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Journal of Chromatography A, 1081 (2005) 72–76
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Assay of acebutolol in pharmaceuticals by analytical capillary isotachophoresis

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Available online 4 May 2005

Abstract

Acebutolol [*N*-{3-acetyl-4-[(2-hydroxy-3-(isopropylamino)propoxy]phenyl} butanamide] is a cardioselective β-blocker with a potent antihypertensive and antiarrhythmic effect. The optimised operational system of electrolytes for the newly developed ITP separation of acebutolol consisted of 10 mM potassium acetate +10 mM acetic acid (pH 4.65) as the leading electrolyte and 10 mM β-alanine with pH ≈4 (adjusted with acetic acid) as the terminating electrolyte. The driving and detection currents were 75 and 20 μA, respectively and the analysis took ≈13 min. Under these conditions the effective mobility of acebutolol was determined as 20.7×10^{-9} m^2 V^{-1} s^{-1}. The calibration dependence was rectilinear in the range 0.14–1.4 mg ml^{-1} of acebutolol base ($r = 0.9995$); relative standard deviation (RSD) values were 1.1% and 1.2% ($n = 6$) when determining 0.42 and 0.98 mg ml^{-1} of acebutolol in a pure standard solution. The method, with the limit of detection (LOD) of 0.04 mg ml^{-1} and limit of quantification (LOQ) of 0.12 mg ml^{-1}, was applied to the assay of acebutolol in Sectral tablets, Acecor tablets, Apo-acebutol tablets (nominal content 400 mg of acebutolol per tablet) and Acebirex tablets (nominal content 200 mg of acebutolol per tablet) with RSD = 0.7–1.7% ($n = 6$). No interference from any excipients present in the tablets was observed. The recoveries ranged from 98.8% to 102.4% as found by the standard addition technique.

Keywords: Capillary isotachophoresis; Acebutolol; Pharmaceutical analysis

1. Introduction

Acebutolol, ACE (Fig. 1) is a cardioselective, hydrophilic β-adrenoreceptor blocking agent with mild intrinsic sympathomimetic activity for use in treating patients with hypertension and ventricular arrhytmias. It is marketed in tablet form for oral administration [1].

The papers published until now have been concerned with the development of rapid and sensitive methods for the separation, identification or determination of acebutolol and several other basic β-blockers in body fluids using gradient high-performance liquid chromatography (HPLC) with photodiode-array UV detection [2], HPLC-mass spectrometry (MS) [3], RP-HPLC using both isocratic and gradient elution [4] or RP-HPLC using solvent modifiers [5]. Gas chromatography with mass spectrometry (GC–MS) was presented for the sensitive detection of beta-blocking agents in human urine recently [6]. Three β-blockers (propranolol, timolol, acebutolol) were separated by capillary electrophoresis (CE) with electrochemical detection [7].

A strategy for rapid screening for the separation of chiral molecules of pharmaceutical interest by normal-phase LC using cellulose/amylose stationary phases was proposed [8]. Capillary electrochromatography (CEC) was used for enantioseparations of ACE and other active substances [9–12]. The super/subcritical fluid chromatography was similarly applied to the chiral separations of β-blockers [13]. The rapid enantiomeric chiral separation of a set of 12 chiral amino-containing pharmaceutical compounds belonging to various therapeutic categories was achieve by capillary electrophoresis with carboxymethylcyclodextrins as selectors [14].

A nonaqueous capillary electrophoresis (NACE) method coupled with either UV or electrospray mass spectrometry (ESI-MS) was described for the simultaneous analysis of seven β-blockers [15]. The enantiomeric resolution of various kinds of basic pharmaceuticals was investigated in NACE systems using an ion-pairing reagent [16].

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doi:10.1016/j.chroma.2005.04.034

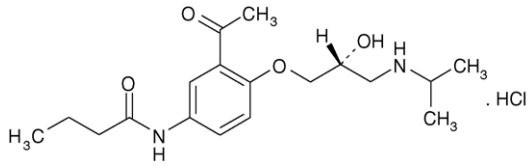

Fig. 1. Chemical structure of acebutolol hydrochloride.

Existing publications concerning the individual determination of acebutolol in pharmaceutical preparations were based on first derivative spectrophotometry, TLC-densitometry and HPLC [17,18], spectrophotometry [19] and GC [20].

To our best knowledge, the ACE has not yet been determined in pharmaceuticals by conventional capillary isotachophoresis. Only the separation of β-blockers oxprenolol, atenolol, timolol, propranolol metoprolol, and acebutolol in human urine by zone electrophoresis with transient isotachophoresis on a poly (methyl methacrylate) chip was investigated recently [21].

The aim of this work was the systematic development and the validation of capillary ITP method with conductimetric detection for the assay of ACE in pharmaceutical formulations.

2. Experimental

2.1. Apparatus

ITP separation performed by using a PC-controlled Villa Labeco EA 100 analyser (VILLA, Spišská Nová Ves, Slovak Republic) operated in a single-column mode. A 160 mm × 0.3 mm (i.d.) analytical capillary made of fluorinated ethylene-propylene copolymer (FEP) and equipped with a conductivity detector was employed. The volume of sampling valve was 30 μl. The data were collected with ITP Win Software, KasComp, Bratislava, Slovakia.

The PHM 220 (Radiometer, France) pH-meter with PHC2401-8 combined glass electrode calibrated with standard Radiometer buffers was employed for pH measurements. The ultrasonic bath Tesson, Tesla Prague, Czech Republic, was used for degassing of solutions.

2.2. Chemicals

Acebutolol hydrochloride was obtained from Sigma–Adrich, Prague, Czech Republic. The dosage forms analysed: Acecor tablets (producer Societa Prodotti Antibiotici Milano, Italy); Apo-acebutol tablets (Toronto, Canada); Sectral tablets (Specia, Nontrouge, France); Acebirex tablets (BTT Erstein, France). The pharmaceuticals were obtained from the pharmacy.

All chemicals (formic, acetic and picolinic acids, tetraethylammonium iodide (TEA), potassium hydrogen carbonate, ε-aminocapronic acid (EACA), β-alanine (BALA), γ-aminobutyric acid (GABA) and methanol were of analytical grade.

A Millipore Milli-Q RG ultra pure water was used for the preparation of the electrolytes and stock solutions.

2.3. Standard and electrolyte solutions

The stock solution (1.4 mg ml^{-1}) of ACE base was prepared by dissolving of ACE·HCl in 1 mM hydrochloric acid. The calibration curve was measured with 0.14–1.4 mg ml^{-1} (0.46–4.6 mM) ACE base solutions (six concentrations, each measured in triplicate).

The leading electrolyte (LE) consisted of 10 mM potassium acetate +10 mM acetic acid (pH 4.65). 10 mM BALA of pH ≈4 (adjusted with acetic acid) was used as the terminating electrolyte (TE). The electrolyte solutions were degassed for 5 min in an ultrasonic bath.

The measurement of the effective mobility of ACE was carried out with 0.5 mM solution of ACE and 0.1 mM TEA as the standard of mobility and calculated from ionic mobilities of K^+, TEA^+ and relative step heights of ACE according to the relevant equation in [22].

2.4. Analysis of tablets

Ten tablets were weighed, crushed and pulverised in an agate mortar, and the weight of the resulting tablet powder equivalent to ≈400 mg of ACE base was transferred into a 100 ml volumetric flask with ≈50 ml of 1 mM HCl and dissolved by applying a 10 min sonication on the ultrasonic bath; thereafter the suspension was diluted to the mark and filtered through a membrane filter (pore size 0.45 mm). The extract subjected to ITP analysis after 10-fold dilution with 1 mM HCl.

2.5. Accuracy test

All the pharmaceutical formulations were initially analysed by ITP as described above; thereafter the samples were treated with known amounts of acebutolol standard at two concentration levels and again subjected to ITP assay. The recoveries of the added amount of ACE were calculated.

3. Results and discussion

3.1. Acid–base properties of the analyte, selection of the electrolyte system and determination of effective mobilities of the drug

The drug under study involves basic amine group which is an important prerequisite for applying any electro-migration method for ITP analysis. According to the earlier literature data [23] the pK_a value of ACE = 9.4 (secondary aliphatic amine group); it means that in aqueous solutions of pH ≤7.4 this compound is practically completely ionised.