Journal of Chromatography A, 1467 (2016) 463–472

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Comparison of liquid and supercritical fluid chromatography mobile phases for enantioselective separations on polysaccharide stationary phases

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ARTICLE INFO

Article history:
Received 25 April 2016
Received in revised form 13 June 2016
Accepted 17 June 2016
Available online 18 June 2016

Keywords:
Chemometrics
Enantiomer separation
Polysaccharide chiral stationary phase
Solvation parameter model
Supercritical fluid chromatography

ABSTRACT

Analysis and production of enantiomerically pure compounds is a major topic of interest when active pharmaceutical ingredients are concerned. Enantioselective chromatography has become a favourite both at the analytical and preparative scales. High-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) are dominating the scene and are often seen as complementary techniques. Nowadays, for economic and ecologic reasons, SFC may be preferred over normal-phase HPLC (NPLC) as it allows significant reductions in solvent consumption. However, the transfer of NPLC methods to SFC is not always straightforward.

In this study, we compare the retention of achiral molecules and separation of enantiomers under supercritical fluid (carbon dioxide with ethanol or isopropanol) and liquid normal-phase (heptane with ethanol or isopropanol) elution modes with polysaccharide stationary phases in order to explore the differences between the retention and enantioseparation properties between the two modes. Chemometric methods (namely quantitative structure-retention relationships and discriminant analysis) are employed to compare the results obtained on a large set of analytes (171 achiral probes and 97 racemates) and gain some understanding on the retention and separation mechanisms. The results indicate that, contrary to popular belief, carbon dioxide – solvent SFC mobile phases are often weaker eluents than liquid mobile phases. It appears that SFC and NPLC elution modes provide different retention mechanisms. While some enantioseparations are unaffected, facilitating the transfer between the two elution modes, other enantioseparations may be drastically different due to different types and strength of interactions contributing to enantioselectivity.

1. Introduction

The production of enantiopure chiral drugs has become a significant concern over the past decades, ever since the awareness of the different bioactivities of enantiomers was raised [1]. While chiral synthesis producing only the target enantiomer is desirable, the development of such methods can be long. On the opposite, enantioselective chromatographic methods are rapidly developed: with current screening strategies offering very short gradient elution and parallel elution on up to 8 columns, suitable chromatographic conditions are often found in barely more than an hour [2]. Furthermore, chiral chromatographic methods are easily scaled up, and are thus useful at all stages of the development of a chiral drug [3]:

(i) At the earliest stages, when small quantities of all stereomers are necessary to evaluate their biological activity.
(ii) During the development of chiral synthesis methods, and when such methods are finally in operation, to evaluate the enantiopurity of the synthetic product.

While enantioselective HPLC was long the preferred method for these tasks, supercritical fluid chromatography (SFC) is now taking the lead [4–8]. This is due to many benefits associated to the use of pressurized carbon dioxide as the major component of the mobile phase. First, CO_2 has a much lower cost than the organic solvents composing the largest portion of normal-phase HPLC mobile phases (hexane or heptane). While the solvent economy at the analytical scale is scarce, it is much more significant at the preparative scale, when dozens of liters of solvent may be used to purify a single enantiomer in sufficient quantities for bioactivity testing. Aside solvent costs, solvent disposal costs also impact the overall economy. Secondly, because CO_2 and co-solvent are separated after the

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http://dx.doi.org/10.1016/j.chroma.2016.06.060
0021-9673/

chromatographic process (CO_2 returns to the gas phase and may be recycled), SFC purified fractions are recovered in the liquid co-solvent and are inherently more concentrated than HPLC fractions. The time and energy expenses to evaporate the solvent and obtain a dry product are thus again lower in SFC. Last but not least, health and safety issues are lessened when CO_2 replaces toxic hexane or heptane, the major solvents used in normal-phase enantioselective HPLC [9].

There are also cases when SFC is preferred to screen for chiral stationary phases, because column equilibration is faster than in HPLC, but the method for large-scale preparative purposes is desired in a liquid phase thus some transposition is necessary [10].

In this paper, we wished to compare the features of enantioselective normal-phase HPLC and SFC with polysaccharide stationary phases, as the latter are the most widely used both for analytical and preparative purposes [11]. One characteristic that is often cited to support the transfer of HPLC methods to SFC is the supposed speed advantage of the latter, due to lower viscosity of the mobile phase allowing for higher flow rates [12–14]. This speed advantage is normally expected to yield increased productivity at the preparative scale, so this point was of principal interest. Besides, the different viscosities usually resulting in higher efficiencies with CO_2-based mobile phases than with conventional liquids, supposedly larger resolution values should be obtained in SFC. This point can be advantageous both at the analytical and the preparative scales, and was our second major interest. Finally, because the replacement of a non-polar liquid alkane (hexane or heptane) by the non-polar pressurized carbon dioxide is usually believed to cause no significant changes in retention and separation behavior, a third aim was the assessment of interactions contributing to retention and enantioseparation in SFC and NP-HPLC modes to illustrate possible differences. For this purpose, quantitative structure-retention relationships (QSRRs) and discriminant analysis (DA) were employed, based on the analysis of a large set of achiral and chiral analytes on a cellulose tris-(3,5-dimethylphenylcarbamate) stationary phase coated on silica.

2. Material and methods

2.1. Stationary phases

The columns selected for this study were all polysaccharide stationary phases: Chiralcel AD-H and Chiralpak IC (Daicel Corp., Japan), with column dimensions 100 mm × 4.6 mm, 5 μm, and Lux Cellulose-1 (Phenomenex, Le Pecq, France) with column dimensions 250 mm × 4.6 mm, 5 μm.

2.2. Chemicals

At Novartis, the solvents used were HPLC-grade ethanol (EtOH) provided by VWR International GmbH (Dietikon, Switzerland), isopropanol (iPrOH) and n-heptane (HPT) provided by Sigma Aldrich Chemie GmbH (Buchs, Switzerland). Carbon dioxide was provided by Carabagas AG (Basel, Switzerland). The enantiomeric pairs were (1) Troeger's base, (2) binaphthol, (3) mandelic methylester, (4) trans-stilbene oxide, (5) flavanone and (6) guaifenesine (Fig. 1).

At Univ Orleans, the solvents used were HPLC-grade ethanol, isopropanol and heptane provided by VWR (Fontenay-sous-Bois, France). Carbon dioxide was provided by Messer (Puteaux, France). Solutions of all probe analytes were prepared in MeOH. For chemometric analyses, 171 achiral compounds were obtained from a range of suppliers. The majority of the 97 racemates were from commercial sources while a small proportion of them were in-house synthesized products whose formulas are confidential.

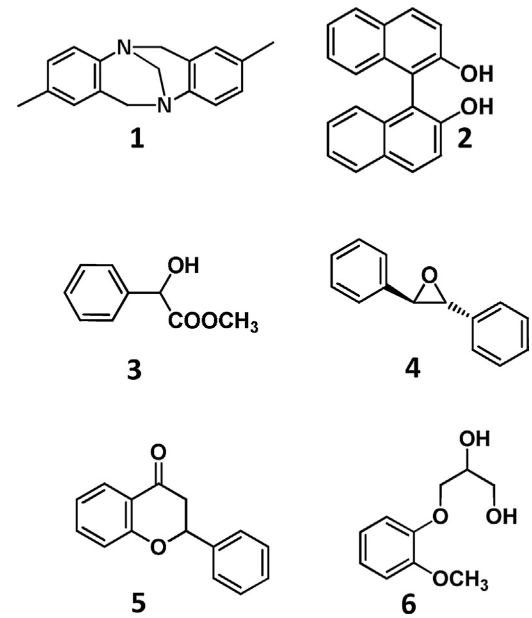

Fig. 1. Structure of the six racemates employed for retention, selectivity and resolution comparisons. (1) Troeger's base, (2) binaphthol, (3) mandelic methylester, (4) trans-stilbene oxide, (5) flavanone and (6) guaifenesine.

Abraham solute descriptors (E, S, A, B and V) used for chemometric analyses were extracted from an in-house database established from all available literature on the solvation parameter model, or, for those compounds that cannot be found in the literature, calculated with the Absolv Webboxes program, based on ADME Boxes version 3.5 (Pharma Algorithms, ACD Labs, Toronto, Canada). Additional descriptors (F and G) were computed in-house with MOE 2009.10 (Chemical Computing Group, Montreal, Canada), and QikProp 2009/08/20 (Schrödinger), as described in previous works [15,16]. The complete tables of analytes and descriptors can be found in supplementary information (Table S1 for achiral analytes and Table S2 for chiral analytes).

2.3. Apparatus and conditions

2.3.1. HPLC analyses

At Novartis, HPLC analyses were conducted on an Agilent 1200 HPLC system (Agilent Technologies AG, Basel, Switzerland).

At Univ Orleans, HPLC analyses were conducted on a LaChrom Merck Hitachi system with L-7100 pump, L-7200 automatic injector, column oven, 5430 Hitachi Chromaster diode-array detector and Sedex 75 evaporative light-scattering detector (Sedere, Orléans, France).

Operating conditions in both cases were as follows: heptane-alcohol 90:10 (v/v), 1 mL/min, 25 °C.

2.3.2. SFC analyses

At Novartis and Univ Orleans, Waters Acquity UltraPerformance Convergence Chromatography™ (UPC^2) systems were used. Operating conditions were as follows: CO_2-alcohol 90:10 (v/v), 25 or 40 °C and 150 bar outlet pressure, with varied flow rates as specified in the main text.

Injection volume was 1 μL for all compounds. Retention factors (k) were calculated based on the retention time t_R, determined using the peak maximum and on the hold-up time t_0 measured on the first negative peak due to the unretained sample solvent.