Journal of Pharmaceutical and Biomedical Analysis 74 (2013) 1–13

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Enantioselectivity of polysaccharide-based chiral selectors in polar organic solvents chromatography: Implementation of chlorinated selectors in a separation strategy

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ARTICLE INFO

Article history:
Received 27 June 2012
Received in revised form
18 September 2012
Accepted 22 September 2012
Available online 17 October 2012

Keywords:
Chiral separations
Polysaccharide-based chiral selectors
Polar organic solvents chromatography
Chlorinated selectors

ABSTRACT

The enantioselectivity of polysaccharide-based chiral stationary phases in polar organic solvents chromatography (POSC) is investigated using both chlorinated and non-chlorinated selectors. A test set of 58 drug compounds was analyzed at the screening conditions of a previously developed separation strategy. Eight stationary phases were screened with eight mobile phases, resulting in 64 chromatographic systems. After selection of the two most successful mobile phases, a preferred testing sequence for the polysaccharide selectors was determined, based on their success rate and complementarity. These stationary phases, screened in the sequence Lux Cellulose-2 > Chiralcel OD-RH > Lux Cellulose 4 > Sepapak-5, each with two mobile phases, showed 48/58 separations (83% of the test set). An attempt was also made to update the existing separation strategy. The existing screening conditions were found to be easily applicable on stationary phases with chlorinated selectors, but the optimization steps appeared to be less successful.

1. Introduction

In the pharmaceutical industry, much emphasis is put on chiral analysis. The reason is the potentially different behavior of the enantiomers of a chiral drug molecule after administration. To cause a therapeutic effect, an administered molecule has to interact with a target receptor. For chiral drug molecules only one enantiomer (the eutomer) will fit properly into this receptor, resulting in the desired therapeutic effect. The other enantiomer (the distomer) can either not interact or can interact less intense with the receptor, which generally causes a lower effect. Occasionally the distomer interacts with other receptors, causing side- or even toxic effects. As a consequence, the enantiomers of drug candidates must be subjected to supplementary investigations during development processes: the eutomer has to be distinguished from the distomer during identification and impurity determinations of the drug substance. For drug products, it should be confirmed that the eutomer is present in the required dose while the distomer level should be analyzed as impurity, as prescribed in the guidelines imposed by the International Conference on Harmonisation (ICH), more precisely in guideline Q6A (decision tree number 5) [1,2]. These guidelines are adopted by the European Medicine Agency (EMA) [3] and by the US Food and Drug Administration (FDA) [4].

Generally, the preferred approaches for chiral separation are the direct methods. This is achieved either by adding a selector to the mobile phase or by introducing, through coating or covalent immobilization, a chiral selector to a chromatographic matrix, and thus creating a chiral stationary phase (CSP). Consequently, these CSPs made liquid chromatography and supercritical fluid chromatography the most applied enantioseparation techniques, especially in an industrial context where both analytical- and preparative-scale separations are needed [5–9].

Different types of chiral entities have been used as selector on CSPs (e.g. Pirkle-type selectors, ligand exchangers, ion exchangers, cyclodextrins, glycopeptide antibiotics, proteins and crown-ether derivatives), but derivatized polysaccharides are by far the most popular [10]. They appear to have the broadest enantioselectivity toward very diverse compounds [11–15]. Amylose- and cellulose-derived selectors have been investigated intensively for several decades. The difference between the two polysaccharide-backbones is situated in the linkage of the glucose units: for amylose it is an α-1,4-glycosidic linkage and for cellulose a β-1,4-glycosidic linkage. These linkages result in helical structures of the polymers. These higher order structures are differently arranged, depending on the polysaccharide type and their substituents [16–18]. The differences in spatial arrangement of the

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http://dx.doi.org/10.1016/j.jpba.2012.09.025

chiral cavities that arise from these helical formations, as well as the substituents on the polysaccharide units, will lead to differences in enantioselectivity. Di-substitution of the phenyl moiety of the derivatized polysaccharide, with electron-donating groups (mainly methyl groups), with electron-withdrawing groups (halogens), or with both, showed an improvement of the overall enantioselectivity. The benefit of these substituents in different positions has led to the development of new polysaccharide-based chiral stationary phases [19–26].

This study will compare the enantioselectivity of two groups of polysaccharide-based CSPs: one with selectors containing only methyl groups and a second that contains chlorinated selectors. The first group contains the selectors amylose *tris*(3,5-dimethylphenylcarbamate), cellulose *tris*(3,5-dimethylphenylcarbamate), amylose *tris*[(S)-α-methylbenzylcarbamate] and cellulose *tris*(4-methylbenzoate); the second cellulose *tris*(3-chloro-4-methylphenylcarbamate)-, amylose *tris*(5-chloro-2-methylphenylcarbamate)-, cellulose *tris*(4-chloro-3-methylphenylcarbamate)- and cellulose *tris*(3,5-dichlorophenylcarbamate) selectors. The enantioselectivities of these selectors in polar organic solvents chromatography (POSC) [27–31], an HPLC mode where the mobile phase consists only of polar organic solvents and which is sometimes also called polar non-aqueous mode, were compared. Mobile phases used are derived from a separation strategy defined earlier [31] and contain either acetonitrile or methanol as main solvent and small amounts (0.1%, v/v) of both an acidic additive, trifluoroacetic acid, and a basic additive, diethylamine. Some mobile phases additionally contain 5% (v/v) of a short-chained alcohol. For optimization purposes the mobile phases were also modified with other additives, i.e. isopropylamine, acetic acid and formic acid. Analyses are performed at 20 °C, but also at 15 °C and 5 °C. After analyzing all results, an attempt was made to update the strategy. The goal is that the updated strategy should remain applicable on a broad range of compounds and result in a higher success rate.

2. Experimental

2.1. Chemicals and reagents

2.1.1. Chiral test set

The test set consists of 58 racemates. The compounds were chosen based on availability in the lab and differences or similarities in molecular and pharmacological properties. The racemic compounds are acebutolol, alprenolol, atenolol, atropine (n = 4), betaxolol, chlorthalidone, ephedrine (n = 2), fenoprofen, ibuprofen, ketoprofen, labetalol (n = 2), mandelic acid, nadolol (n = 3), naproxen, naringenin, oxazepam, pindolol, praziquantel, promethazine, sulpiride, suprofen, tetramisole and warfarin (all from Sigma–Aldrich, Steinheim, Germany), acenocoumarol and dimethindene (from Novartis, Basel, Switzerland), nimodipine, nisoldipine and nitrendipine (Bayer, Leverkusen, Germany), leucovorin (n = 2) and oxprenolol (Cynamid Benelux, Brussels, Belgium), propranolol and verapamil (Fluka, Neu-Ulm, Switzerland), ambucetamide (Janssen Pharmaceutica, Beerse, Belgium), bopindolol (Sandoz, Holskirchen, Germany), carvedilol (Boehringer, Mannheim, Germany), esmolol (Du Pont de Nemours, Saconnex, Switzerland), flurbiprofen (ICN Biomedicals, Ohio, USA), mebeverine (Duphar, Amsterdam, the Netherlands), metoprolol (Astra Hassle AB, Lund, Sweden), nicardipine (UCB, Brussels, Belgium), sotalol (Merck, Darmstadt, Germany), terbutaline (Astra Draco, Lund, Sweden), bupranolol, carazolol, salbutamol, salmeterol, bisoprolol, methadone, carbinoxamine, chlorphenamine, hexobarbital, isothipendyl, mepindolol, meptazinol, mianserin, propiomazine, procyclidine and tertatolol were gifts from different sources.

As indicated above (n = 2, 3 or 4), some compounds have more than one asymmetric center which implies 2^n possible stereoisomers (with n the number of asymmetric centers). In practice, they consist of enantiomers, diastereomers and isomers with internal symmetry axes. Separations of these compounds already have been developed with different techniques and according to the literature, only two stereoisomer peaks can be expected for atropine (n = 4) [32], ephedrine (n = 2) [33] and leucovorin (n = 2) [34], while four stereoisomers are expected for labetalol (n = 2) [35] and nadolol (n = 3) [35].

All compounds were dissolved in the main solvent of the mobile phase, i.e. acetonitrile (ACN) or methanol (MeOH) (both HPLC-grade, from Fisher Scientific, Loughborough, Leicestershire, UK). When solubility problems occurred, mixtures of 50/50 (v/v) acetonitrile/methanol were used, except for leucovorin, which was dissolved in MilliQ-water (in-house prepared with a Millipore MilliQ-System, Milford, MA, USA).

The concentration of the samples was about 250 μg ml^{-1}. Dissolution of the samples was accelerated by placing them in an ultrasonic bath (Branson 5210, Danbury, CT, USA).

2.1.2. Mobile and stationary phases

Isopropanol (IPA), ethanol (EtOH) and butanol (ButOH) (all HPLC-grade from Fisher Scientific) were also used as mobile-phase components. Diethylamine (DEA) and trifluoroacetic acid (TFA) (both from Sigma–Aldrich, Steinheim, Germany) were used as basic and acidic mobile phase additives, respectively. Acetic acid (Merck), formic acid and isopropylamine (both Sigma–Aldrich) were also tested. The non-chlorinated CSPs that are used are commercialized as Chiralpak® AD-RH, Chiralcel® OD-RH, Chiralpak® AS-RH and Chiralcel® OJ-RH (all from Chiral Technologies Europe (Illkirch, France)), containing amylose *tris*(3,5-dimethylphenylcarbamate), cellulose *tris*(3,5-dimethylphenylcarbamate), amylose *tris*[(S)-α-methylbenzylcarbamate] and cellulose *tris*(4-methylbenzoate), respectively, as selectors. The particle size of all columns was 5 μm with column dimensions of 15 cm × 0.46 cm and 25 cm × 0.46 cm. The screening experiments have been performed on columns with both dimensions.

In addition, four chlorinated CSPs were tested. These stationary phases use cellulose *tris*(3-chloro-4-methylphenylcarbamate), amylose *tris*(5-chloro-2-methylphenylcarbamate), cellulose *tris*(4-chloro-3-methylphenylcarbamate) and cellulose *tris*(3,5-dichlorophenylcarbamate) as selectors. They were initially commercialized as Sepapak®-2, Sepapak®-3, Sepapak®-4 and Sepapak®-5, respectively, and were purchased from Sepaserve (Münster, Germany). In the meantime, the names of some columns were changed: Sepapak®-2 became Lux™ Cellulose 2, Sepapak®-3 changed to Lux™ Amylose 2 and Sepapak®-4 was renamed to Lux™ Cellulose 4 [36]. They are now commercialized by Phenomenex® (Torrance, CA, USA). The columns all have dimensions of 25 cm × 0.46 cm and particle sizes of 5 μm. An overview of the used mobile and stationary phases is given in Table 1.

2.2. Chromatographic conditions

The chromatographic system is a Merck-Hitachi HPLC system (Tokyo, Japan) consisting of a L-7100 pump, a programmable autosampler L-7200 with a 100 μl loop, an L-7400 UV detector, a D-7000 interface and an L-7360 column oven. All chromatographic data were managed with the D-7000 HPLC System Manager software (Merck-Hitachi, 1994–2001, version 4.1). The injection volume was 5 μl. Isocratic elution was performed with a flow rate of 0.5 ml min^{-1}, following the conditions of the original strategy. The compounds were analyzed at a column temperature of 20 °C and detected at 220 nm.