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Separation of nadolol racemates by high pH reversed-phase preparative fixed-bed chromatography: Comparison of C18 materials

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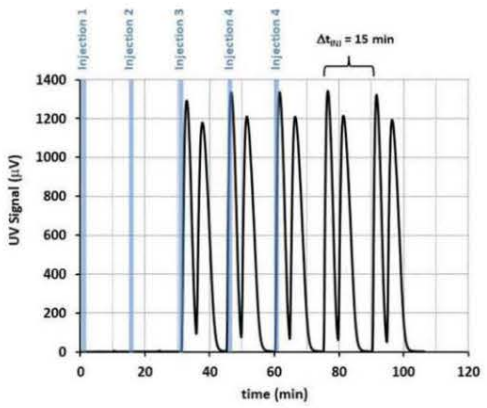

Fixed-Bed technology will be used for the multicomponent preparative separation of a pharmaceutical beta-blocker chiral drug. New strategies using different achiral stationary phases will be presented. Nadolol is a quaternary mixture of equal amounts of four stereoisomers and will be used as case-study. A new methodology for the design, optimization and experimental implementation of the multicomponent separation will be introduced, including the use of three different achiral adsorbents, the screening and choice of the best adsorbent-solvent combination, taking in account the final preparative separation using the fixed-bed technology. Extensive experimental and simulation results will be presented, including solvent screening, measurement of equilibrium adsorption isotherms, breakthrough measurements, and fixed-bed (Azura prep HPLC unit) experimental preparative separation using C18 columns under reversed-phase mode.

Introduction

One of the main goals of the pharmaceutical industry nowadays, is to have more safe and efficient drugs. The purification of chiral pharmaceutical drugs is getting the interest from the industrial companies, particularly after the international regulations. Currently, more than 40% of marketed drugs have chiral active ingredients and almost half of these drugs are marked as racemic mixture.

Nadolol is one representative beta-blocker pharmaceutical drug prescribed worldwide for relieve of several diseases mainly related with the cardiovascular system. However, like other pharmaceutical drugs, it is also related with some severe risks, such as depression, insomnia and cardiovascular failure, among others. Some authors refer that these side effects could be related to the fact that nadolol drug is still marketed as a mixture of equal amounts of its four stereoisomers. Additionally, there are studies referring that some therapeutic effects of this drug are related to only one of the four stereoisomers. Despite the growing pressure of the international regulation agencies for pharmaceutical drugs' safety, pure single nadolol stereoisomers are still no commercially available.

The nadolol pharmaceutical drug represents a very interesting case-study of multicomponent chiral separation since it is composed by four stereoisomers, being two pairs of enantiomers. In this way, it introduces the possibility of alternative strategies, using different kind of separation sequences and techniques, the use of different packings (chiral and achiral stationary phases), and the correspondent mobile phase optimization at both normal and reversed phase modes [1-3].

The design of the complete separation of nadolol stereoisomers asks for a global experimental and simulation methodology considering both the characterization and the optimization of each separation step and its sequences, to achieve the four nadolol components pure.

The present work will scope on optimizing the enantiomers separation of nadolol using different achiral C18 adsorbents. For this case, an extensive set of experiments were carried out using achiral C18 columns, such as, XBridge, Shield and XSelect, all the three achiral adsorbents obtained from Waters. The experimental work focus on screening of mobile phase composition, solubility of nadolol racemates using different pure solvents and solvent mixtures, pulses under analytical and preparative conditions, equilibrium adsorption isotherms and breakthrough measurements. Additionally, experimental results will include the preparative separation by fixed-bed chromatography using an Azura Prep LC unit equipped with two 250 mL/min pump heads and a XBridge Prep OBD C18 10 µm (250x30 mm) column with a 10 µm particle size diameter [2]. Experimental results presented in this work will stress the advantage of using an intermediate step based on achiral reversed-phase liquid chromatography to perform the separation of the two racemates of nadolol.

Materials and methods

The mixture of the four nadolol stereoisomers was obtained from Sigma-Aldrich (Schnelldorf, Germany). The HPLC-grade solvents, ethanol, acetonitrile and the basic modifier diethylamine (DEA) were obtained from Fluka (Bunchs, Switzerland). Three types of analytical (4.6mm ID x 250mm L; particle size diameter of 5 µm) and preparative (19mm ID x 100mm L; particle size diameter of 10 µm) Waters C18 achiral columns were used: XBridge, Shield and XSelect, all obtained from Waters. The columns' efficiency characterization, screening of the mobile-phase composition, loading experiments, 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). The analytical pulses of nadolol were carried out on a Knauer analytical HPLC system. 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. The preparative separation of nadolol stereoisomers was carried out on an Azura Fixed-Bed preparative HPLC system from Knauer (See Fig. 1).

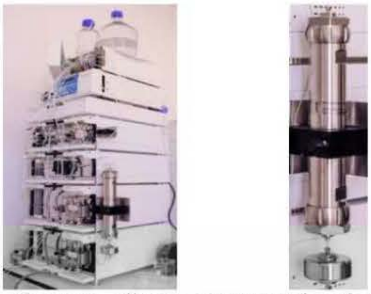

Figure 1. The Azura Pilot Prep HPLC (Brigantia EcoPark).

The system was equipped with two preparative HPLC pumps P2.1L model with 250 mL/min pump heads, and one UV/VIS detector UVD2.1L model set at 270 nm wavelength. This preparative system was equipped with a Waters XBridge Prep C18 column of preparative diameters (30 mm ID x 250 mm L and particle size diameter of 10 µm). A flow-rate between 25 and 75 mL/min was used with this preparative column.

Results

The set of experimental and simulation results will include the screening of the mobile phase composition using the tree types of achiral adsorbents. Several reversed-phase solvents based on ethanol-water mixtures were tested in terms of resolution and dispersion, by means of loading pulses. Results presented will include the experimental measurement of the equilibrium adsorption isotherms (Fig. 2) and breakthroughs experiments for all the three types of adsorbents and using the most promising solvent compositions.

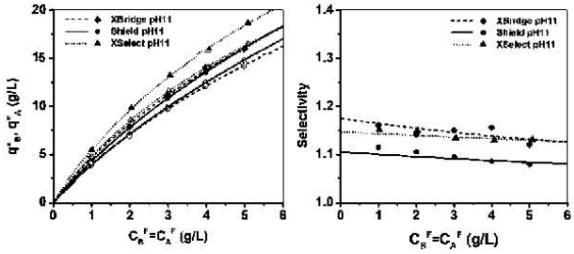

Figure 2. Comparison between experimental and model results for the adsorption equilibrium isotherms (left) and selectivity (right) for the two pairs of nadolol racemates, as a function of their feed concentrations, using 30%ethanol/70%water mobile phase composition with 0.005% diethylamine (pH=11) and the three different C18 Waters columns: XBridge (diamonds), Shield (circles) and XSelect (triangles). All fittings use the linear + Langmuir competitive model.

Modelling and its validation is a crucial step to the accurate equilibrium and kinetic data estimation. Some simulation results for the preparative separation of the nadolol racemates by simulated moving bed technology will be also presented (See Fig. 3). Finally, some experimental results concerning the preparative separation of nadolol racemates using the Azura Fixed-Bed preparative HPLC system will be also presented.

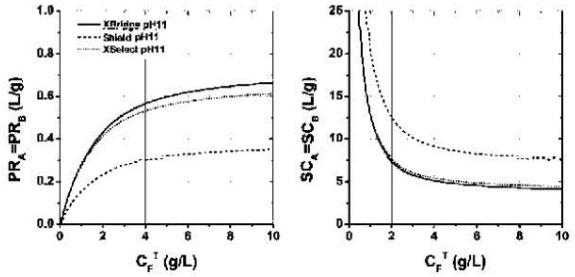

Figure 3. SMB productivity (left) and solvent consumption (right) for the separation of nadolol racemates using the XBridge (solid lines), Shield (dashed lines) and XSelect (dotted lines) columns using a 30%ethanol/70%water with 0.005%diethylamine as mobile phase (pH=11) as a function of the nadolol feed concentration.

Conclusions

The optimization of preparative fixed-bed chromatography depends on the proper choice of the mobile phase composition. The separation of nadolol racemates was studied using different ethanol/water compositions with three different achiral C18 Waters materials (XBridge, XSelect and Shield) at both analytical and preparative scales. The design of the preparative separation process was studied, by means of loading pulses, the measurement of the adsorption equilibrium isotherms, breakthrough experiments using a 30%ethanol/70%water mobile phase composition.

A linear+Langmuir model was found to describe well the adsorption behavior. Breakthrough experiments were also performed to validate the equilibrium model and to predict axial dispersion and mass transfer resistance. The equilibrium data was also used to predict the operating conditions for future extra simulated moving bed (SMB) operation.

Additional experiments were carried out on a fixed-bed preparative system in order to optimize the separation of nadolol racemates. A mobile phase composition of 20%ethanol/80%water/0.1%diethylamine was selected to perform a sequential five-injection experiment to confirm the viability of fixed-bed operation for obtaining pure nadolol racemates.

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