Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 1201–1209

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Comparison of chiral electrophoretic separation methods for phenethylamines and application on impurity analysis

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ARTICLE INFO

Article history:
Received 30 April 2010
Received in revised form 21 June 2010
Accepted 22 June 2010
Available online 30 June 2010

Keywords:
Enantioseparation
Ephedrine derivatives
Microemulsion electrokinetic chromatography
Sulfated β-cyclodextrin
Impurity analysis

ABSTRACT

A chiral microemulsion electrokinetic chromatography method has been developed for the separation of the enantiomers of the phenethylamines ephedrine, *N*-methylephedrine, norephedrine, pseudoephedrine, adrenaline (epinephrine), 2-amino-1-phenylethanol, diethylnorephedrine, and 2-(dibutylamino)-1-phenyl-1-propanol, respectively. The separations were achieved using an oil-in-water microemulsion consisting of the oil-component ethyl acetate, the surfactant sodium dodecylsulfate, the cosurfactant 1-butanol, the organic modifier propan-2-ol and 20 mM phosphate buffer pH 2.5 as aqueous phase. For enantioseparation sulfated β-cyclodextrin was added. The method was compared to an already described CZE method, which made use of *heptakis*(2,3-di-*O*-diacetyl-6-*O*-sulfo)-β-cyclodextrin (HDAS) as chiral selector. Additionally, the developed method was successfully applied to the related substances analysis of noradrenaline, adrenaline, dipivefrine, ephedrine and pseudoephedrine monographed in the European Pharmacopoeia 6.

1. Introduction

Phenethylamines interfere with the peripheral nervous system and are able to induce the release of noradrenalin (norepinephrine), so that they act as oral vasoconstrictors and bronchodilators [1–3]. For example ephedrine, methylephedrine, norephedrine and pseudoephedrine are some of the active components of ephedrae herba [4,5]. Often they are used as ingredients of cold medicines and anorectics because of their aforementioned sympathomimetic qualities. Analysis of ephedra alkaloids is of interest for food, e.g. tea, forensic and pharmaceutical applications [6]. Most of drugs are chiral. Since the biological activity, toxicology and pharmacokinetics of the enantiomers of a chiral drug can be different, it is important to ensure the enantiomeric purity by means of a chiral separation method [1,2,7–10] (Fig. 1).

Based on the principles of electrophoresis, the capillary zone electrophoresis was designed to separate analytes in a small capillary due to their size-to-charge ratio.

Beside the development of many other related techniques, in 1991 [11] the microemulsion electrokinetic chromatography (MEEKC), using mainly oil-in-water (O/W) microemulsions (ME), was invented. Hence, separation of uncharged and hydrophobic substances was made available, because the oil droplets of the ME act as pseudostationary phase and solubilize many hydrophobic analytes. In MEEKC, the chromatographic separation is based on the partition of the analytes between the oil droplets and the buffer solution. For stabilization of the oil droplets surfactants like SDS, and cosurfactants, often short chain alcohols (e.g. 1-butanol), are used. The micelles have a negative charge on the surface because the hydrophilic sulfate head group of the surfactant remains in the aqueous buffer solution, whereas the hydrocarbon tails position in the oil core [12–16].

Application of CDs in CE is a useful technique for resolution of enantiomers and for determination of the enantiomeric excess [17,18]. However, for separation of enantiomers, chiral modifiers such as the cyclodextrins and their derivatives were added to the running buffer [9,10,19–24]. By derivatisation of a native CD many variations are given for enantioseparation of a wide range of chiral compounds. For enantioseparation both hydrophobic and ionic interactions are as necessary as sterically effects and hydrogen bonds [9,25].

In the case the enantiomeric purity of a drug has to be evaluated by means of MEEKC, a chiral selector has to be added to the ME. To form a chiral BGE, chiral oils like (*S*)-(+)-2-octanol [26–28], chiral surfactants like dodecoxycarbonylvaline [29–31] or chiral cosurfactants like (*S*)-2-hexanol [30,32] can be applied. As already mentioned, in CE the mostly used chiral selectors are cyclodextrins, which work in different CE-methods. Good results occurred,

Abbreviations: HDAS, *heptakis*(2,3-di-*O*-diacetyl-6-*O*-sulfo)-β-cyclodextrin; ME, microemulsion; MEEKC, microemulsion electrokinetic chromatography; O/W, oil-in-water; sulf. β-CD, sulfated β-cyclodextrin.

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doi:10.1016/j.jpba.2010.06.025

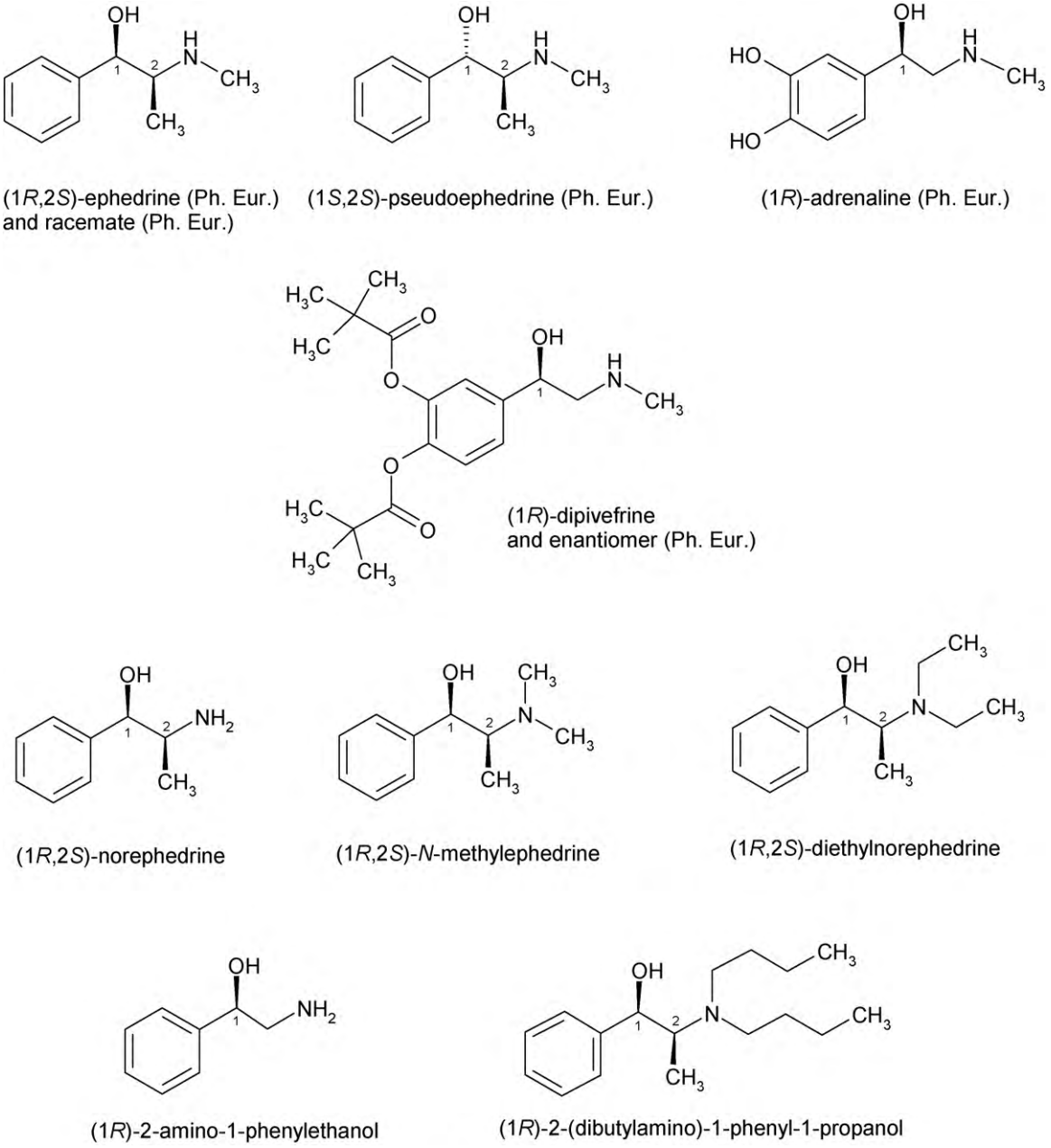

Fig. 1. Structural formulae of the chiral phenethylamines.

e.g. with a dimethyl-β-CD-modified CZE for enantioseparation of 1-aminoindan [9], with a CD-modified MEKC for the determination of isochromene derivatives using hyrdroxypropyl-β-CD [33] and with a CD-modified MEEKC, e.g. for phenylalanine analogues [34] and tropa alkaloids [35].

The purpose of this work was the development and validation of a chiral MEEKC method for the enantioseparation of the aforementioned phenethylamines in clinical use and the comparison of the MEEKC results with results obtained with CD-modified CZE, using *heptakis*(2,3-di-*O*-diacetyl-6-*O*-sulfo)-β-CD [36,37] and dimethyl-β-CD [38], respectively, as chiral selector. Additionally, it was checked, whether CD-modified MEEKC method is appropriate for impurity-profiling of the chiral phenethylamines, as it is typically performed in international pharmacopoeias.

2. Materials and methods

2.1. Instrumentation

All MEEKC separations were performed on a Beckman Coulter P/ACE System MDQ (Fullerton, CA, USA), equipped with an UV-detector measuring at 200 nm. The uncoated fused silica capillaries purchased from BGB Analytik (Schloßböckelheim, Germany) had an internal diameter of 50 μm, an effective length of 40 cm and a total length of 50.2 cm.

The pH of the buffer systems was determined by means of a PHM 220 Lab pH meter (Radiometer Copenhagen, Lyon, France). For the preparation of the ME a 2510-Branson-Sonicator (Heinemann, Ultraschall- und Labortechnik, Schwäbisch Gmünd, Germany) was used.