Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1141 (2007) 287–294
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Ketopinic acid and diisoproylideneketogulonic acid as chiral ion-pair selectors in capillary electrophoresis Enantiomeric impurity analysis of *S*-timolol and 1*R*,2*S*-ephedrine

Ylva Hedeland, Jenni Lehtinen, Curt Pettersson *

Division of Analytical Pharmaceutical Chemistry, Uppsala University, Biomedical Centre, Box 574, SE-751 23 Uppsala, Sweden

Received 16 May 2006; received in revised form 30 November 2006; accepted 1 December 2006
Available online 21 December 2006

Abstract

1*S*,4*R*-(+)-ketopinic acid [(+)-KPA] has been introduced as a chiral selector for the separation of pharmacologically active amines by non-aqueous capillary electrophoresis (NACE). (+)-KPA gave enantioresolution for most of the compounds previously separated by 2*R*,3*S*,4*R*,5*S*-(−)-2,3:4,6-di-*O*-isopropylidene-2-keto-L-gulonic acid [(−)-DIKGA], but with a reversed migration order. A complete enantioresolution (Rs = 4.2) was obtained for timolol, a compound that could not be resolved using (−)-DIKGA as the selector. Thus, (+)-KPA was evaluated for the enantiomeric purity determination of *S*-timolol. A method based on pre-concentration by transient isotachophoresis (tITP) provided a limit of detection (LOD) of 0.2% *R*-timolol in *S*-timolol samples. Because of the lack of enantioresolution of ephedrine when (+)-KPA was used as the selector, a method with (−)-DIKGA has been developed and validated for determination of the enantiomeric purity of the 1*R*,2*S* enantiomer. The method gave good precision and accuracy with an LOD (S/N = 3) of 0.033% for the enantiomeric impurity 1*S*,2*R*-ephedrine.

Keywords: Diisoproylideneketogulonic acid; Enantiomeric purity; Ephedrine; Ketopinic acid; Timolol; Non-aqueous capillary electrophoresis

1. Introduction

A majority of the chiral drugs introduced on the market today are enantiomerically pure. In 2003, 82% of these drugs were based on a single enantiomer [1]. Thus, there is a strong need for efficient, simple and rapid analytical methods for the determination of enantiomeric impurity. According to the guidelines from the US Food and Drug Administration (FDA), the presence of the unintended enantiomer in a stereochemically pure drug should be treated in the same way as any impurity [2]. However, technical limitations sometimes make it impossible to obtain the same limits of quantification and qualification as for the achiral impurities [2]. According to the ICH guidelines, the impurities above 0.05 or 0.1% (depending on dose) of the active substance in new products should be identified and reported to the authorities [3].

Nuclear magnetic resonance (NMR) with chiral shift reagents [4], polarimetry [4] and dual circular dichroism with simultaneous ultraviolet detection [5] and voltammetry/amperometry with enantioselective sensors [6] have been applied for enantiomeric impurity determinations without a preceding separation step. However, separation techniques are especially suitable for chiral analysis in complex samples, as they only require a small amount of the sample and because these techniques enable efficient isolation of the enantiomers from the matrix to be carried out prior to detection. Mass spectrometric, spectrophotometric and fluorimetric detectors are often used for the subsequent determination of the separated enantiomers.

The major separation technique for the determination of chiral purity is still high-performance liquid chromatography (HPLC) with chiral stationary phases [4,7], but gas chromatography (GC) [4,8], supercritical fluid chromatography (SFC) [9] and capillary electrophoresis (CE) [10,11] are also used. CE has a higher separation efficiency compared to conventional HPLC, which theoretically makes it possible to improve resolution and shorten the analysis time. The detection limit is often higher in CE than in LC owing to the lower loading capacity of CE and the use of on-column detection. This might complicate the analysis of the impurities present in just low amounts. The detection limit in CE is often improved by the use of a

* Corresponding author. Tel.+46 18 4714340; fax: +46 18 4714393.
E-mail address: Curt.Pettersson@farmkemi.uu.se (C. Pettersson).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.12.013

pre-concentration technique, for example, manipulating the electrophoretic velocity of the analyte in the injection zone or by trapping the analyte on to a pseudo stationary phase. Several different techniques such as pH mediated stacking [12], use of a dynamic pH junction [13] and different sweeping techniques [14], field amplified sample stacking (FASS) [15–17], transient isotachophoresis (tITP) [18], the use of low temperature zones [19] and low- and high conductivity zones [20] have been developed for pre-concentration as alternatives to chromatographic techniques [21]. For recent reviews on on-line pre-concentration in CE, see the following references [22,23]. It should be stressed that the use of a stacking technique to improve the detection limit increases the complexity of the system, which might affect the robustness of the method. For a review on enantiomeric purity determinations by CE, see the article by Blomberg and Wan [10].

The first publications on enantiomeric separations in non-aqueous CE (NACE) appeared in 1996 [24–27]. This means of performing chiral separations facilitates the use of selectors with a low solubility in water and may improve the enantioselectivity of the chiral counter-ions without or with only a low enantioselectivity in aqueous solvents [25], since the lower dielectric constants in organic solvents promote the ion-pair formation. The most frequently used chiral selectors in NACE are the cyclodextrins or the cyclodextrin derivatives [28]. Enantiomeric separations using ion-pairing [24,25] or ligand-exchange have also been demonstrated in NACE [28,29].

The present study had two main objectives. The first of these was to evaluate 1*S*,4*R*-(+)-ketopinic acid [(+)-KPA] as a chiral counter-ion in NACE. The chiral resolution capacity of (+)-KPA for enantiomeric amines was compared with the previously used counter-ion 2*R*,3*S*,4*R*,5*S*-(−)-2,3:4,6-di-*O*-isopropylidene-2-keto-L-gulonic acid [(−)-DIKGA] [30]. The second objective was to give examples of potential applications of ion-pair selectors in CE by developing two analytical methods for the determination of the enantiomeric impurity in *S*-timolol and in 1*R*,2*S*-ephedrine. The method using (+)-KPA was applied to impurity determination of *R*-timolol. The enantiomeric impurity of 1*S*,2*R*-ephedrine in an Efedrin injection solution was evaluated using (−)-DIKGA as the chiral ion-pair selector.

2. Experimental

2.1. Chemicals

The chiral selector (1*S*,4*R*)-7,7-dimethyl-2-oxobicyclo [2.2.1]heptane-1-carboxylic acid [(+)-ketopinic acid] was obtained from Sigma–Aldrich (St. Louis, MO, USA) and (−)-DIKGA from Fluka (Buchs, Switzerland). Ethanol (EtOH, 99.7%) was bought from Solveco (Stockholm, Sweden) and methanol (MeOH, 99.99%) from Fischer Chemicals (Zurich, Switzerland). *rac*-Atenolol hydrochloride, *R*-atenolol hydrochloride, *S*-atenolol hydrochloride, *rac*-isoprenaline hemisulfate, *R*-isoprenaline bitartrate, *S*-isoprenaline bitartrate, *rac*-pindolol, *rac*-propranolol hydrochloride, *R*-propranolol hydrochloride, *S*-propranolol hydrochloride, *rac*-sotalol and *S*-timolol maleate were supplied by Sigma (St. Louis, MO, USA). *R*-pindolol and *S*-pindolol were gifts from AstraZeneca (Mölndal, Sweden). *R*-timolol maleate, 1*R*,2*R*-ephedrine hydrochloride [(−)-pseudoephedrine] and 1*S*,2*S*-ephedrine hydrochloride [(+)-pseudoephedrine] were from the European Directorate for the Quality of Medicines (EDQM) (Strasbourg, France). *rac*-Bambuterol hydrochloride and *rac*-terbutaline sulfate were gifts from Astra Draco (Lund, Sweden). 1*R*,2*S*-(−)-ephedrine and 1*S*,2*R*-(+)-ephedrine were from Fluka. The structures of the selectors (counter-ions) and the solutes are shown in Fig. 1A and B, respectively. The electro-osmotic flow (EOF) marker mesityl oxide was from Sigma. Sodium hydroxide, potassium hydroxide, hydrochloric acid, triethanolamine and sodium acetate were supplied by Merck (Darmstadt, Germany). The coating chemicals acrylamide, ammonium persulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine were obtained from Bio-Rad Labs. (Hercules, CA, USA) and γ-methacryloxypropyltrimethoxysilane was bought from Sigma. Efedrin (Batch 5A208A) produced by Merck (Stockholm) were obtained from Apoteket (Stockholm). All chemicals used were of analytical grade or better. The water was purified in a Milli-Q water system (Millipore, Bedford, MA, USA).

2.2. Instrument and procedures

The instrument used to perform the CE was an HP^{3D}CE instrument from Hewlett-Packard (Waldbronn, Germany) equipped with a UV-diode array detector. The data was recorded using the HP Chemstation software Version A 07.01. Fused silica capillaries (50 μm i.d., 365 μm o.d.) were obtained from Micro-Quartz (Munich, Germany). Polyacrylamide coated capillaries were prepared according to the coating procedure published by Hjertén [31]. About 0.2 cm of the polyimide coating was burned off on both ends of the capillary, in order to avoid sample carry-over. The capillary length was 32 cm, with an effective length of 23.5 cm (unless otherwise stated). Before first use, the capillaries were conditioned with 0.1 M sodium hydroxide, water, 0.1 M hydrochloric acid, water and ethanol (15 min and 35 mbar for each step). All samples were dissolved in MeOH. The electrophoresis was carried out at 30 kV and the temperature was set to 25.0 °C. Between the analyses that were performed using the same background electrolyte (BGE), the capillary was flushed with the BGE for 5 min (1 bar). When exchanging the BGEs, the capillaries were flushed with the new electrolyte for 20 min. Mesityl-oxide (0.1%, v/v, dissolved in MeOH) was used as a marker for the electro-osmosis. Solutions of isoprenaline were protected from light and prepared daily owing to their chemical instability.

2.2.1. Method development for S-timolol

The enantiomers of timolol were detected at 220 nm on the cathodic side in all experiments. In the FASS experiments, a plug of pure EtOH was injected (35 mbar in 1 s) before injection of the sample solution. The sample (*S*-timolol 0.5 mM) was injected electrokinetically at a constant voltage (8 kV) over 10 s.

The tITP conditions used in this study were based on the work by Shibabi [18]. The capillary was first flushed with the BGE