Journal of Chromatography A, 1515 (2017) 109–117

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Chiral stationary phase optimized selectivity liquid chromatography: A strategy for the separation of chiral isomers

Ravindra Suryakant Hegade [a], Maarten De Beer [b], Frederic Lynen [a,*]

[a] Separation Science Group, Department of Organic and Macromolecular Chemistry, Faculty of Sciences, Ghent University, Krijgslaan 281-S4 Bis, B-9000 Ghent, Belgium
[b] AmatsiSEPS, Technologiepark 4, B-9052 Ghent, Belgium

ARTICLE INFO

Article history:
Received 26 April 2017
Received in revised form 21 July 2017
Accepted 24 July 2017
Available online 2 August 2017

Keywords:
Chiral separation
Column coupling
Separation of enantiomers
Stationary phase optimized selectivity liquid chromatography (SOSLC)
High performance liquid chromatography

ABSTRACT

Chiral Stationary-Phase Optimized Selectivity Liquid Chromatography (SOSLC) is proposed as a tool to optimally separate mixtures of enantiomers on a set of commercially available coupled chiral columns. This approach allows for the prediction of the separation profiles on any possible combination of the chiral stationary phases based on a limited number of preliminary analyses, followed by automated selection of the optimal column combination. Both the isocratic and gradient SOSLC approach were implemented for prediction of the retention times for a mixture of 4 chiral pairs on all possible combinations of the 5 commercial chiral columns. Predictions in isocratic and gradient mode were performed with a commercially available and with an in-house developed Microsoft visual basic algorithm, respectively. Optimal predictions in the isocratic mode required the coupling of 4 columns whereby relative deviations between the predicted and experimental retention times ranged between 2 and 7%. Gradient predictions led to the coupling of 3 chiral columns allowing baseline separation of all solutes, whereby differences between predictions and experiments ranged between 0 and 12%. The methodology is a novel tool allowing optimizing the separation of mixtures of optical isomers.

1. Introduction

Most therapeutic drugs contain one or more chiral centers and are used either as discrete enantiomerically pure molecules or as racemic mixtures. Regulatory agencies recommend individual assessment of the activity of each enantiomer for racemic drugs and promote the development of new chiral drugs as single enantiomers [1]. Accordingly, the availability of tools allowing improved separation of all stereoisomers related to the pharmaceutical solutes could be significant both from an analytical and a preparative point of view. High performance liquid chromatography (HPLC) and Supercritical fluid chromatography (SFC) employing chiral stationary phases (CSPs) are broadly used for the separation of mixtures of enantiomers. Although many types of stationary phases have been commercialized, the polysaccharide phases based on derivatized cellulose and amylose are mostly employed. The versatility and reliability of such phases derives from the isocyanate based derivatization of the hydroxyl functions on the saccharide, leading to the incorporation of aromatic groups containing varying side group functionalities on the carbohydrate backbone. This increases the chiral selectivity, the applicability and also the stability of such stationary phases. The various commercialized sets of polysaccharide columns allow for automated screening for the most effective separation of chiral pairs, whereby for typical small molecule drugs successful separation can usually be obtained on at least one of the stationary phases, providing various mobile phase compositions are thereby screened as well [2]. Although this strategy is successful and broadly applied, it is aimed at finding the columns providing the best separation of one chiral pair at a time and it fails to ensure that the most suitable conditions for the separation of a mixture of chiral pairs or of a mixture of all stereoisomers of therapeutic agent has been indeed been found. A solution to this problem can be found through the coupling of columns containing different chiral phases or it can also be addressed via the combination of both chiral and achiral columns.

However, thus far the reported coupled columns approaches including chiral phases have been exclusively employing trial and error strategies. In this way Kristensen et al. combined achiral normal and reversed phase columns with a chiral glycoprotein type of column for the separation of the enantiomers of methadone and metabolites thereof in serum and urine [3,4]. Armstrong and coworkers coupled two whelk type columns whereby the selectiv-

* Corresponding author.
E-mail address: frederic.lynen@ugent.be (F. Lynen).

http://dx.doi.org/10.1016/j.chroma.2017.07.078
0021-9673/

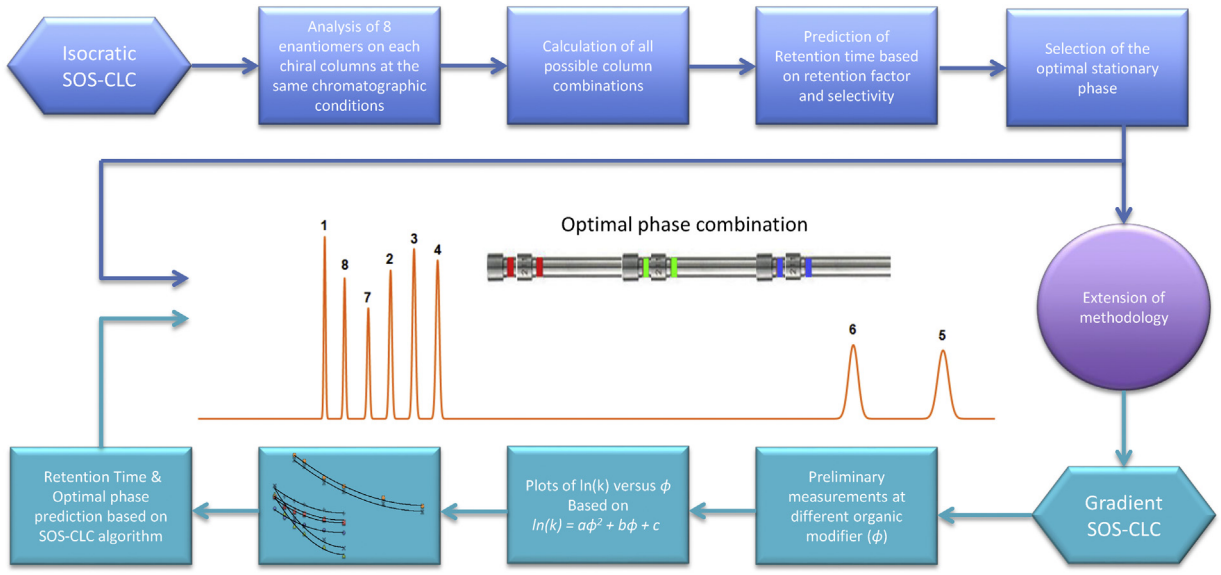

Fig. 1. Organogram depicting the general strategy regarding both the isocratic and the gradient chiral Stationary-Phase Optimized Selectivity Liquid Chromatography approaches.

ity of enantiomers of alkoxy substituted esters of phenylcarbamic acid could be optimized through adjustment of the composition of a make-up flow added before the second column [5]. Lindner and coworkers combined a chiral anion-exchanger CSP with reversed phase for the separation of the enantiomers of thyroxine and of triiodothyronine [6]. The chiral separation of the enantiomers of homocysteine, cysteine, and methionine has, for example, also been reported whereby a C18 phase was coupled with a chirobiotic TAG column [7].

As an alternative to these ad hoc methods, the translation of the Stationary phase Optimized Selectivity Liquid chromatography (SOSLC) towards chiral separations, offers a novel perspective as it allows for rational design of the separation whereby individual peaks can, under ideal circumstances, literally be positioned in the chromatograms according to the user's wishes. As shown in Fig. 1, this strategy predicts the retention of the solutes of a mixture on any possible combination of columns providing the retention on the individual phases is known. As the number of column combinations rapidly increases when a number of stationary phases and segment lengths are at once disposal, the methodology is ideally suited for in silico based prediction of retention times and automated ranking thereof according to e.g. the resolution of the critical pair for the shortest analysis time [8,9]. Alternatively ranking can also be performed in such a way that one peak is maximally separated from its neighbors for improved preparative purification [10]. The SOSLC methodology, which was originally developed for isocratic reversed phase analysis, was commercialized in 2005 [11]. The approach has since its inception been improving in various ways, whereby now algorithms are available for gradient analysis, [12,13] it has been shown that the methodology can be used on conventional HPLC columns [14] and the concepts also proved applicable on compressible phases as used in supercritical fluid chromatography [10]. As thus far no normal phase HPLC approaches have been reported employing the SOSLC approach and as there is need for improved tools for the resolution of complex chiral mixtures, the potential of the methodology is studied in this work on all possible combinations of up to 5 widely available commercial chiral columns. Both the isocratic and the gradient variants were investigated allowing broad assessment of chiral SOSLC.

2. Experimental

2.1. Chemicals and reagents

The racemates of *trans*-stilbene oxide (TSO), hexobarbital (HXL) and 4-phenyl-1,3-dioxane (4PD) were purchased from Chimica (Geel, Belgium), Sigma–Aldrich (Steinheim, Germany) and TCI (Zwijndrecht, Belgium). 1,2,3,4-tetrahydro-1-napthol (THN) and (*S*)-(+)-1,2,3,4-Tetrahydro-1-naphthol and HPLC grade hexane and 2-propanol were also obtained from Sigma–Aldrich. HPLC grade ethanol was purchased from Acros Organics (Geel, Belgium). The concentration of the stock solutions of TSO, THN, HXL and 4PD were 10 mg/mL (in hexane), 2 mg/mL (in hexane), 5 mg/ mL (in ethanol) and 2 mg/mL (in ethanol), respectively. The above stock solutions of TSO, TPN, HXL and 4PD were further diluted with hexane to a final concentration of 100 μg/mL, 400 μg/mL, 500 μg/mL and 400 μg/mL respectively, such as to obtain solutions containing the individual solutes, the racemates or a mixture of the eight solutes.

2.2. Instrumentation

An Agilent 1260 HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler, quaternary pump, solvent degasser, column oven and a variable wavelength detector (VWD) was used for all analyses. Five Phenomenex (USA) LUX columns (50 × 4.6 mm column with 3 micrometer particles) were used containing the amylose 2, cellulose 1, cellulose 2, cellulose 3 and the cellulose 4 phases. A short pre-column (4 × 3.0 mm) packed with the Amylose 2 was used before all columns. The mobile phases were composed of hexane and 2-propanol. Detection was performed at 210 nm for the isocratic and at 220 nm for the gradient measurements. The column oven was set at 25 °C, injection volumes comprised 2 μL, and in all analyses the flow rate was set at 0.5 mL/min. A system dwell time of 124.2 s was measured as a necessary requirement allowing correct prediction of the retention times. As not all solutes were available as enantiomerically pure standards, pure enantiomers were obtained by preliminary semi-preparative separation of 10 μL injections of 1000 μg/mL solutions on the used analytical columns. The absolute configuration of each solute was confirmed by optical rotation measurements on a Polarimeter (Perkin-Elmer 241, Germany) of