Journal of Chromatography A, 1216 (2009) 5140–5146

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Enantioseparation of paroxetine intermediate on an amylose-derived chiral stationary phase by supercritical fluid chromatography

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ARTICLE INFO

Article history:
Received 30 January 2009
Received in revised form 20 April 2009
Accepted 22 April 2009
Available online 3 May 2009

Keywords:
Enantioseparation
Supercritical fluid chromatography
Paroxetine intermediate
Chiralpak AD

ABSTRACT

The enantioseparation of *trans*-3-ethoxycarbonyl-4-(4′-fluorophenyl)-1-methyl piperidine-2,6-dione (**3**), which is one of the important racemic precursors of *trans*-(−)-paroxetine, has been investigated using supercritical fluid chromatography on a Daicel Chiralpak AD column. Supercritical CO_2 modified with methanol, ethanol and 2-propanol were used as mobile phase. The influence of type and concentration of alcohol modifier on retention factor, enantioselectivity and resolution were studied. Among methanol, ethanol and 2-propanol, 2-propanol was proved to be the most favorable modifier, and 9.5% (v/v) of 2-propanol was the preferred concentration at which racemate **3** could be separated with resolution of 15.86 and retention factor of 6.323. The effects of pressure and temperature were investigated at 9.5% (v/v) of 2-propanol in the pressure range of 12–24 MPa and temperature range of 303.15–318.15 K. It was found that the lower pressure and temperature were favorable to the enantioseparation. Using van't Hoff plot, the isoenantioselective temperature was calculated to be 410 K. The enantioseparation process was "enthalpically driven" under experimental conditions. Finally, the retention factors were satisfactorily correlated by a simplified lattice–fluid model with average absolute relative deviation (AARD%) of both enantiomers smaller than 1.76%.

1. Introduction

trans-(−)-Paroxetine (**1**, Fig. 1), (3*S*, 4*R*)-3-[(1,3-benzodioxol-5-yloxy)methyl]-4-(4′-fluoro-phenyl)piperidine, is a selective 5-hydroxytryptamine (5-HT) reuptake inhibitor currently used as an antidepressant [1], which is used in the treatment of social anxiety disorder, obsessive compulsive disorder, panic disorder, and generalized anxiety disorder [2]. Clinical studies show that the drug is as effective as tricyclic antidepressants, but has much less side effect [3,4]. The molecular structure of paroxetine contains two chiral centers in the piperidine ring, resulting in two pairs of enantiomers, i.e., the *cis* and *trans* forms.

Most of the manufacturing routes developed towards the preparation of this compound, marketed as a single enantiomer, involve the key intermediate, racemic mixtures of *trans*-4-(4′-fluorophenyl)-3-hydroxymethyl-1-methylpiperidine (**2**), which can be obtained from *trans*-3-ethoxycarbonyl-4-(4′-fluorophenyl)-1-methylpiperidine-2,6-dione (**3**) by a reduction step (Fig. 1). The optically pure enantiomers of **2** and **3** can be obtained by chiral resolution using a biocatalytic method [5] or diastereoisomeric crystallization of salts with chiral acids such as (−)-di-*p*-toluoyltartaric acid or (+)-2′-nitrotartranilic acid [6,7]. However, such enantioseparation processes are time-consuming and uneconomical. So, the cost of production can be considerably reduced if racemate **3** is resolved prior to performing the reduction step [8].

Historically, high-performance liquid chromatography (HPLC) has been one of the most widely used techniques in the area of chiral separation. However, more recently, supercritical fluid chromatography (SFC) has emerged as a powerful alternative and in some cases as a complementary technique [9–11]. Supercritical fluids, especially supercritical CO_2, possess properties that are intermediate between those of liquids and gases, and the tunability of these properties by a proper choice of operating conditions. The higher diffusivity as compared to those of liquids results in better separation efficiencies, and the lower viscosity offers lower pressure drops at preparative conditions where columns are usually operated at higher flow rates [12]. The specific properties of supercritical CO_2 have made it good candidate for use as mobile phase in chromatographic applications.

To our knowledge, the SFC enantioseparation of racemic compound **3** has not previously been published. The aim of this work is to study the enantioseparation of this racemic compound using chiral SFC. Among the variety of commercially available chiral stationary phases (CSPs), polysaccharide derivatives are chiral selectors with a wide range of applicability in SFC. In particular, 3,5-dimethylphenylcarbamates of amylose (Chiralpak AD) is one of the most popular and successful CSPs, achieving a high efficiency for a broad-spectrum of racemic compounds [12–18].

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doi:10.1016/j.chroma.2009.04.069

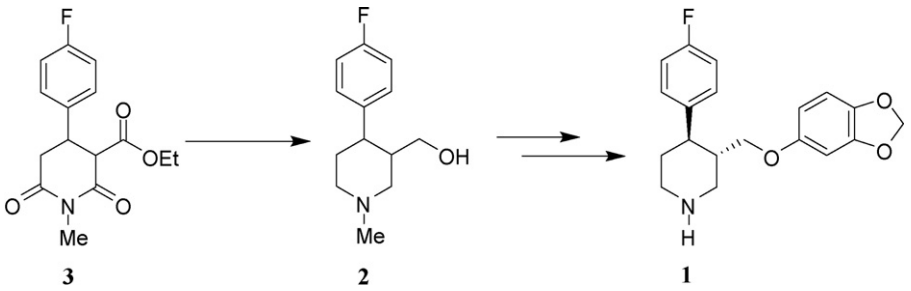

Fig. 1. Synthetic pathway of *trans*-(−)-paroxetine (**1**).

In this work, a study of enantioseparation of racemic compounds **3** on Chiralpak AD by SFC is presented. The effects of modifier, pressure and temperature on retention factor, enantioselectivity and resolution were evaluated. A simplified lattice model was used to describe the effect of density and temperature on retention factor.

2. Experimental

2.1. Materials

Carbon dioxide with purity of 99.99% was purchased from Minxing Gas Co. (Hangzhou, China). Chromatographic grade methanol and 2-propanol were obtained from Tedia (Fairfield, OH, USA). Analytical grade anhydrous ethanol was obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). The individual *trans*-(+)-(3*R*, 4*S*) and racemic compound **3** were kindly supplied by Zhejiang Chiral Medicine Chemicals Co. (Hangzhou, China). 1,3,5-Tri-*tert*-butylbenzene (TTBB) was purchased from Aldrich (Milwaukee, WI, USA). The racemate **3** sample was prepared in methanol with a concentration of 0.596 mg/mL. All materials were used without further purification.

2.2. Instrumentation

The supercritical fluid chromatograph from JASCO (Hachioji, Japan) equipped with a PU-2080-CO_2 carbon dioxide pump, a PU-2080 modifier pump, a CO-2065 column oven, an AS-2059-SF autosampler with a 5 μL loop, a UV-2075 UV–Vis detector, a BP-2080 backpressure regulator and a LC-Net II/ADC data collector. The system was controlled by ChromNAV 1.09.02 software.

The Chiralpak AD column (250 mm × 4.6 mm, 5 μm) was packed with amylose tris(3,5-dimethylphenylcarbamate) coated on a silica gel support, and obtained from Daicel (Tokyo, Japan).

2.3. Chromatographic method

The mobile phase investigated in this work was methanol, ethanol or 2-propanol modified supercritical CO_2. When the modifier, pressure or temperature was changed, the equilibration time was not less than 15 min and the baseline was monitored to confirm the shift. The detection wavelength was set at 220 nm. The injection volume was 5 μL.

Retention factor k was calculated from Eq. (1)

$$k = \frac{(t_R - t_0)}{t_0} \quad (1)$$

where t_R is the retention time measured at peak maximum, each retention time of enantiomers was the mean values of not less than two replicate experiments; t_0 is the dead time.

The value of resolution R_s was given by the software and calculated according to Eq. (2)

$$R_s = \frac{2(t_{R2} - t_{R1})}{(w_1 + w_2)} \quad (2)$$

where w is the peak width at the base of the peak, subscripts 1 and 2 represent the former and the latter eluted enantiomers of compound **3**, respectively.

3. Results and discussion

Since the mobile phase flow rate has essentially no influence on the enantioselectivity for a particular chiral stationary phase and compound, the flow rate in all experiments was selected to be 2.0 mL/min based on the results of our pre-experiments.

In the following study, the pressure values were all measured behind the column. The modifier concentrations were calculated based on the flow rates of CO_2 and modifier at the pump head, which were measured at 263.15 K and room temperature, respectively. The dead time has been determined by TTBB at different flow rates using 9.5% (v/v) 2-propanol as modifier. It was found that TTBB was eluted just after the first baseline perturbation, and the difference between their retention times was smaller than 2%. For convenience, the dead time was determined by the retention time of the first baseline perturbation.

3.1. Effect of modifier

Though the solubility of racemic compound **3** in supercritical CO_2 determined by a dynamic method is about 15–18 mg/mL at 15 MPa and 308.15 K [19], it cannot be eluted by pure supercritical CO_2 under this condition even the elution time is up to 60 min. This may be due to the stronger interaction of the polar sites of compound **3** with the stationary phase than with the nonpolar CO_2. Therefore, the appropriate modifier is necessary for the enantioseparation of racemic compound **3**.

Alcohols are the most popular modifier used in chiral SFC. According to literature [20], the alcohol modifiers can not only compete with the solute for hydrogen bonding to the carbamate of CSP, but also cause changes of the steric environments of the chiral cavity by being adsorbed to the chiral cavities or achiral polar function groups, affecting the retention of enantiomers and the selectivity of enantioseparation. Since methanol, ethanol and 2-propanol are benign solvents to solubilize compound **3** because of their strong hydrogen bond donating ability, they are selected as modifiers in this study.

A variety of mobile phase compositions were investigated by changing the type and concentration of alcohol modifiers. The experimental data are listed in Table 1. It was shown that racemic compound **3** can well be enantioseparated with resolutions higher than 5.3 at all mobile phase compositions. The enantioseparation of racemic compound **3** on Chiralpak AD by HPLC using hexane modified with ethanol or 2-propanol as mobile phase has been reported