Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 288–294
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Fast generic chiral separation strategies using electrophoretic and liquid chromatographic techniques

Hasret Ates, Debby Mangelings, Yvan Vander Heyden *

Department of Analytical Chemistry and Pharmaceutical Technology, Vrije Universiteit Brussel-VUB, Laarbeeklaan 103, B-1090 Brussels, Belgium

Received 10 October 2007; accepted 9 December 2007
Available online 23 December 2007

Abstract

Because of the large number of commercially available chiral selectors both for electrophoretic and chromatographic techniques, the experimental possibilities to separate enantiomers are numerous. As a result, the development of a proper separation method for a chiral molecule is challenging. Generic separation strategies can present a useful approach for chiral method development. This paper overviews some strategies that have been developed earlier for different electrophoretic and chromatographic techniques. These strategies can be used either for impurity determination of enantiomers or for the enantioseparation of racemic mixtures. They are not only generic, i.e. applicable on diverse molecules, but are also fast, i.e. requiring only a limited number of experiments to reach a decision.

Keywords: Chiral separations; Generic separation strategies; Method development; Capillary electrophoresis; Liquid chromatography

1. Introduction

In the last decades, chirality has become a major concern in pharmaceutical industry. New chiral drug substances have to be stereochemically identified before being marketed since enantiomers can differ in pharmacological, pharmacokinetic and toxicological properties when exposed to chiral environments such as biological systems, e.g. the human body [1–3]. In such systems, one enantiomer (the eutomer) will be therapeutically active while the other, the distomer, can be less active or inactive, it can have an opposite or even toxic effect. Obviously the separation of enantiomers is gaining importance at almost every level of pharmaceutical drug development and quality assurance [4].

Several analytical techniques can be used to separate enantiomers but the most frequently used in pharmaceutical analysis are capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) [5,6]. Separations can be performed either directly or indirectly. The indirect approach uses a reaction of an enantiomerically pure chiral derivatization agent with the enantiomers to form diastereomers. These can be separated with any conventional achiral separation system since diastereomers differ in physico-chemical properties. The direct approach for chiral separations consists of the formation of transient diastereomeric complexes between the enantiomers and a chiral selector. Chiral selectors can either be added to the mobile phase or the background electrolyte, or bonded onto a chromatographic support creating a chiral stationary phase (CSP).

The fact that nowadays many chiral selectors are available makes the selection of proper analytical conditions to obtain an acceptable result for a given analyte, challenging. Often the search for suitable conditions is still a trial-and-error approach which can be extensively time-, money- and labor consuming. To avoid these drawbacks, fast generic chiral separation strategies have been created [7–13]. A fast generic strategy allows developing separations for large sets of substances with different molecular structure in a limited number of experiments.

In this paper we present an overview of such strategies in CE and in HPLC. For the latter technique, strategies in normal-phase liquid chromatography (NPLC), reversed-phase liquid chromatography (RPLC) and polar organic solvent chromatography (POSC) have been defined. These strategies can be considered complementary and are used as alternatives when one fails to separate a given compound.

The first step in the strategies is a screening where the analytes are examined according to a limited number of conditions.

* Corresponding author. Tel.: +32 2 477 4734; fax: +32 2 477 4735.
E-mail address: yvanvdh@vub.ac.be (Y. Vander Heyden).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.12.015

After evaluating the resolution (Rs), which is the most important response for chiral separations, some optimization steps can be considered when desired. When compounds have a Rs > 1.5 after screening, i.e. are baseline separated, usually no further optimization is needed but the strategies allow optimizing responses such as analysis time or peak shape. When 0 < Rs < 1.5 after screening, i.e. when the peaks are only partly separated, some optimization should be performed to increase the resolution. However when Rs = 0 after screening, a limited number of additional experiments will be done but when the separation does not improve, the applied technique or mode is changed.

When peaks become baseline separated (Rs > 1.5) after execution of the additional experiments, again there is the possibility to optimize further if desired. If not, the method development stops here. However, when still no good enantioseparation is achieved, which means that Rs < 1.5, each strategy suggests to switch to another separation technique or mode.

2. Capillary electrophoresis

Capillary electrophoresis is a technique that provides several advantages for chiral separations, such as high efficiency, a fast equilibration time and its ability to add various chiral selectors to the background electrolyte (BGE). Another important advantage of CE is the low consumption of reagents and sample during analysis which makes it possible to test new and/or expensive compounds and selectors.

The proposed separation strategy in CE is based on the use of three highly sulphated cyclodextrins (HSCDs), i.e. HS-α-CD, HS-β-CD and HS-γ-CD [14]. Its general set-up is shown in Fig. 1. This set-up was based on experimental and literature knowledge [7,13]. The selectors are screened at one set of conditions and in the sequence: (1) HS-γ-CD, (2) HS-β-CD and (3) HS-α-CD. For the experimental conditions of the screening step the phosphate buffer has a concentration of 50 mM and is set at a pH of 2.5. The cyclodextrin concentration is 5% (w/v). The applied electric field has a strength of 300 V/cm while the capillary temperature is 25 °C. The sequence of the selectors is based on their observed enantioselectivity. HS-γ-CD is the selector that shows enantioselectivity for most compounds, followed by the β and α forms [14].

Depending on the obtained results, several optimization steps are defined as shown in Fig. 1. The first one is the optimization of the Rs. This step (optimization 1) will be used when the compound is partly but not baseline separated at screening conditions. Depending on the analysis time at screening conditions, two different experimental designs are proposed. If the analysis time is less than 15 min, a 2^3 full factorial design with one central point must be carried out. This means that three factors will be examined at two levels followed by one experiment defined at central conditions. The factors considered are the cyclodextrin concentration that varies from 2.5 over 6.25 to 10% (w/v), the pH of the BGE that is increased from 2.5 to 3.25 and 4 and the percentages of methanol in the BGE which will be added ranging from 0% over 7.5% to 15%.

If the analysis time is more than 15 min, the design is a 2 × 3 factorial design, where the concentration of CD and the pH of the BGE are the factors, the latter examined at two levels (2.5 and 4) while the former is examined at three levels (2.5%, 5% and 10% (w/v)).

For nearly baseline separated peaks, the strategy provides a second optimization possibility, which aims at improving the efficiency and the peak shape (optimization 2). This optimization also applies a 2^3 full factorial design. The voltage (250 V/cm and 350 V/cm), temperature (15 °C and 25 °C) and ionic strength of the BGE (25 mM and 75 mM) are now the factors. For compounds that are baseline separated, a third optimization step (optimization 3) can be executed. The choice exists between a specific migration time optimization (performing a 2^3 full factorial design) and a global optimization (performing a 2^{4-1}

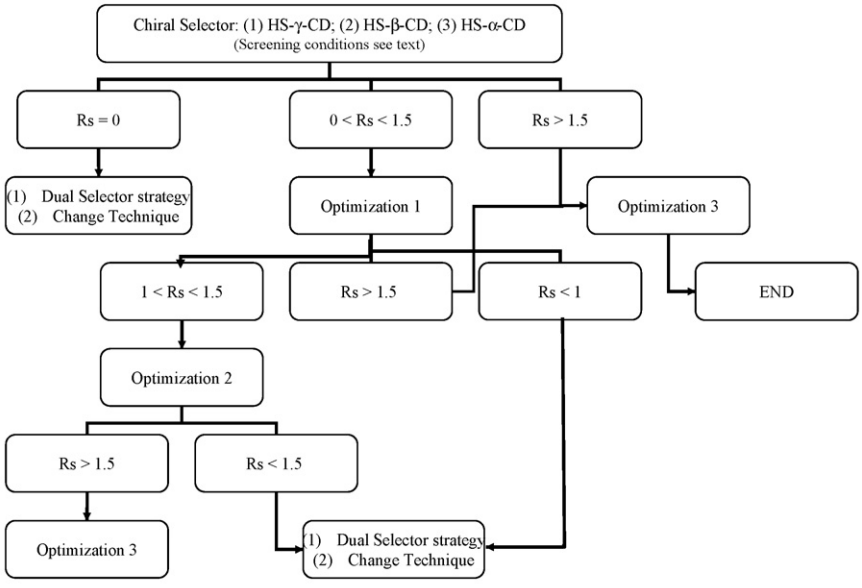

Fig. 1. General CE strategy.