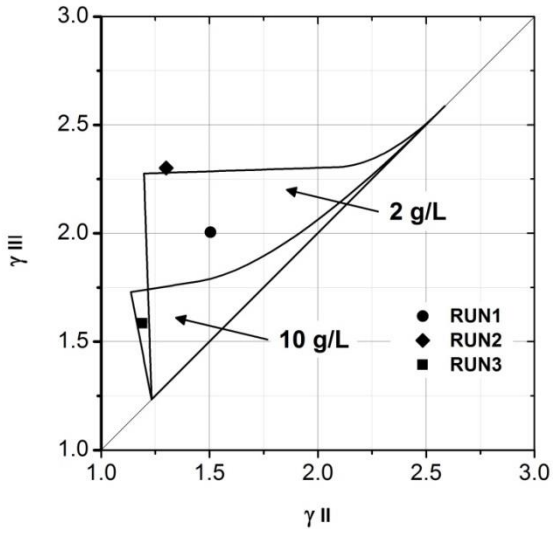

Figure 4.14. *SMB separation regions and experimental obtained conditions for the 100%methanol:0.1%diethylamine and two different racemic feed concentrations (2 and 10 g/L). Points represent the experimental γ_{II} and γ_{III} values obtained in the three SMB operations. RUN1 and RUN2 (2 g/L) and RUN3 (10 g/L).*

Table 4.2 presents the main SMB experimental operating parameters: switching time, the internal and external flow-rates, the corresponding true moving bed (TMB) gamma values and the performance parameters of each one of the three SMB runs.

Table 4.2. SMB experimental values (using a maximum system pressure drop of 40 bar).

RUN	C_F^T (g/L)	Internal flow-rates (mL/min)	External flow-rates (mL/min)	TMB γ values	Performance parameters
1	2.02	$Q_I^* = 35.5$ $Q_{II}^* = 11.0$ $Q_{III}^* = 13.2$ $Q_{IV}^* = 8.3$ $t^* = 3.15\ min$	$Q_E = 27.2$ $Q_F = 2.2$ $Q_X = 24.5$ $Q_R = 4.9$ $Q_{REC} = 8.3$	$\gamma_I = 7.08$ $\gamma_{II} = 1.50$ $\gamma_{III} = 2.00$ $\gamma_{IV} = 0.89$	$PUX = 100.0\%$ $RCX = 100.0\%$ $PR = 0.31\ g/(L.hr)$ $SC = 27.71\ L/g$
2	2.02	$Q_I^* = 35.5$ $Q_{II}^* = 10.1$ $Q_{III}^* = 14.5$ $Q_{IV}^* = 7.7$ $t^* = 3.15\ min$	$Q_E = 26.9$ $Q_F = 4.4$ $Q_X = 24.9$ $Q_R = 6.8$ $Q_{REC} = 8.1$	$\gamma_I = 6.97$ $\gamma_{II} = 1.29$ $\gamma_{III} = 2.30$ $\gamma_{IV} = 0.75$	$PUX = 100.0\%$ $RCX = 90.9\%$ $PR = 0.59\ g/(L.hr)$ $SC = 15.61\ L/g$
3	10.04	$Q_I^* = 30.0$ $Q_{II}^* = 15.5$ $Q_{III}^* = 18.3$ $Q_{IV}^* = 11.4$ $t^* = 1.95\ min$	$Q_E = 18.7$ $Q_F = 2.8$ $Q_X = 14.5$ $Q_R = 7.0$ $Q_{REC} = 11.3$	$\gamma_I = 3.23$ $\gamma_{II} = 1.18$ $\gamma_{III} = 1.58$ $\gamma_{IV} = 0.60$	$PUX = 99.5\%$ $RCX = 97.6\%$ $PR = 1.98\ g/(L.hr)$ $SC = 3.13\ L/g$

For a feed concentration of 2 g/L were obtained, for the more retained stereoisomer in the extract outlet stream, purities and recoveries values of 100%. However, this separation presents a relatively low productivity value (0.31 g/(L.hr)) and a high solvent consumption

(27.7 L/g). A second run using the same feed concentration was carried out, enhancing the productivity (near the double) and decreasing the solvent consumption (near the half) but committing the recovery (100% extract purity and 90.9% extract recovery). Another SMB run was performed using a considerable higher feed concentration of 10 g/L (Run 3) and an improvement of performance parameters were observed. For the more retained stereoisomer in the extract outlet stream, a purity of 99.5%, and a recovery of 97.6% was obtained, with a productivity value of 1.98 g/(L.hr) and a solvent consumption of 3.13 L/g.

Figures 4.15, 4.16 and 4.17 shows the transient evolution on the concentration of nadolol stereoisomers species in the extract and raffinate outlet streams during the SMB operation for the three runs.

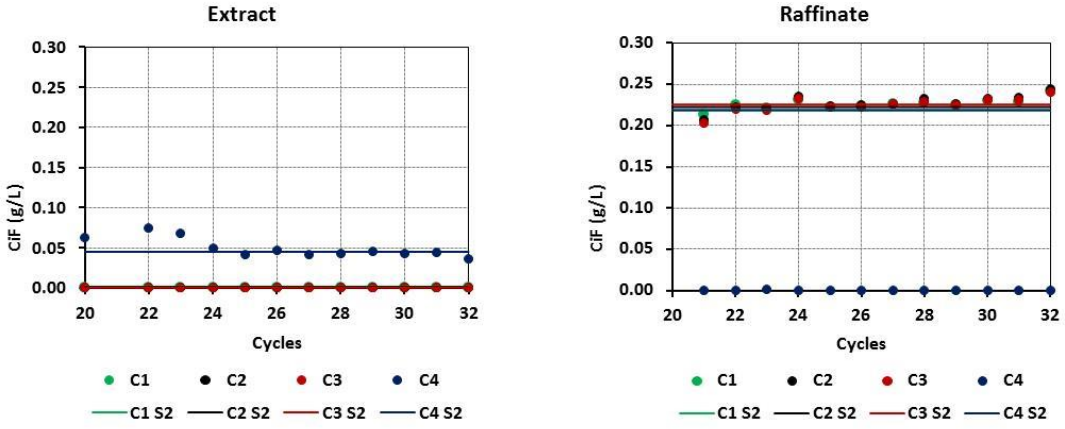

Figure 4.15. *Comparison between the simulation and experiential transient concentration of extract (left) and raffinate (right) streams for four nadolol stereoisomers, and Run 1 (using a 2.02 g/L of feed concentration).*

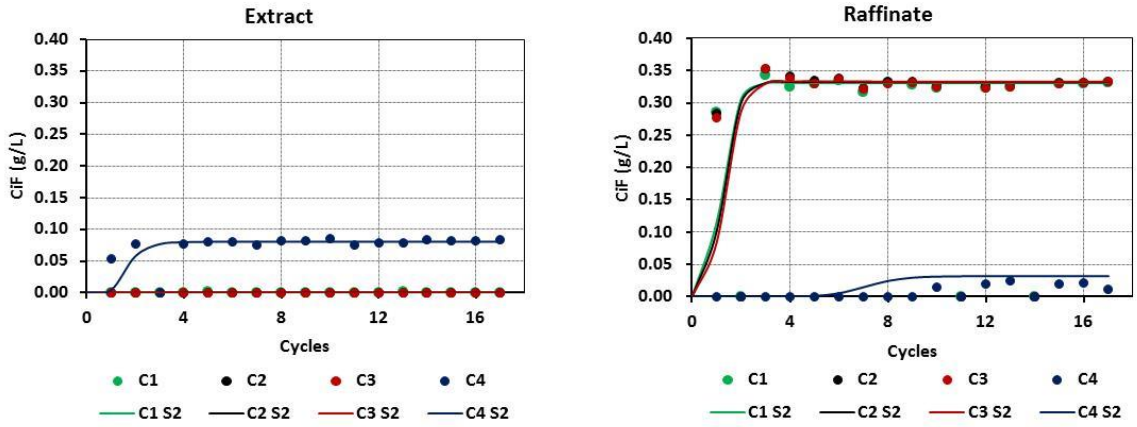

Figure 4.16. *Comparison between the simulation and experiential transient concentration of extract (left) and raffinate (right) streams for four nadolol stereoisomers, and Run 2 (using a 2.02 g/L of feed concentration).*

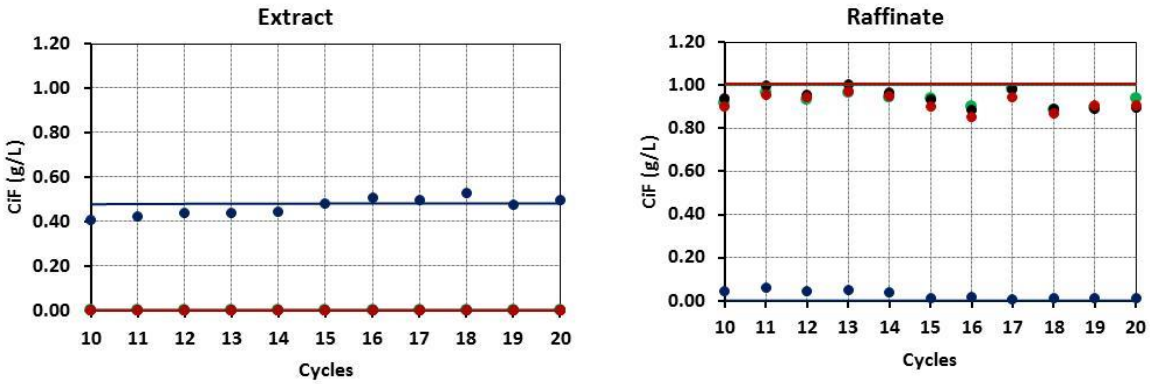

Figure 4.17. *Comparison between the simulation and experiential transient concentration of extract (left) and raffinate (right) streams for four nadolol stereoisomers, and Run 3 (using a 10.03 g/L of feed concentration).*

Both extract and raffinate outlet streams were collected during the last 10 cycles of each SMB run. The samples were named as "X1" and "R1" for run1," X2" and "R2" for run2 and "X3" and "R3" for run3. The samples were then analysed in the analytical HPLC Knauer system using the analytical chiral column. The experimental chromatograms obtained for all samples are presented in Figure 4.18.

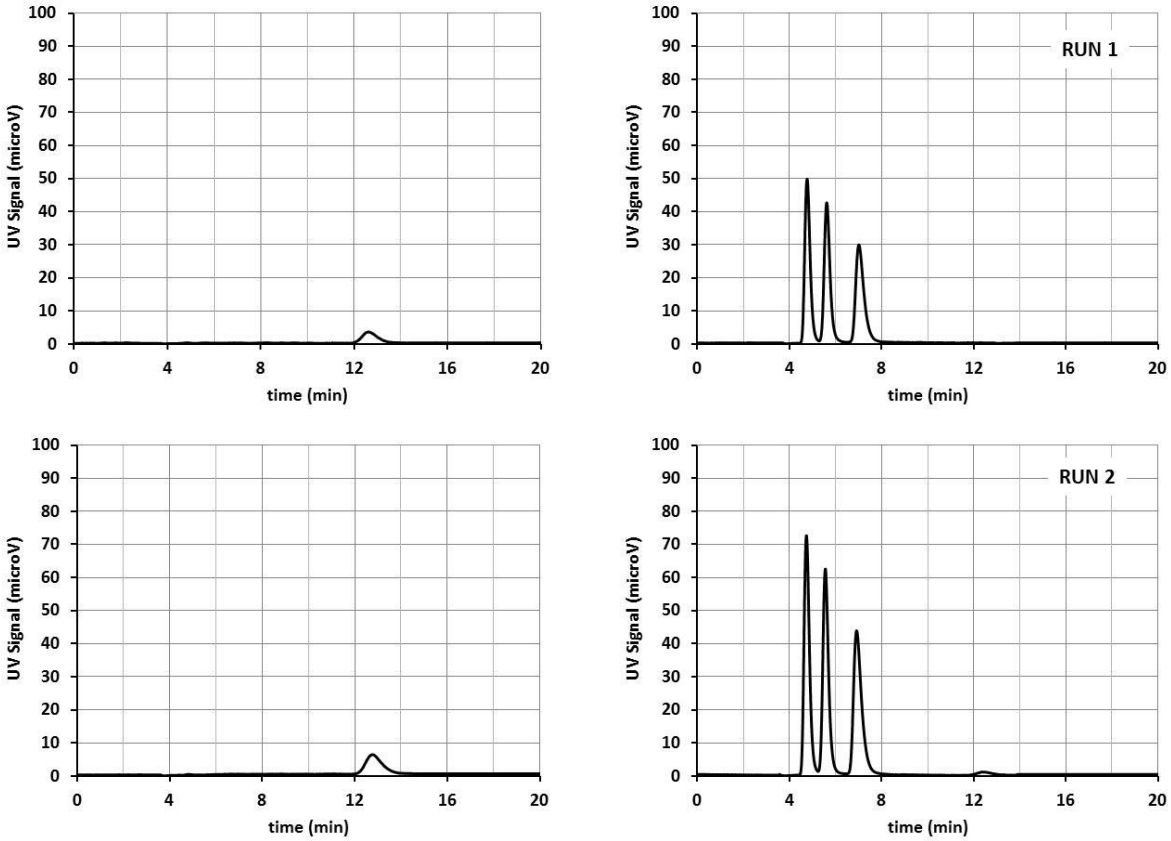

Figure 4.18. *HPLC analysis for all extract (left figures) and raffinate (right figures) outlet streams collect during the final 10 cycles of each SMB run.*

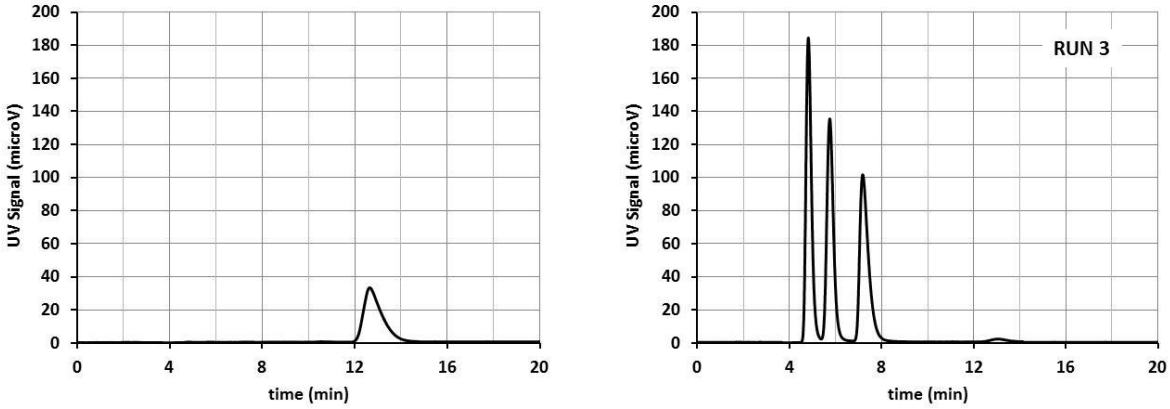

Figure 4.18. (Cont.) *HPLC analysis for all extract (left figures) and raffinate (right figures) outlet streams collect during the final 10 cycles of each SMB run.*

Is possible to confirm almost 100% purity obtained in the extract outlet stream for all the 3 SMB runs (Figure 4.18 and Table 4.2). The chromatogram obtained for raffinate samples collected during the cyclic steady-state of run1 present on contamination (PUR=100%). However, raffinate samples collect during run2 or run3 presents some contamination with the most retained stereoisomer, and then complete separation cannot be considered at the raffinate outlet stream. These conclusions can be confirmed observing the final experimental results presented in Table 4.2 for the 3 SMB runs.

4.7 Use of SMB Extract and Raffinate samples for additional adsorption isotherm and breakthrough measurements

The extract and the raffinate samples obtained in the three SMB experimental runs presented before were distillated under vacuum using rotary vapor equipment. After distillation, both extract and raffinate concentrated samples were used to additionally study the adsorption behavior through adsorption equilibrium isotherm and breakthrough measurements using these 1+2+3 and 4 “pure” feeds. The equilibrium experimental results obtained using these SMB outlets are presented in Figure 4.19.

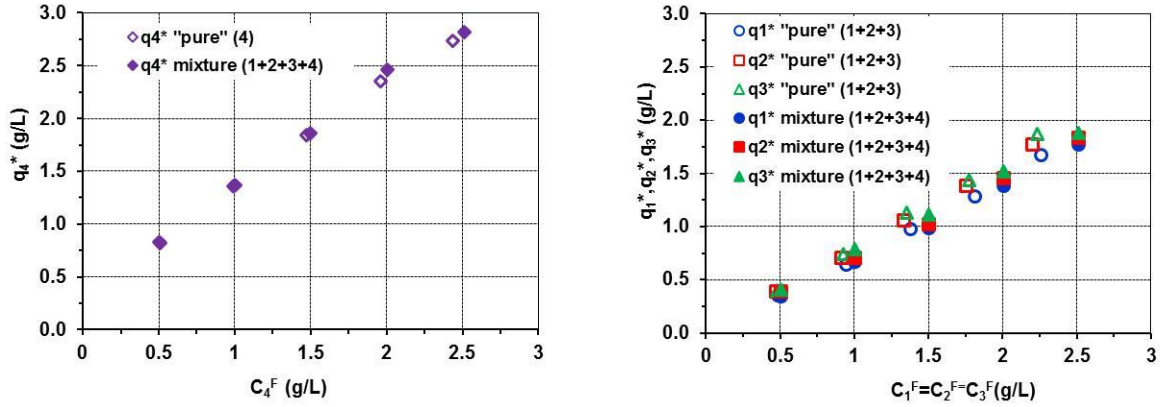

Figure 4.19. *Competitive adsorption isotherm using the extract (left) and raffinate (right) SMB outlet streams after solvent distillation. Comparison between experimental adsorption behavior using racemic mixtures (1+2+3+4; closed points) and "pure" feeds (4 and 1+2+3 and 4; open points) using 100%MeOH:0.1%DEA mobile phase.*

The experimental results presented in Fig. 4.19 show that there is no meaningful differences in the adsorption behaviour for the most retained stereoisomer (component 4), considering the racemic mixture (1+2+3+4) and the "pure" SMB outlets (4 alone). However, for the three less retained enantiomers alone (1+2+3) it can be observed a light increase in the amount adsorbed in the solid phase when compared with the situation of racemic mixture (1+2+3+4), as it can be observed in Figure 4.19 (right). This can be explained by the former adsorption equilibrium isotherm model presented in Equation 3.5 and Section 4.4. Since component 4 is not present, the denominator of the Langmuir term of Equation 3.5 will have a lower value (because C_4=0) and, so, q_1^*, q_2^* and q_3^* will be slightly higher. The same will occur for component 4 alone (C_1=C_2=C_3=0), but, in this case, since b_1, b_2 and b_3 are significantly smaller than b_4, the diminishing of the denominator of the Langmuir term is not so intense and, so, there are no significant differences in q_4^* alone and in the racemic mixture (Figure 4.19, left).

The former adsorption model was used to describe the adsorption behaviour found for both "pure" SMB outlets. Figure 4.20 presents a reasonable agreement between the new data and the former model except for a slightly discrepancy at high concentrations.

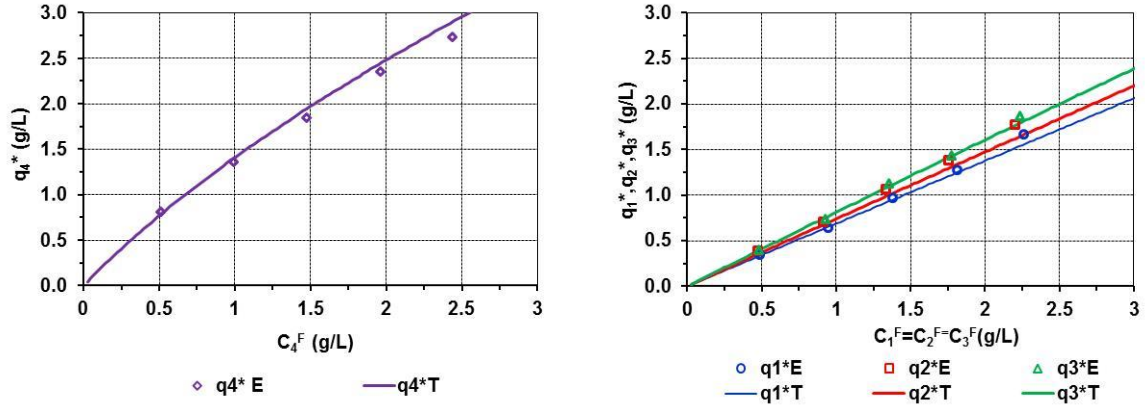

Figure 4.20. *Competitive adsorption isotherm using the extract (left) and raffinate (right) SMB "pure" outlet streams. Comparison between experimental (points) and the former linear+Langmuir model, LLG6, presented in Section 4.4 (lines).*

After the adsorption isotherm measurements, a saturation-regeneration breakthrough was also performed using the SMB extract and raffinate outlets. These experimental results, as well as the simulation of the fixed-bed adsorption behavior using the former LLG6 model (Section 4.4), are presented in Figure 4.21 (using, as feed, the recovered SMB raffinate outlet) and in Figure 4.22 (using, as feed, the recovered SMB extract).

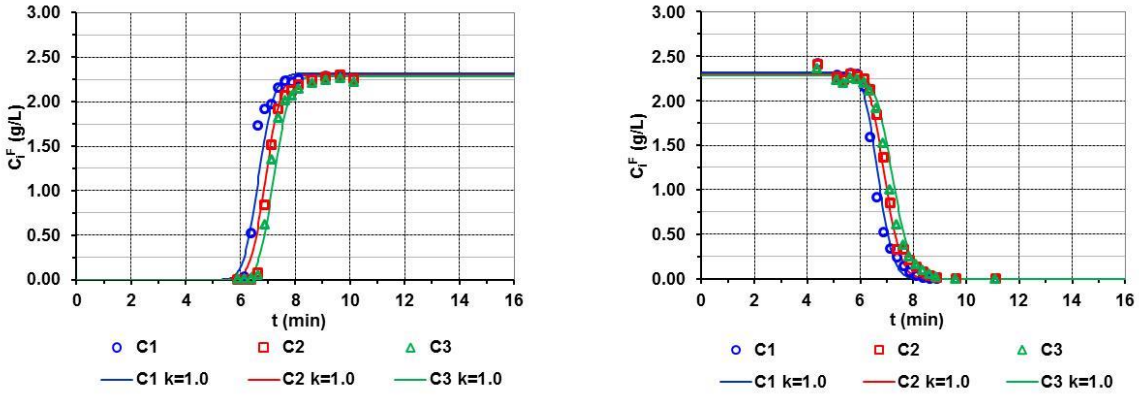

Figure 4.21. *Saturation (adsorption) and regeneration (desorption) curves for SMB raffinate (feed concentration of 2.32 g/L of component 1, 2.30 g/L of component 2 and 2.28 g/L of component 3). Comparison between experimental (points) and simulation (lines) results. Mobile phase 100%metanol:0.1DEA; flow rate: 5 mL/min. Model parameters used: ε = 0.4, Pe=1000; k = 1s^{-1}; LLG6 adsorption isotherm model parameters, as presented in Section 4.4.*

It can be concluded that the former LLG6 adsorption equilibrium isotherm model describes well the fixed-bed adsorption behaviour. A very interesting experimental and simulation result was obtained when compared to Figure 4.13. Figure 4.21 do not present any "roll-up" phenomenon, typical of the competition under preparative chromatography, since the fourth component is not present anymore.

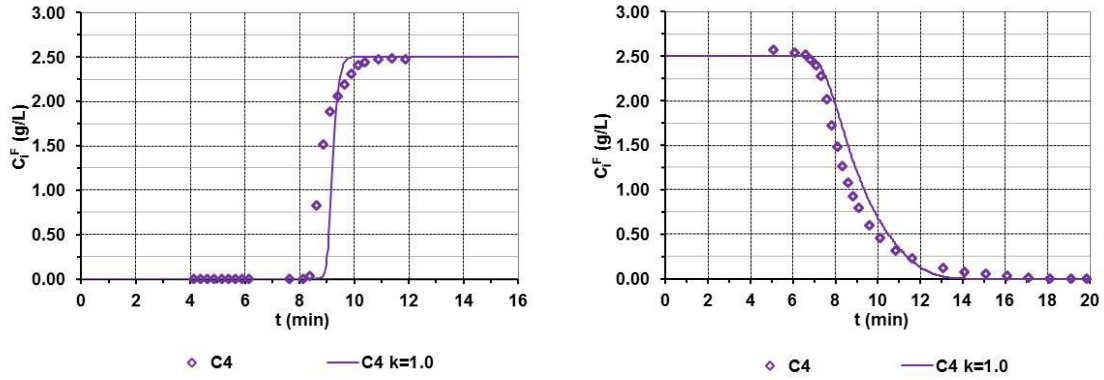

Figure 4.22. *Saturation (adsorption) and regeneration (desorption) curves for SMB extract (feed concentration of 2.50 g/L of component 4). Comparison between experimental (points) and simulation (lines) results. Mobile phase 100%metanol:0.1DEA; flow rate: 5 mL/min. Model parameters used: ε = 0.4, Pe=1000; k = 1s^{-1}; LLG6 adsorption isotherm model parameters, as presented in Section 4.4.*

Results presented in Figure 4.22 shows a light difference between experimental points and the predicted adsorption behaviour (lines). This can be due to the not so good prediction of the adsorption at high concentrations as shown in Figure 4.20 for component 4. It should be noticed that a new fitting using all the adsorption equilibrium data (joining the racemic and the SMB "pure" extract and raffinate outlet streams) was implemented but no better results were obtained. The overall fitting could better describe the adsorption of component 4 alone at high concentrations (Figure 4.20) but with a worst description of the same component when in the racemic mixture (Figure 4.13).

Chapter 5

Conclusions and future work

The screening of mobile phase composition for the preparative separation of nadolol stereoisomers was carried out using different solvents mixtures and compositions on a Chiralpak® IA chiral stationary phase. Additionally, Chiralpak® AD material was also used to compare to the results published for the same nadolol separation (Ribeiro *et al.*, 2013). For this task of the master thesis, eight columns with preparative dimensions (SMB columns) were home-made packed.

Several different mobile phase mixtures and compositions were identified as promising solvents for the (1+2+3)/4 pseudo-binary preparative separation. From all of the possible solvents, a set of seven candidates were selected and presented, using ethanol, methanol, heptane, acetonitrile and tetrahydrofuran mixtures (all with 0.1% diethylamine). Additional loading pulses experiments were also carried out in order to better understand the adsorption behavior at preparative and non-linear conditions (high feed concentrations). The selectivity and retention data were taken into account to justify the selection of a 100%methanol:0.1%DEA solvent composition to perform fixed-bed and adsorption equilibrium isotherm measurements. A linear+Langmuir model was found to describe well the adsorption behaviour. Breakthrough experiments were also performed to validate the equilibrium model and to predict Peclet number and mass transfer coefficient. The equilibrium data was also used to predict the initial operating conditions to perform the SMB separation.

The experimental SMB pseudo-binary-separation was carried out using feed concentrations of 2 and 10 g/L. The complete separation of the most retained stereoisomer was achieved in Run 1 (PUX and PUR=100%) with a productivity of 0.31 g/(L.hr) and a solvent consumption of 27.71 L/g. With a new Run 2 using 2 g/L, we succeeded to increase productivity to 0.59 g/(L.hr) and a solvent consumption of 15.61 L/g obtaining of 100% extract purity and 90.9% extract recovery. Another SMB run was performed using a considerable higher feed concentration of 10 g/L (Run 3) and an improvement of performance parameters were observed. For the more retained stereoisomer in the extract outlet stream, a purity of 99.5%, and a recovery of 97.6% was obtained, with a productivity value of 1.98 g/(L.hr) and a solvent consumption of 3.13 L/g.

During the SMB operation, the extract and raffinate outlet streams were collected to perform further equilibrium and fixed-bed experiments. These experiments allowed studying the adsorption competition behaviour of the most component in the initial mixture.

For future work, it is now possible to explore others promising mobile phase compositions that were identified in this work. As an example, we can suggest the 25%methanol:75%acetonitrile:0.1%DEA mobile phase composition as a promising solvent composition to perform the SMB separation. The obtained results for this mobile phase composition shows less retention time for the last retained compound, and therefore can promote better SMB performances. Other types of chiral stationary phases, other types of SMB operation and SMB cascade system can also be explored to achieve the complete separation of the four nadolol stereoisomers.

For instance, this search grope presented an oral communication on "Strategies for Multicomponent Separation of Nadolol Stereoisomers by Preparative Liquid Chromatography" at the XXIV Encontro Nacional da SPQ (Ribeiro *et al*., 2015) where the JO presses (a SMB related technique that allows real ternary separation) was applied by simulation to separate (1+2+3) nadolol ternary mixture obtained experimentally at the raffinate of the SMB operation presented in this master thesis.

Also, other stationary phases can be used to achieve different preparative paths for the complete separation of nadolol stereoisomers. One system under study is the use of a C18 non-chiral stationary phase to achieve the separation of the two pairs of enantiomers of nadolol (2+3)/(1+4) using SMB operation and reversed-phase mode (using an ethanol/water mobile phase)

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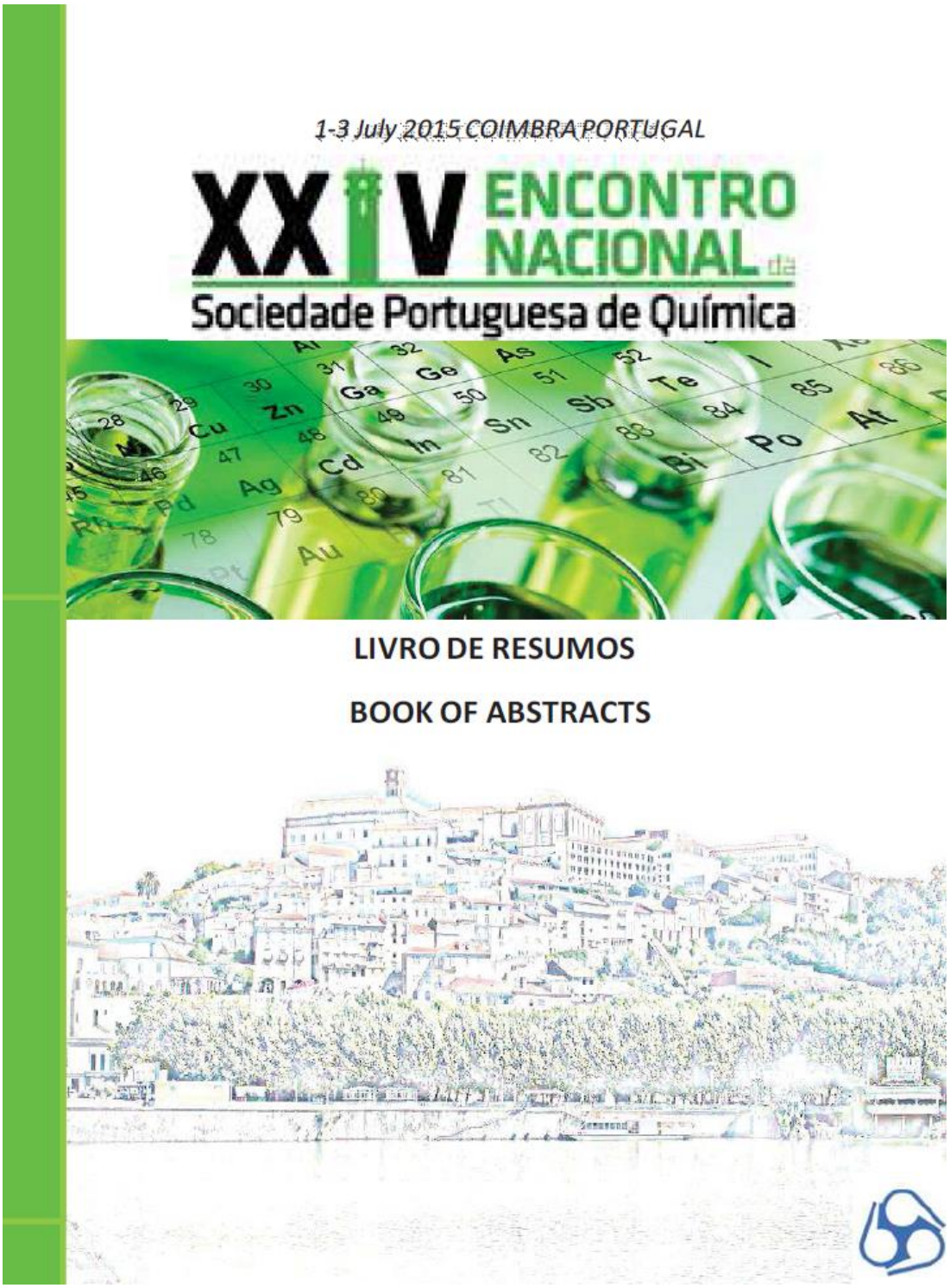
APPENDIX

Outputs of the master thesis work

APPENDIX A

XXIV Encontro Nacional da Sociedade Portuguesa de Química, 01/07/2015 a 03/07/2015, Coimbra, Portugal



Poster communication:

P-76 SEPARATION OF NADOLOL STEREOISOMERS BY LIQUID CHROMATOGRAPHY USING CHIRALPAK IA CHIRAL STATIONARY PHASE

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The separation of nadolol stereoisomers on CHIRALPAK® AD at both analytical and preparative scales was recently reported by Ribeiro et al. [1]. CHIRALPAK® AD is an amylose-based chiral stationary phase (CSP) and is produced by physical coating of the chiral polymer on a matrix. However, due to their coated nature, this CSP can only be used with a limited range of solvents such as the polar (e.g. alcohols) or non-polar solvents (e.g. alkanes). Immobilization of a polysaccharide-derivative on the support is an evolutionary strategy to make a CSP compatible with the whole range of organic solvents, which will consequently extend its application scope. CHIRALPAK® IA is a CSP containing amylose 3,5-dimethylphenylcarbamate immobilized onto silica gel [2].
This work will present a complete methodology concerning experimental, modelling and simulation results. Both the CHIRALPAK® AD and CHIRALPAK® IA CSPs will be evaluated using the nadolol stereoisomers separation as case study. The selection of the proper CHIRALPAK® IA/solvent combination for preparative operation will be fully study taking into account a complete screening strategy [3]. Additional results will include the measurement of the chiral mixture solubility's, equilibrium adsorption data and fixed bed (breakthroughs) experiments. The complete screening will lead to the choice of the better solution for the pseudo-binary separation of the nadolol most retained stereoisomer. Finally, experimental results will be presented for the preparative separation using a Simulated Moving Bed (SMB) pilot unit. The results obtained will be compared with previous published work using the CHIRALPAK® AD adsorbent.

Acknowledgements: Financial support by the Portuguese R&D foundation FCT (Fundação para a Ciência e a Tecnologia) and European Community through FEDER (project PTDC/EQU-EQU/119025/2010) is gratefully acknowledged. This work was co-financed by FCT/MEC and FEDER under Program PT2020 (Project UID/EQU/50020/2013).

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APPENDIX B

XXIV Encontro Nacional da Sociedade Portuguesa de Química, 01/07/2015 a 03/07/2015, Coimbra, Portugal

Oral communication:

OC-44 STRATEGIES FOR MULTICOMPONENT SEPARATION OF NADOLOL STEREOISOMERS BY PREPARATIVE LIQUID CHROMATOGRAPHY

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The Simulated Moving Bed (SMB) technology is receiving an increasing interest as an alternative technique for the production of fine chemicals and pharmaceuticals. However, the classic SMB process is limited to the separation of binary (or pseudo-binary) mixtures or to the recovery of one single component from a multicomponent mixture. Several configurations have been proposed in order to extend the SMB technology to the separation of multicomponent mixtures by using a cascade of SMBs in series or other complex SMB related techniques like multi-zone SMB, intermittent SMB and JO processes. The JO technology allows the separation of ternary mixtures through a cyclic process constituted by two discrete steps. [1,2]

Nadolol is a pharmaceutical drug marketed as a mixture of its four stereoisomers and its prescription is related with some severe risks such as heart failure. The nadolol stereoisomers will be used in this work as a case study for the development of chromatographic strategies for multicomponent separation.

Recently, our research group reported the pseudo-binary separation of nadolol stereoisomers by SMB chromatography [3]. A SMB pilot unit with Chiralpak AD chiral stationary phase (CSP) was used to obtained the more retained stereoisomer 100% pure in the extract and the mixture of the other three stereoisomers being co-eluted in the raffinate.

This work will show how different strategies for multicomponent separation can be implemented, using different CSP, solvent compositions and SMB related techniques, namely:

a) The use of a different CSP, the Chiralpak IA, allowing the use of a wider range of solvents and therefore better separation performances than Chiralpak AD;

b) To achieve a final ternary separation, using the mixture of the three stereoisomers co-eluted in the raffinate, previously referred, as the feed for a subsequent JO process;

c) The separation of the two pairs of nadolol enantiomers using an achiral C18 material, followed by two parallel classic SMB binary enantioseparation processes.

Acknowledgements: Financial support by the Portuguese R&D foundation FCT (Fundação para a Ciência e a Tecnologia) and European Community through FEDER (project PTDC/EQU-EQU/119025/2010) is gratefully acknowledged. This work was co-financed by FCT/MEC and FEDER under Program PT2020 (Project UID/EQU/50020/2013).

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APPENDIX C

III Encontro de Jovens Investigadores do Instituto Politécnico de Bragança, 11/11/2015 a 13/11/2015, Bragança, Portugal

Oral communication:

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Submission

Thank you for your submission. Your submission ID number is 10. Please write this number down and include it in any communications with us.

Below is the information submitted.

Submission ID: 10

Title: Separation of Nadolol Stereoisomers by Liquid Chromatography using Chiralpak IA Chiral Stationary Phase

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Topic(s): Tecnologias

Keywords: Chiral liquid chromatography; Immobilized chiral stationary phase; Nadolol stereoisomers; Screening of solvents.

Abstract: Chiralpak® IA adsorbent is used for the analytical, preparative and simulated moving bed (SMB) liquid chromatography of nadolol, a pharmaceutical drug marketed as a mixture of four stereoisomers. The experimental results include: solubility measurements, incorporation of a basic additive into the solvent, screening of solvent composition for both the complete baseline resolution (analytical separation) of all the four nadolol and preparative and SMB pseudo-binary separation of the most retained stereoisomer. Experimental results show that baseline separation of all the four stereoisomers of nadolol can be achieved using an alcohol/hydrocarbon or alcohol/acetonitrile solvent mixture. The 10%ethanol/90%acetonitrile is presented as the one that presents better resolutions and lower retentions. If the main goal is to perform the preparative pseudo-binary separation (the complete separation of the more retained enantiomer), pure ethanol, pure methanol, alcohol/acetonitrile and alcohol/tetrahydrofuran mixtures will allow better separation results. The 100%methanol:0.1%diethylamine solvent composition was selected to perform the experimental SMB separation. Experimental results also include equilibrium adsorption isotherm and breakthroughs measurements followed by SMB experimental operation. Using a 10 g/l total feed concentration, the more retained stereoisomer is recovered at the extract outlet stream with 99.5% purity, obtaining a system productivity of 1.98 gl-1h-1 and requiring a solvent consumption of 3.13 l/g of product. Comparing hereby results, with ones recently presented by Ribeiro et al. (2013) and Jermann et al. (2015), this work show that Chiralpak® IA chiral adsorbent is an interesting alternative to Chiralpak® AD for the separation of nadolol stereoisomers at both analytical and preparative scales.

Comments: Mestrado em Engenharia Química
Preferência: Comunicação oral (em Inglês)

APPENDIX D

XXI Encontro Galego Português de Química, 18/11/2015 a 20/11/2015, Pontevedra, Espanha

Participante/participant:
ANTONIO RIBEIRO
Resumo/Abstract:

Preparative Separation of Multicomponent Mixtures by Simulated Moving Bed Liquid Chromatography

Autores/Authors:

A. Ribeiro[1], N. Graça[2], R. Arafah[1], A. Rodrigues[2], L. Pais[1]

Tópico/Topic:
INGENIERÍA Y QUÍMICA INDUSTRIAL
Tipo de apresentação/Presentation Type:
ORAL
Código de Comunicación: **IND01**

Caro Antonio:
Temos o prazer de informar que o seu resumo acima referenciado foi selecionado para Oral e será publicado no livro de resumos. .
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Já pode realizar a inscrição e o pagamento correspondente, o dantes possível. Caso necessite de alguma informação adicional contacte por favor a comissão organizadora.

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XXI ENCONTRO GALEGO PORTUGUÉS DE QUÍMICA

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Encontro Galego - Portugués de Química

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Oral communication:

Preparative Separation of Multicomponent Mixtures by Simulated Moving Bed Liquid Chromatography

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Direct racemic resolution of enantiomers by means of liquid chromatography using chiral stationary phases is nowadays a very popular technique. This popularity is mainly due to development of new chiral stationary phases and also by exploring and developing new and more efficient modes of operation. The use of chiral liquid chromatography through the simulated moving bed (SMB) technology has gained a renewed interest as an alternative technique for the production of fine chemicals and pharmaceuticals.

The classic SMB process is a continuous process to separate binary (or pseudo-binary) mixtures or to recover one single component from a multicomponent mixture. Several modified SMB processes have been introduced to separate multicomponent mixtures. Among then, the cascade SMB, the intermittent SMB, the JO processes and other complex multi-zone SMB related techniques, are often applied to the separation of multicomponent mixtures. The JO technology allows the separation of ternary mixtures through a cyclic process constituted by two discrete steps [1].

ORAL

Nadolol is a pharmaceutical drug marketed as a mixture of four stereoisomers, used to treat cardiovascular diseases. However, its prescription is also related with some severe risks such as heart failure. It is well known that pure enantiomer separation is important to control chiral drugs safety. Recently, our research group reported the pseudo-binary separation of nadolol by SMB chromatography [2]. Using the classic SMB mode of operation, the complete separation of nadolol stereoisomers was achieved using Chiralpak AD chiral stationary phase (CSP). The more retained stereoisomer was collected 100% pure in the extract and a mixture of the other three stereoisomers was collected in the raffinate. In this work, we will present different strategies for multicomponent separation, using different solvent compositions, other CSP and SMB related techniques. Namely, (a) The use of Chiralpak IA, that comparing to AD CSP, allows the use of a wider range of solvents and therefore better separation performances; (b) The use of the JO process to achieve a final ternary separation, using the mixture of the three stereoisomers that co-eluted in the raffinate in the separation previously referred and (c) The separation of the two pairs of nadolol enantiomers using an achiral C18 material, followed by two parallel classic SMB binary enantioseparation processes.

Acknowledgements
Financial support by the Portuguese R&D foundation FCT (Fundação para a Ciência e a Tecnologia) and European Community through FEDER (project PTDC/EQU-EQU/119025/2010) is gratefully acknowledged. This work was co-financed by FCT/MEC and FEDER under Program PT2020 (Project UID/EQU/50020/2013) and by QREN, ON2 and FEDER (Project NORTE-07-0162-FEDER-000050).

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[1] V.G. Mata and A.E. Rodrigues, J. Chromatogr. A **2001**, 939, 23-40.
[2] Ribeiro, A.E.; Rodrigues A.E.; Pais, L.S.; *Chirality* **2013**, *25*, 197-205.

APPENDIX E

9º Encontro Nacional de Cromatografia, 05/01/2016 a 09/01/2015, Lisboa, Portugal

Assunto: Registration update: 9º Encontro Nacional de Cromatografia & XVI COLACRO
De: Sociedade Portuguesa de Quimica <eventos@chemistry.pt>
Data: 15-10-2015 11:28
Para: ANTÓNIO MANUEL ESTEVES RIBEIRO <aribeiro@ipb.pt>

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1. Personal Information
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Full name: ANTÓNIO MANUEL ESTEVES RIBEIRO
Scientific name: A.E. Ribeiro
Email: aribeiro@ipb.pt
Date and Time: 15/10/2015 11:21
IP: 188.83.73.189

2. Abstract Submission
Abstract #4078
Authors:
* L.S. Pais
* A.E.Ribeiro
* R.S.Arafah
* A.E.Rodrigues
Title: SEPARATION OF NADOLOL STEREOISOMERS BY FIXED-BED AND CONTINUOUS PREPARATIVE LIQUID CHROMATOGRAPHY USING C18 COLUMNS
Topic:
Preferred type of presentation: Oral communication
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Proposed for Oral communication: (Submitted)

SEPARATION OF NADOLOL STEREOISOMERS BY FIXED-BED AND CONTINUOUS PREPARATIVE LIQUID CHROMATOGRAPHY USING C18 COLUMNS

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Continuous preparative liquid chromatography is nowadays a well-established technology used for the separation of a wide range of chemical mixtures. Among these techniques, the simulated moving bed (SMB) technology has gained an increasing interest to the industry in the production of fine chemicals and pharmaceuticals. This growing is due to the development of new and more versatile stationary phases, as well as new operating schemes for SMB and other continuous chromatographic processes.

In recent years, the authors have focused in the preparative separation of chemical drugs by chiral SMB chromatography. Different case studies have been considered, including the separation of non-steroidal anti-inflammatory drugs (ketoprofen and flurbiprofen enantiomers) [1-4], and the pseudo-binary separation of nadolol stereoisomers, a beta-blocker pharmaceutical drug [5]. While the first two case studies are typical examples of binary chiral mixtures (a pair of enantiomers), the last is an example of a quaternary mixture, composed by two pairs of enantiomers. This considerably increases the complexity and the difficulty of the separation process, asking for new strategies for the complete resolution of all the four components.

Experimental and simulation results have been recently presented considering a first step of a pseudo-binary separation by SMB (the more retained component being obtained pure in the extract and the other three co-eluting in the raffinate), followed

by a ternary separation through a JO process [6]. This work introduces a different strategy using an achiral C18 stationary phase under reversed-phase mode to perform a first SMB separation step. The C18 achiral adsorbent allows the separation of the two pairs of nadolol diastereomers, i.e., the first racemate (composed by the nadolol compounds 2 and 3) co-eluting in the raffinate, and the second racemate (composed by the nadolol compounds 1 and 4) to be obtained in the extract SMB stream. After this preliminary achiral separation step, two parallel SMB runs must be carried out using a chiral stationary phase to achieve the complete separation of all the four nadolol stereoisomers.

Acknowledgements:

Financial support by the Portuguese R&D foundation FCT (Fundação para a Ciência e a Tecnologia) and European Community through FEDER (project PTDC/EQU-EQU/119025/2010) is gratefully acknowledged. This work was co-financed by FCT/MEC and FEDER under Program PT2020 (Project UID/EQU/50020/2013) and by QREN, ON2 and FEDER (Project NORTE-07-0162-FEDER-000050).

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[4] A. Ribeiro, P. Gomes, L. Pais, A. Rodrigues, Chirality, 2011, 23, 602.

[5] A. Ribeiro, A. Rodrigues, L. Pais, Chirality, 2013, 25, 197.

[6] A. Ribeiro, N. Graça, R. Arafah, E. Gheysens, A. Rodrigues, L. Pais, in XXIV Encontro Nacional da Sociedade Portuguesa de Química, Coimbra, Portugal, 1-3 July, 2015 (oral communication).

APPENDIX F

12th International Conference on the Fundamentals of Adsorption, 29/05/2016 to 03/06/2016, Friedrichshafen, Germany

Assunto: 12th International Conference on the Fundamentals of Adsorption Abstract submission (PN:2348)
De: Andrea Köhl <koehl@dechema.de>
Data: 12-10-2015 15:27
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Topics: 5. Liquid phase and gas phase adsorption processes

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Kind regards

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Proposed for Oral communication: (Submitted)

STRATEGIES FOR MULTICOMPONENT SEPARATION OF NADOLOL STEREOISOMERS BY SIMULATED MOVING BED AND JO PROCESSES

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The Simulated Moving Bed (SMB) chromatography is an interesting alternative technique for the production of fine chemicals and pharmaceuticals. The pioneer classical SMB concept was designed for the separation of binary mixtures or to the recovery of one single component from a multicomponent mixture. In recent years, this technology has undergone through several important technical developments, allowing the exploitation of better preparative separation performances. The introduction of a wide range of new and more powerful preparative stationary phases allied to the development of new and more versatile strategies and modes of SMB operation are now a reality. Several configurations have been proposed in order to extend the SMB technology to the separation of multicomponent mixtures by using a cascade of SMBs in series or other complex SMB related techniques like multi-zone SMB, intermittent SMB and JO processes [1-3].

Nadolol is a pharmaceutical drug marketed as a mixture of its four stereoisomers and its prescription is related with some severe risks such as heart failure. This four component mixture will be used as a case study for the development of chromatographic strategies for multicomponent separation. Recently, our research group reported the pseudo-binary separation of nadolol stereoisomers by SMB chromatography [4]. A SMB pilot unit with Chiralpak AD chiral stationary phase was used to obtained the more retained stereoisomer 100% pure. A different strategy was also recently published based on a three column intermittent SMB unit [5].

A new methodology for the design, optimization and experimental implementation of the multicomponent separation will be introduced, including the use of different chiral and achiral adsorbents, the screening and choice of the best adsorbent-solvent combinations, and the use of different SMB operating modes and strategies.

Acknowledgements: Financial support by the Portuguese R&D foundation FCT (Fundação para a Ciência e a Tecnologia) and European Community through FEDER (project PTDC/EQU-EQU/119025/2010) is gratefully acknowledged. This work was co-financed by FCT/MEC and FEDER under Program PT2020 (Project UID/EQU/50020/2013) and by QREN, ON2 and FEDER (Project NORTE-07-0162-FEDER-000050).

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APPENDIX G

Article to be submitted to Chirality Journal (Wiley, United States)

"Separation of Nadolol Stereoisomers by Liquid Chromatography using Chiralpak IA Chiral Stationary Phase"

António E. Ribeiro, Rami S. Arafah, Alírio E. Rodrigues and Luís S. Pais