Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1098 (2005) 166–171
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Separation and determination of norepinephrine, epinephrine and isoprinaline enantiomers by capillary electrophoresis in pharmaceutical formulation and human serum

Shoulian Wei [a,b], Guanqun Song [c], Jin-Ming Lin [a,c,*]

[a] *Department of Chemistry, Tsinghua University, Beijing 100084, China*
[b] *Department of Light Industry and Chemistry, Zhaoqing University, Zhaoqing 526061, China*
[c] *Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China*

Received 9 May 2005; received in revised form 10 August 2005; accepted 10 August 2005
Available online 6 September 2005

Abstract

A capillary electrophoresis method with ultraviolet (UV) detection was developed and optimized for the enantiomer separation of norepinephrine (NE), epinephrine (EP) and isoprenaline (IP) using dual cyclodextrins (CDs) of 2-hydroxypropyl-β-CD (HP-β-CD) and heptakis (2,6-di-o-methyl)-β-CD (DM-β-CD) as chiral selectors. Optimal separation was obtained using a running buffer of 50 mM phosphate containing 30 mM HP-β-CD and 5 mM DM-β-CD at pH 2.90 and a field strength of 20 kV in 45 cm × 75 μm (40 cm effective length) uncoated capillary. The UV absorbance detection was set at 205 nm. A 0.1% (w/w) polyethylene glycol or 0.1% (v/v) acetonitrile was used to enhance the detection sensitivity. There was a wide and excellent linear calibration graph for each enantiomer in the range 1.0×10^{-3} to 1.0×10^{-6} M and the detection limit (S/N = 3) was found from 8.5×10^{-7} to 9.5×10^{-7} M. The method has been applied for the determination of isoprenaline in isoprenaline hydrochloride aerosol and to the analysis of serum samples. The recoveries of NE and EP in serum and IP in drug were ranged from 90 to 110%. The relative standard deviations of all the analyte peaks were less than 2.8% for migration time and less than 4.8% for peak area.

Keywords: Capillary electrophoresis; Cyclodextrin; Enantiomer separation; Norepinephrine (NE); Epinephrine (EP); Isoprinaline (IP)

1. Introduction

Isoprenaline is symphatomimetic drugs with potent β_2-adrenoceptor widely used in the treatment of respiratory diseases [1]. It is also used illegally as growth promoters in the liver stock industry [2]. Its residue can be toxic to humans. Epinephrine (EP) and norepinephrine (NE), component of neural transmission media, have important effect on the transmission of nerve impulses and exhibit vasoconstriction and blood pressure elevation. Many diseases such as Parkinson's are related to the concentration of EP and NE in blood as well as urine. Pharmaceutically, they are widely used for treatment of neutral disorder [3]. These drugs are usually administrated as the racemate, which exhibit very different pharmacological effects. For example, the activity of L-EP is ten times stronger than its isomer D-EP [4]. Therefore, L-forms are usually used in the local anesthetic or ophthalmic solution. In order to monitor the effect of drugs given, to control the enantiomeric purity of the drugs administered and to study the drug pharmacokinetic and pharmacodynamic properties, it is desired and important to develop the analytical method that can effectively separate and determine these enantiomers.

A number of analytical methods have been reported to determine EP, NE and isoprenaline (IP) enantiomers [5–19]. Among them, high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) are the most widely used separation techniques. For example, the enantiomer separation of both NP and EP by HPLC was reported after derivatization [5,6] or directly separation [7–9]. However, these HPLC methods suffer a few drawbacks, such as by-products derived disturb the detection of the derivatives in the chromatogram, lack of general applicability, high consumption of samples and less robust.

CE is a powerful enantiomer separation and quantitation technique that often provides higher separation efficiency, shorter

* Corresponding author. Tel.: +86 10 62841953; fax: +86 10 62841953.
E-mail address: jmlin@mail.tsinghua.edu.cn (J.-M. Lin).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.08.038

analysis time, lower operation cost, more robust and wider applicability. CE with the direct method using β-cyclodextrin (β-CD) or derivatized-CD as chiral selectors was reported to be effective for enantiomer separations of NE, EP and IP. Quang and Khaledi [10] reported the use of β-CD and tetraalkylammonium for the separation of NE, EP and IP enantiomers. However, only partial separation was obtained for NE and EP. Gahm and Stalcup [11] and Yang et al. [12] investigated the enantiomer separation of NE, EP and IP using sulphated β-CD as chiral selector. The resolutions of NE and EP were insufficient and the peak of each enantiomer tailed. Male and Luong [13] demonstrated DM-β-CD as chiral selector to separate NE, EP and IP, but the separation of NE and EP could not be improved. Dong and Sun [14] and Liu and Nussbaum [15], respectively, reported baseline separation of IP by using HP-β-CD, or DM-β-CD, CM-β-CD as chiral selector. Garcia-Ruiz and Marina [16] and Cucinotta et al. [17] studied the enantiomer separation of NE and EP by using permethylated-β-CD or 6-O-succinil-β-CD as chiral selector, however, the tested of NE and EP enantiomers could not be completely resolved. Recently, Schwarz and Hauser [18] demonstrated a combination of CM-β-CD and 18-crown-6 as chiral selector to separate the enantiomers of NE and EP, but the baseline separation of the two forms of NE was not successful. Therefore, in spite of intensive investigates in the field of enantiomer separation of NE, EP and IP, the resolution achieved for NE or EP was insufficient to permit practical use. To the best of our knowledge, until now no application to biofluids, enantiopurity testing or commercial formulations have been reported. To suit practical use, a further improvement of the resolution is required.

In this study, we attempted to improve the separation of enantiomers of NE and EP by using HP-β-CD or HP-β-CD combination of other selectors. In additional, organic modifier has been tried and found effective in order to increase the method detection sensitivity. The developed assay method was also validated for sensitivity, precision, linearity, recovery, and reproducibility. It has been successfully applied to the analysis pharmaceutical formulation and human serum samples.

2. Experimental

2.1. Reagents

(±)-Norepinephrine L-bitartrate hydrate (99%) (NE), (R)-(−)-norepinephrine hydrochloride bitartrate salt, (±)-epinephrine hydrochloride (EP) and (±)-isoprenaline hydrochloride (IP) (structural formulate was shown in Fig. 1) were purchased from Sigma Aldrich (St. Louis, MO, USA). Isoprenaline hydrochloride aerosol was obtained from Penglai Nuokang Pharmaceutical Factory (Shandong, China). β-Cyclodextrin (β-CD) was obtained from Fluka (Buchs, Switzerland). Methyl-β-cyclodextrin (Me- β-CD) and dimethyl-β-cyclodextrin (DM-β-CD) were from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). Hydroxypropyl-β-cyclodextrin (HP-β-CD, >97%) was purchased from Acros Organics. Polyethylene glycol was obtained from Shanghai Chemical Reagent Co. (Shanghai, China). Tris (hydroxymethyl) aminomethane (Tris), tetramethylammoniumhydroxide solution (TMAH), phosphoric acid, acetone (≥99.5%) and triethanolamine (TEA) were purchased from Beijing Chemical Factory (Beijing, China). Acetonitrile and ethylene glycol were from Tianjing Chemical Factory (Tianjing, China). All other reagents used were of analytical grade purity. Water for preparation of sample and buffer solution was deionized by a Milli-Q purification system with a 0.2-μm fiber filter (Barnstead, CA, USA).

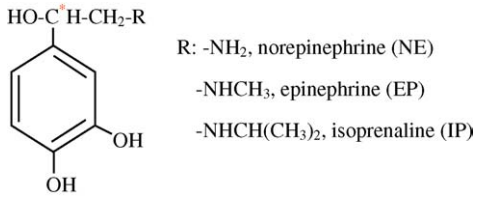

Fig. 1. Chemical structures of norepinephrine, epinephrine and isoprinaline.

2.2. Apparatus and analytical procedure

All experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis system (Beckman, Fullercon, CA, USA) equipped with a photodiode array detection system. Sample detection was performed at 205 nm. The electropherograms were recorded and integrated by an IBM personal computer with 32-karat software version 4.0 (Beckman). An uncoated fused-silica capillary (Yongnian Optic Fiber Factory, Heibei, China) of 75 μm I.D. and 40 cm effective length was used for separation. The capillary temperature was maintained at 25 °C by the cooling system of the CE instrument. The new capillary was flushed successively for 30 min with 1 M NaOH, 10 min with 1 M HCl, 10 min with water to activate and clean the silica wall and then equilibrated with the operating buffer for 10 min. After every running the capillary was preconditioned with 1 M NaOH for 2 min, water for 2 min, the running buffer for 2 min. At the beginning of each day and whenever the buffer solution was changed, the capillary was rinsed with 1 M NaOH for 5 min, water for 2 min, and the running buffer for 2 min. Samples were injected with pressure at 0.8 psi for 10 s and separated at 20 kV (1 psi = 6894.76 Pa). Standard solutions of EP and IP were prepared by dissolving each of them in water to give a concentration of 10^{-3} M and 7×10^{-4} M, respectively. Finally, dilutions of this solution in water were made in order to obtain the required concentration. The background electrolyte (BGE) was prepared by mixing a 50 mM phosphoric acid, 30 mM HP-β-CD, 5 mM DM-β-CD, 0.1% (w/w) polyethylene glycol in water and pH adjusted to 2.90 with pH meter (IQ Scientific Instruments, Shanghai, China) by adding TEA or TMAH, 0.5 M Tris solution.

2.3. Sample preparation

The determination of the IP in the isoprenaline hydrochloride aerosol required a dilution of the aerosol in a final volume of 1.0 mL with water. Different sample solutions were prepared by diluting different amounts of the aerosol