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


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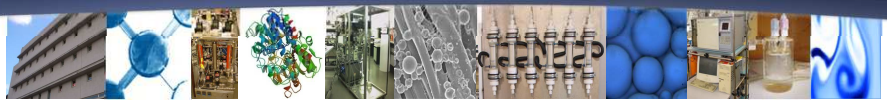


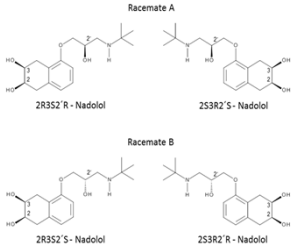
## INTRODUCTION

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.

**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; in fact, two pairs of enantiomers. Nadolol is presently marketed as an equal mixture of the four stereoisomers, designated as the diastereomers "racemate A" and "racemate B" . Racemate A is a mixture of stereoisomers 2 and 3 and racemate B is a mixture of stereoisomers 1 and 4.




## METHODOLOGY & EQUIPMENT

### METHODOLOGY

A. Screening of solvent (mobile phase) composition using preparative C18 achiral columns (XBridge, Waters) and selection of the most promising mobile phase compositions for binary preparative separations;

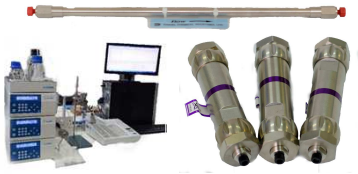
B. The adsorption equilibrium isotherm data is obtained experimentally and fitted to a model to describe the adsorption behaviour. This is a very critical step, since a weak description of the adsorption equilibrium data will also lead to a weak description of the adsorption process and to a wrong optimization of the final separation process at preparative scale;

C. The hydrodynamic and kinetic data (Peclet number and mass transfer resistances) are estimated throughout breakthrough experiments. This step is also used to validate the adsorption equilibrium isotherm model previously selected;

D. The SMB separation is carried out at pilot scale. This step needs the estimation of the SMB operating conditions, using simulation and the concept of separation regions.

### HPLC APPARATUS

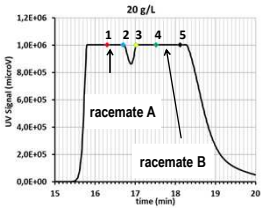
- Jasco HPLC System containing a PU-1580 pump, an UV-1575 multiwavelength detector set at 270 nm, a polarimeter set at sensitivity 64 and a manual injector Rheodyne with 20µL, 100 µL and 1000 µL loops.
- Preparative Knauer HPLC system equipped with a Smartline UV detector 2520 set at 270 nm wavelength, two Smartline 1050 pumps with 50 mL pump heads, a manual injection valve and two different loops (100 and 1000 µL)
- Two types of C18 preparative columns were used: a 10 µm SiliaChrom XT C18 (20mm x 150mm) and a 10 µm Waters XBridge C18 (19mm x 100mm). For the analysis of single stereoisomers an analytical chiral column was used: 5 µm Chiralpak IA column (250x4.6 mm).



## EXPERIMENTAL RESULTS

### SiliaChrom XT C18 preparative column

40Ethanol:60Water:0.2DEA
(feed: 20 g/L, loop 1000 µL, Q= 5 mL/min)

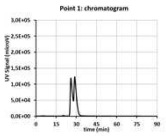



### Peak identification (samples 1 and 4)

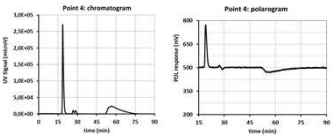
Identification of samples 1 and 4 using a Chiralpak IA chiral column and with UV and polarimeter detections

| Sample | Collecting time |
|---|---|
| 1 | [16min 20sec – 16min 35sec] |
| 4 | [17min 40sec – 17min 55sec] |

Sample 1: racemate A (stereoisomers 2 and 3)

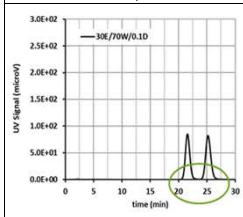



Sample 4: racemate B (stereoisomers 1 and 4)



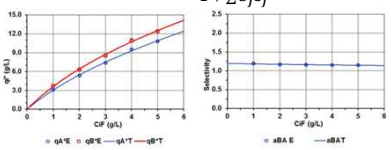

**30Ethanol:70Water:0.1DEA**

**Nadolol pulses**
(feed: 2g/L, loop: 100 µL, Q= 5 mL/min)




**Adsorption Equilibrium Isotherms**
(linear + Langmuir model)

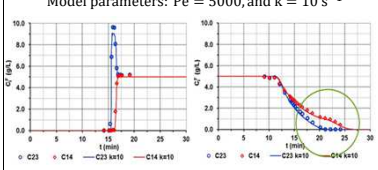
$$q_i^* = mC_i + \frac{Qb_iC_i}{1+\sum b_jC_j}$$



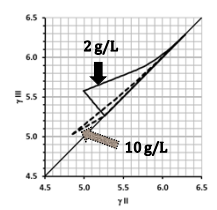

Model parameters: m=1.0007; Q=25.37 g/L;
$b_A = 9.883 \times 10^{-2}$ L/g; $b_B = 1.259 \times 10^{-1}$ L/g

**Breakthrough Experiments**
(saturation and regeneration)

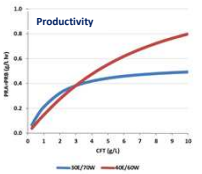
Model parameters: Pe = 5000, and k = 10 $s^{-1}$




**SMB Separation Regions**
(for feed concentration of 2 and 10 g/L)

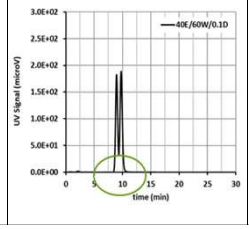


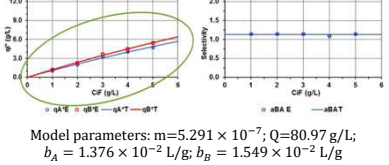

**SMB System Productivity and Solvent Consumption:**
comparison between 30Ethanol:70Water and 40Ethanol:60Water




**40ethanol:60Water:0.1DEA**

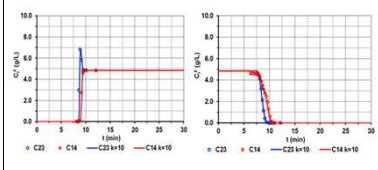
**Waters XBridge C18 preparative column**

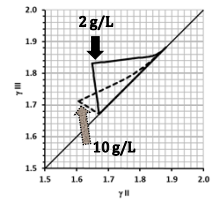


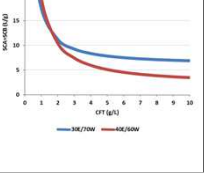




Model parameters: m=$5.291 \times 10^{-7}$; Q=80.97 g/L;
$b_A = 1.376 \times 10^{-2}$ L/g; $b_B = 1.549 \times 10^{-2}$ L/g

Model parameters: Pe = 5000 and k = 10 $s^{-1}$




## CONCLUSIONS & FUTURE WORK

- **The C18 column is able to separate the two racemates of nadolol stereoisomers: the less retained peak contains the racemate A (2+3 stereoisomers) and the more retained peak contains the racemate B (1+4 stereoisomers).**
- **Ethanol/Water solvent mixtures were used to implement the separation of nadolol racemates under reversed phase mode. Two mobile phase compositions were tested: 30/70 and 40/60 Ethanol/Water, both with 0.1DEA.**
- **The 40Ethanol:60Water solvent mixture allows better separation performances, particularly at high feed concentrations: more linear adsorption equilibrium isotherms, with less retention times and similar selectivity when compared with 30Ethanol:70Water.**
- **For feed concentrations higher than 3 g/L, the SMB system productivity is higher using 40Ethanol:60Water. Using a feed concentration of 10 g/L with 40Ethanol:60Water, the system productivity is 1.6 times and the solvent consumption is half the ones obtained with 30Ethanol:70Water.**

**Future Work: Taking into account these results, experimental SMB separation of the two nadolol racemates A and B will be carried out using Waters XBridge C18 columns and 40Ethanol:60Water:0.1DEA mobile phase composition.**


References:

[1] A. Ribeiro, N. Graça, L. Pais, A. Rodrigues, Sep. Purif. Technol. 2008, 61, 375.
[2] A. Ribeiro, N. Graça, L. Pais, A. Rodrigues, Sep. Purif. Technol. 2009, 68, 9.
[3] A. Ribeiro, P. Gomes, L. Pais, A. Rodrigues, Sep. Sci. Technol. 2011, 46, 1726.
[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).



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