Short communication

Optimization of an electrolyte system for analysis of ethambutol in pharmaceutical formulations by capillary zone electrophoresis using complexation with copper(II)

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ABSTRACT

An alternative methodology for the determination of ethambutol by capillary zone electrophoresis (CZE) under direct UV detection at 262 nm, using acetic acid/sodium acetate buffer solution (pH 4.6) containing copper(II) sulphate to form the ethambutol–copper(II) complex, within analysis time of 2.5 min is proposed. The optimum CE conditions for the background electrolyte were established performing experiments of a 3^2 factorial design. Complex formation was evidenced by the UV batochromic shift and the $[CuETB]^0$ and $[CuETB]^{2+}$ chemical structures were indicated by LC–MS analysis. After some validation parameters have been performed, such as linearity ($r = 0.999$), selectivity (comparison between slope of the calibration curve of the external standard and calibration curve of the standard addition), area precision (RSD%: <2.13 for ETB and <1.94 for 2A1B), recovery mean (101.7% for ETB and 99.95% for 2A1B) and quantification limit (mg L^{-1}: 10.17 for ETB and 19.70 for 2A1B), the method was successfully applied to ETB analysis in pharmaceutical formulation samples. It is possible to determine the presence of the 2A1B impurity at concentrations of less than 1% ETB content.

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1. Introduction

Ethambutol (ETB) is a synthetic antimycobacterial agent with substantial activity against *Mycobacterium tuberculosis*, *Mycobacterium bovis*, and most strains of *Mycobacterium kansaii*. ETB is effective adjunct therapy for the treatment of tuberculosis (TB) but is not indicated as a monotherapy. The standard first-line treatment against active TB is a combination of the rifampicin, isoniazid, pyrazinamide and ETB drugs given in combination over a period of 6–9 months [1,2]. ETB degrades to 2-amino-1-butanol (2A1B) and permitted amounts of this degradant should be less than 1% of the ETB·2 HCl content [3].

Owing to the importance of ETB in TB treatment, the development and optimization of selective, sensitive, reproducible and fast analytical methods are necessary. However, the direct determination of ETB by UV detection is difficult because of its low molar absorptivity. Since this behavior inhibits HPLC applications, auxiliary procedures such as derivatization and complexation are necessary [4–8]. Lacroix et al. attempted to circumvent these dif-

ficulties by measuring the 270 nm absorbance of the ETB–copper sulfate complex. However, their technique required the use of two analytical columns and the synthesis of the internal standard [5]. Gamberini and Ferioli proposed the use of chemical derivatization with phenylethylisocyanate and the subsequent measurement of the 254 nm absorbance, but their result presented low sensitivity [6]. Chenevier et al. also proposed the use of chemical derivatization with phenylethylisocyanate, in order to increase the sensitivity under UV detection at 200 nm within analysis time of 15 min [7]. Ion-pair reversed phase liquid chromatography to permit the formation of the cationic $\{[Cu_2EBT]^{2+}(SO_4)^{2-}\}$ complex in solution was developed by Jiang et al. The complexation was performed in the column after CuSO$_4$ addition to the mobile phase and pH adjustments using diluted hydrochloride acid. This methodology was suggested for potential application in the monitoring of raw material and the quality control of pharmaceutical formulations [8]. Other detections mode for HPLC such as mass spectrometric and fluorescence has been described for ETB analysis in biologic fluids [9–12].

Regarding CE procedures, Ragonese et al. proposed using a Box–Behnken design for the optimization of the electrolyte system, which was useful for the separation of ETB and 2A1B by UV detection at 200 nm within 4 min. However, in spite of its impor-

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tance, this methodology presents low sensitivity being necessary the use of high ETB concentrations of the order of magnitude of 10^3 mg L^{-1} to obtain satisfactory analytical signal [13]. Hsieh et al. carried out another approach through the simultaneous analysis of ETB and methoxyphenamine using CE under electrochemiluminescence detection in 8 min [14].

However, there is still ample space in the literature for ETB analysis by CE under UV–vis detection. Within this context, a simple and fast methodology for ETB analysis by CZE is proposed. The method was successfully applied to ETB analysis in pharmaceutical formulation samples for which it is possible to determine the presence of the 2A1B impurity at concentrations of less than 1% ETB content.

2. Experimental

2.1. Chemicals and solutions

All chemicals were analytical grade. Acetic acid, sodium hydroxide and copper(II) sulphate pentahydrate were purchased from Vetec (Rio de Janeiro, RJ, Brazil), ethambutol dihydrochloride (ETB·2HCl) from Genix Indústria Farmacêutica (Goiás, GO, Brazil) and 2-amino-1-butanol from Sigma (St. Louis, MO, USA).

Aqueous acetic acid/sodium acetate buffer (HAc/NaAc) (pH 4.6) (buffer solution-BS) stock solution containing a concentration of 100.0 mmol L^{-1} and aqueous copper(II) sulphate pentahydrate (CuSO$_4$·5H$_2$O) stock solution containing 40.00 mmol L^{-1} were used for electrolyte solution preparation.

Aqueous ETB stock solution containing 20.00 mmol L^{-1} was used during CE procedures, such as method optimization and sample quantification, after adequate dilution in the background electrolyte (BGE).

UV–vis electronic spectra were obtained under the following conditions: **ETB**: 2.45×10^{-4} mol L^{-1}; **Cu^{2+}**: 2.45×10^{-4} mol L^{-1}; **2A1B**: 5.61×10^{-4} mol L^{-1}; **Cu–ETB**: mixture containing 2.45×10^{-4} mol L^{-1} of ETB and 2.45×10^{-4} mol Cu^{2+}; **Cu-2A1B**: mixture containing 2.61×10^{-4} mol L^{-1} of 2A1B and 2.45×10^{-4} mol of Cu^{2+}. All solutions were diluted in 60.00 mmol L^{-1} of BS.

2.2. Sample solutions

Six ethambutol dihydrochloride tablets were individually weighed and ground to homogeneously fine powders. The powder corresponding to 12.50 mg of active ingredient for each tablet was weighted and dissolved in 25.00 mL of water in a volumetric flask. After 5 min of sonication, the suspensions were filtered through a 0.45 µm millipore filter (São Paulo, SP, Brazil) in order to obtain clear solutions. The samples were diluted to 50.00 µg mL^{-1} in the BGE.

2.3. Cu–ETB crystallization

86.90 mg ETB·2HCl and 159.0 mg of CuSO$_4$·5H$_2$O were added to an aqueous solution containing 60.00 mmol L^{-1} of BS. The solution was maintained at room temperature until crystal formation. The crystals obtained were dissolved in a solution containing methanol and water (1:1, v/v) before analysis by liquid chromatography–mass spectrometry (LC–MS).

2.4. Instrumentation

The experiments involving separation optimization were conducted in a CE system (HP3d CE, Agilent Technologies, Palo Alto, USA) equipped with a DAD set at 262 nm, a temperature control device, maintained at 25 °C, and data acquisition and treatment

software (HP ChemStation, rev A.06.01). Samples were injected hydrodynamically (30 mbar 5 s) and the electrophoretic system was operated under normal polarity and constant voltage conditions of +25 kV. For all experiments, a fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) 48.5 cm (40 cm effective length) × 75 µm ID × 375 µm OD was used.

The experiments involving chemical characterization by liquid chromatography–mass spectrometry (LC–MS) were carried out with a Waters model ZQ-LC/MS 2000.

The absorption spectra measurements were made in a double-beam in time UV–vis spectrophotometer system (model UV-1601PC, Shimadzu, Kyoto, Japan) using quartz regular cells of optical path equal to 1.0 cm.

2.5. Analytical procedures

When a new capillary was used, it was conditioned by a pressure flush of 1.00 mol L^{-1} NaOH solution (30 min), deionized water (5 min) and electrolyte solution (10 min). In between runs, the capillary was replenished with 0.20 mol L^{-1} NaOH solutions (2 min), deionized water (2 min) and fresh electrolyte solution (3 min, pressure flush).

3. Results and discussion

3.1. Study of the ethambutol–copper(II) complex formation

Based on the complexation study of ETB with copper(II) performed by Jiang et al., a acetic acid/sodium acetate buffer solution (pH 4.6) was used in order to maintain the pH in the 4.0–5.0 interval, because according to the literature the recovery and sensitivity were poor with a pH under 4.0 and over 5.0 owing to the occurrence of cupric hydroxide precipitation [8]. Fig. 1 shows the electronic UV–vis spectra for copper(II) sulphate, ETB, 2A1B, Cu–ETB and Cu-2A1B. Analyzing the spectra, it is possible to observe a bathochromic shift on comparison of the absorption bands for free copper(II) (250 nm) and Cu–ETB (262 nm), which suggests complexation occurrence. It is also possible to note a small change in the absorption band of Cu-2A1B in comparison with 2A1B. Based on the UV–vis spectral analysis, the wavelength at 262 nm was selected for the optimization study by CE.

A complementary study by LC–MS was performed in order to evaluate the Cu–ETB complex formed. The results obtained indicate the presence of two main compounds: one with *m/z* 266.2 and
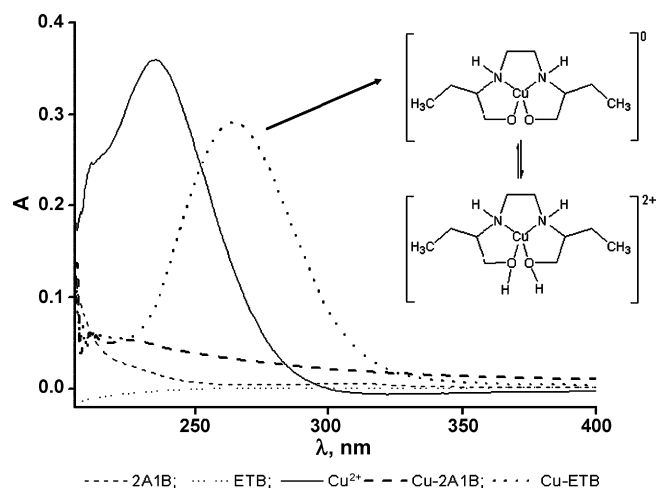

Fig. 1. Electronic UV–vis spectra for copper(II) sulphate, ETB, 2A1B, Cu–ETB and Cu-2A1B.