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Response surface methodology for the determination of the design space of enantiomeric separations on cinchona-based zwitterionic chiral stationary phases by high performance liquid chromatography

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ABSTRACT

Quality-by-Design approach for enantioselective HPLC method development surpasses Quality-by-Testing in offering the optimal separation conditions with the least number of experiments and in its ability to describe the method's Design Space visually which helps to determine enantiorecognition to a significant extent. Although some schemes exist for enantiomeric separations on Cinchona-based zwitterionic stationary phases, the exact design space and the weights by which each of the chromatographic parameters influences the separation have not yet been statistically studied. In the current work, a screening design followed by a Response Surface Methodology optimization design were adopted for enantioseparation optimization of 3 model drugs namely the acidic Fmoc leucine, the amphoteric tryptophan and the basic salbutamol. The screening design proved that the acid/base additives are of utmost importance for the 3 chiral drugs, and that among 3 different pairs of acids and bases, acetic acid and diethylamine is the couple able to provide acceptable resolution at variable conditions. Visualization of the response surface of the retention factor, separation factor and resolution helped describe accurately the magnitude by which each chromatographic factor (% MeOH, concentration and ratio of acid base modifiers) affects the separation while interacting with other parameters. The global optima compromising highest enantioresolution with the least run time for the 3 chiral model drugs varied extremely, where it was best to set low % methanol with equal ratio of acid-base modifiers for the acidic drug, very high % methanol and 10-fold higher concentration of the acid for the amphoteric drug while 20 folds of the base modifier with moderate %methanol were needed for the basic drug. Considering the selected drugs as models for many series of structurally related compounds, the design space defined and the optimum conditions computed are the key for method development on cinchona-based chiral stationary phases.

1. Introduction

Quality by design (QbD) can be defined as the systematic approach to method development starting with predefined objectives and emphasizing product and process understanding as well as process control, based on sound science and quality risk management. Quality must be built in the design and should not be expected to improve by additional inspection via experimentation [1]. QbD offers the advantage of extracting maximum information through the application of statistics and mathematics to the experimental data obtained from a carefully selected experimental design. In contrast, development of chiral separation methods has been approached primarily by the "Quality by Testing (QbT)" approach for long decades-and still is- which tests product quality at the end of the experimental process leading to equivocal results, because it only verifies there are no significant changes in method response such as retention and resolution, after small but deliberate changes in the method variables [1,2]. By this approach, QbD is sacrificed which allows accurate investigation of critical process parameters, such as % organic modifier, concentration of additives, temperature, flow rate which have an impact on the chromatographic critical quality attributes, such as retention and resolution in a multitude of enantiorecognition processes involved in a constantly growing number of chiral stationary phases (CSP). Several methods have been reported, attempting to apply QbD in the field of

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achiral separations using special DoE HPLC softwares [3–5] but also using versatile statistical softwares [6–8]. It was sometimes possible to use reversed phase (RP) DoE modelling softwares when the linear solvent strength theory associated with separations in the RP mode was strongly controlling enantiorecognition to explore predictability of chiral separations on a variety of stationary phases [9–11]. However, when other types of interactions are involved in enantiorecognition, no marketed software is currently available to predict for new observations based on a number of experimental runs. Statistical softwares such as Minitab®, Unscrambler®, Statistica® and many others represent the tools of choice to use with the significant variety of enantiorecognition theories associated with CSPs [12].

A variety of chiral stationary phases are nowadays available for enantiomer separation of all kind of chiral structures with polysaccharides and macrocyclic antibiotics being the most popular ones. Among the most recently commercialized CSPs are the Chiralpak ZWIX(+) and ZWIX(-) phases [13,14]. Obtained by fusion of chiral anion-exchanger (quinine and quinidine carbamate) and chiral cation-exchanger ((*S,S*)- and (*R,R*)-trans-2-aminocyclohexanesulfonic acid) selector moieties, they cover applicability profiles of a wide range of chiral compounds comprising chiral acids, chiral bases and amphoteric chiral compounds. They have in their individual chiral selector structures cationic amino group and anionic sulfonate functional groups. Enantiorecognition and optimization of separation on Chiralpak ZWIX has not been previously explored using experimental design [15]. However the enantioresolving unit 2,6-diisopropylphenyl carbamate derivative of quinine was used to model ketoprofen enantioseparation by molecular dynamics [16], aliphatic 3-hydroxyalkanoic acids were successfully separated by a QbT approach with acceptable run time and enantioresolution [17]. A rich experimental work was reported to propose a scheme and optimize organic/ aqueous contents, acid and base modifiers for the separation of amino acids and small peptides on Chiralpak ZWIX(+) based on systematic experiments [18–20]and a description of chromatographic conditions for separation of secondary amino acids and other model compounds [21,22] using QbT approach was reported. From these studies it becomes clearly evident that separation factors and resolution are influenced by a number of experimental factors which would make a systematic optimization by DoE worthwhile and more efficient. The ultimate goal would be to achieve full baseline resolution under robust conditions.

Thus, the aim of the presented work is to use DoE to establish the design space (DS) for chiral separation on ZWIX columns for 3 model drugs differing in their acid-base character (Fmoc-leucine, tryptophan and salbutamol). Such an approach reveals the multidimensional combination and interaction of mobile phase input variables and readily leads to separation conditions that demonstrate a good quality with respect to selectivity and retention. The regression equations controlling these 2 chromatographic responses are to be computed for each drug to show the significance of each chromatographic factor on retention and resolution as a step ahead in the exploration of the enantiorecognition on these CSPs.

2. Experimental

2.1. Chemicals and reagents

Racemic tryptophan (TRYP), salbutamol sulfate (SALB) and *N*-[(9H-fluoren-9-yl-methoxy)carbonyl]-L-leucine were purchased from Sigma Aldrich (Taufkirchen, Germany). Racemic *N*-[(9H-fluoren-9-yl-methoxy)carbonyl]-DL-leucine (Fmoc-Leu) was synthesized from L-leucine and *N*-(9-Fluorenylmethoxycarbonyloxy)succinimide (Fmoc-OSu) (both from Sigma Aldirch). Acetonitrile (ACN) and methanol (MeOH) used for HPLC were HPLC gradient grade and purchased from J.T. Baker (Avantor Performance Materials, Deventer, Netherlands). Acidic and basic modifiers comprising acetic acid (HOAc), formic acid (FA), trifluoroacetic acid (TFA), heptafluorobutyric acid (HFBA), diethylamine (DEA), triethylamine (TEA), *N,N*-diisopropylethylamine (DIPEA) were of analytical grade and purchased from Sigma Aldrich. Ammonia solution (AMM) (7N in methanol) was obtained from Sigma Aldrich.

2.2. Instrumentation and chromatographic conditions

The HPLC system consisted of an Agilent 1100 series liquid chromatograph equipped with a vacuum degasser, a binary pump (0.8 mL/min), a thermostated column compartment (25 °C), an autosampler (20 μL) and a ultraviolet detector (230 nm) all from Agilent Technologies (Waldbronn, Germany). ChemStation data acquisition software was used for data acquisition and data processing. The chiral column used was a prototype of CHIRALPAK ZWIX (+) (Chiral Technologies Europe, Illkirch, France)(250 × 4.6 mm i.d., 5 μm). Samples were dissolved at 0.15 mg/ml in methanol.

2.3. Statistics

Screening and optimization designs were established with Minitab 17 (Minitab Inc., USA).

3. Results and discussion

3.1. Screening and optimization designs

3.1.1. The 2^m4^n screening design

In the first instance it was of interest to figure out the most important factors in a screening design which then allows their systematic study in an optimization design. The DoE screening approach aims to include all factors influencing retention and resolution of the 3 racemates on the ZWIX (+) column. It has been reported that type and percentage of organic solvents (MeOH, ACN), as well as type and concentration of acid and base modifiers are the key factors to control separation on ZWIX columns, besides the common factors such as temperature and flow rate [23]. Common screening designs (such as the fractional factorial design) have problems to consider both categorical (e.g. type of additive) and continuous variables (e.g. molarity of additive). An experimental design which can deal which such a situation is the 2^m4^n screening design which was therefore employed in this study. Herein, there are m four-level factors and n two-level factors where p is the degree of fractionation and 4m2n-p is the number of runs. In the given example, we have 2 four-level factors (i.e. type of acid and type of base each 4 different types) and 3 two-level factors (i.e. percentage of methanol in acetonitrile-based mobile phase, molarity of acid and molarity of base). This 2^m4^n screening design was necessary to determine the most versatile acid-base modifiers of the mobile phase that would result in some enantiomeric resolution for all compounds. The experimental runs are summarized in (Fig. 1) Table 1.

Enantiomer separations were observed for the distinct model compounds under various conditions (Table 1). However, it also turned out that several conditions caused peak splitting and distorted peaks being not at all suitable for the given purpose. This indicates that the type and concentration of acid/base additives are of utmost importance and a delicate fine tuning may be necessary in order to achieve good peak shapes and furnish optimized separations. Overall, the chromatograms of the 16 different mobile phases

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