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Article

Ethambutol Analysis by Copper Complexation in Pharmaceutical Formulations: Spectrophotometry and Crystal Structure

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Um método alternativo para determinação de etambutol (ETB) por espectrofotometria UV em formulações farmacêuticas por complexação com Cu(II) foi otimizado usando um planejamento fatorial completo 3^2, tendo como variáveis Cu(II) e tampão aquoso de acetato de sódio em pH 4,6. A estequiometria predominante do complexo em solução aquosa tamponada nesse pH e a constante de formação do complexo foram obtidas usando o método de Job e o diagrama de Scatchard, respectivamente. A estrutura cristalina do complexo de monocristal foi obtida por difração de raios X. O método espectrofotométrico foi aplicado na análise de formulação farmacêutica sendo encontrado: 408,0 ± 11,9 mg de ETB·2HCl, faixa de recuperação de 100,9-104,0% e limites de detecção e quantificação de 0,8 e 2,8 mg L^{-1}, respectivamente. O método espectrofotométrico ao ser comparado com o método por eletroforese capilar de zona não evidenciou diferenças significativas no intervalo de 95% de confiança.

An alternative method for ethambutol (ETB) determination by UV spectrophotometry in pharmaceutical formulations using ETB complexation with Cu(II) was optimized employing a 3^2 full experimental design having Cu(II) and aqueous acetic acid/sodium acetate buffer at pH 4.6 as variables. The predominant complex stoichiometry in aqueous buffer solution at this pH and formation constant were obtained by using Job's method and Scatchard diagram, respectively. The complex crystalline structure of monocrystal was obtained by X-ray diffraction. The developed method was applied to the pharmaceutical formulation analysis being found: 408.0 ± 11.9 mg of ETB·2HCl, recovery range of 100.9-104.0% and detection and quantification limits of 0.8 and 2.8 mg L^{-1}, respectively. The spectrophotometric method was compared to zone capillary electrophoresis and no significant difference was found within the 95% confidence interval.

Keywords: ethambutol, complexation, spectrophotometry, crystal structure

Introduction

Nowadays, tuberculosis (TB) is becoming a worldwide problem. This contagious disease is transmitted through the air and it is caused by the bacterium *Mycobacterium tuberculosis*, which can attack different organs of the human body. However, it most commonly affects the lungs, accounting for more than 75% of the cases.[1] According to the World Health Organization (WHO), more than two billion people are infected with TB bacilli and a total of 1.77 million people died from TB in 2007.[2] Nowadays, the first line TB treatment is based on four drugs: isoniazid, rifampicin, pyrazinamide and ethambutol (ETB), which are available in cheap generic forms and are effective if taken as prescribed.[3] These drugs complement each other and are used in various combinations, which are very important to prevent the emergence of multiple drug-resistant organisms, resulting in ineffective treatment.[3] In this context, ETB is an important component in TB treatment (Figure 1). This drug is an aminoalcohol, which affects the biosynthesis of mycolic acids, an important class of compounds frequently found in the cell walls of a group of bacteria known as Mycolata taxon.[4] The probable mechanism of action of ETB involves the inhibition of

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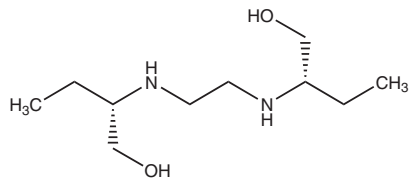

pK_a: 9.59 and 14.43

Figure 1. ETB chemical structure.

the arabinofuranosyl transferases important enzymes that promote the polymerization of arabinose into the arabinan domain of arabiongalactan which together with peptidoglycan are essential components in the cell wall of the *Mycobacterium tuberculosis*.

Due to the importance of ETB in TB treatment, different analytical methods such as UV-Visible spectrophotometry,[5] atomic absorption spectrometry,[5] potentiometry,[5] high performance liquid chromatography (HPLC)[6,7] and capillary electrophoresis (CE)[8-11] have been reported. As for spectrophotometric analysis, direct ETB determination in UV range is not feasible due to low molar absorptivity, thus requiring derivatization. Hassan and Shalaby[5] proposed ETB spectrophotometric analysis by means of complexation reaction between copper phosphate and ETB in medium of borate buffer pH 9.2 and read the absorbance of the final solution at 640 nm. This method involves steps such as heating, cooling and filtration before absorbance reading, making sample preparation procedure long and arduous. Within this context, the present work studies the development and optimization of a fast and simple alternative spectrophotometric method for ETB determination in pharmaceutical formulations at 262 nm using fast complexation with Cu(II) in pH 4.6.

Experimental

Chemicals and solutions

All chemicals were of analytical grade. Acetic acid, sodium hydroxide, copper(II) sulphate pentahydrate and copper(II) nitrate were purchased from Vetec (Duque de Caxias-RJ, Brazil), ethambutol dihydrochloride (ETB·2HCl) from Genix Indústria Farmacêutica (Anápolis-GO, Brazil).

Aqueous acetic acid/sodium acetate buffer (HAc/NaAc) (pH 4.6), stock solution containing a concentration of 50.0 and 100.0 mmol L^{-1} and aqueous copper(II) sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) stock solution containing 5.0 and 50.0 mmol L^{-1} were used in the preparation of solutions for the spectrophotometric study and for the capillary zone electrophoresis (CZE), respectively.

Aqueous ETB·2HCl stock solution containing 2.5 mol L^{-1} was used for method optimization and sample quantification, after adequate dilution in appropriate concentration of buffer and Cu(II).

Aqueous ETB·2HCl stock solution and aqueous $CuSO_4$ stock solution containing 1.0 mol L^{-1} were used during procedures for Job's method and Scatchard diagram.

CuETB crystallization

The compound CuETB was prepared as follows: 0.0560 g of ETB·2HCl was dissolved in ethanol and 3.0 mol L^{-1} of HAc/NaAc buffer solution. The pH of final solution was 5.0. It was added to this solution an ethanolic solution of $Cu(NO_3)_2$ containing 0.1326 g by diffusion. After 4 months (under rest at room temperature), suitable blue single crystals were obtained. The elemental analyses for C, H and N were carried out in a Perkin-Elmer 2400 analyzer (Waltham, Massachusetts, USA), and the results for the obtained CuETB were as follows: calculated: C, 33.24%; H, 5.58%; N, 11.63; found: C, 33.51%; H, 6.87%; N, 12.79%.

Sample preparation

Twenty ETB·2HCl tablets were individually weighed and ground to homogeneously fine powders. The powder corresponding to 7.0 mg of active ingredient for each tablet was weighed and dissolved in 10.00 mL of water in a volumetric flask. After 10 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 44.2 mg L^{-1} of ETB·2HCl in 0.5 mmol L^{-1} of $CuSO_4$ and 5.0 mmol L^{-1} of HAc/NaAc buffer before spectrophotometric and CE analysis.

Instrumentation

The measurements of absorption spectra 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.

Single crystal X-ray data were collected using a Bruker Kappa CCD diffratometer with Mo-K_α radiation (λ = 0.71073 Å) at room temperature (298 K). Data collection, reduction and cell refinement were performed by COLLECT. EVALCCD and DIRAX programs.[12-14] The structures were solved and refined using SHELXL-97.[15] An empirical isotropic extinction parameter x was refined, according to the method described by Larson.[16]

A multiscan absorption correction was applied.[17] The structures were drawn by ORTEP-3 for Windows[18] and Mercury[19] programs. The $C_{10}H_{24}O_5N_3ClCu$ crystal data: M = 365.31 g mol^{-1}, orthorhombic, a = 6.7029(5) Å, b = 13.929(1) Å, c = 18.094(1) Å, V = 1689.4(2) Å^3, T = 298 K, space group $P2_12_12_1$, Z = 4, μ(Mo-K_α) 1.470 mm^{-1}, d_{calc} = 1.436 g cm^{-3}, 9232 reflections measured, 3643 unique (R_{int} = 0.052) which 2986 were used to refine 181 parameters, final $R(F)$, $wR(F^2)$ and S 0.043, 0.094 and 1.067, respectively. CCDC 769526 contains the supplementary crystallographic data for CuETBb compounds. These data can be obtained free of charge as informed in reference.[20]

The experiments involving separation were carried out in a CE system (HP3d CE, Agilent Technologies, Palo Alto, CA, USA) equipped with a DAD set at 262 nm, a temperature control device, maintained at 25 ºC, 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 (40.0 cm effective length) × 75 μm ID × 375 μm OD was used.

The dissolution of samples and standards were carried out in a Unique ultrasonic cleaner, USC (2850) model, power of 120 W (São Paulo-SP, Brazil) - ultrasom.

Analytical procedures

Spectrophotometric measurement was performed by using aqueous solution of HAc/NaAc buffer and Cu(II) as reference in the same concentrations used to standards and sample dilution.

As for CE, when a new capillary was used, it was conditioned by a pressure flush of 1.0 mol L^{-1} NaOH solution (30 min), deionized water (5 min) and electrolyte solution (60.0 mmol L^{-1} of HAc/NaAc and Cu(II) of 5.0 mmol L^{-1}) (10 min). Between runs, the capillary was replenished with 0.2 mol L^{-1} NaOH solutions (2 min), deionized water (2 min) and fresh electrolyte solution (3 min, pressure flush).

Software

Microsoft Excel 2007 was used to calculate matrices and to make a response surface graphic. A normality test (Shapiro–Wilk test), a homogeneity of variance test (Levene statistic) and an independent sample t-test were performed using SPSS 8.0 for windows.

Results and Discussion

Absorbance signal optimization

After confirming the formation of complex CuETB,[7,10] a spectrophotometric study was made to maximize the absorbance signal. A 3^2 experimental design involving HAc/NaAc (pH 4.6) buffer and Cu(II) with triplicate in central point as factors was performed. The factors were evaluated in three concentration levels: 60.0, 32.5 and 5.0 mmol L^{-1} for HAc/NaAc buffer and 0.5, 0.3 and 0.1 mmol L^{-1} for Cu(II). Table 1 shows the experimental design matrix with responses and Figure S1 (supplementary information S1) the electronic spectra obtained.

Table 1. Experimental design 3^2 and responses

Experiment	Cu(II)	HAc/NaAc buffer	Absorbance
1	−1	−1	0.259
2	−1	0	0.212
3	−1	+1	0.188
4	0	−1	0.588
5	0	0	0.523
6	0	+1	0.468
7	+1	−1	0.746
8	+1	0	0.732
9	+1	+1	0.652
10	0	0	0.541
11	0	0	0.535
12	0	0	0.525

Cu(II) (mmol L^{-1}): (−1) 0.1; (0) 0.3; (+1) 0.5.
HAc/NaAc buffer (mmol L^{-1}): (−1) 5.0; (0) 32.5; (+1) 60.0.

By analyzing the results of 3^2 experimental design the CuETB complexation at levels (-1) of HAc/NaAc buffer and (+1) of Cu(II) was found to lead to the highest absorbance signal. Thus, the optimum condition found was 5.0 mmol L^{-1} of buffer HAc/NaAc and 0.5 mmol L^{-1} of Cu(II).

The response data obtained for absorbance are given in Table 1. By using a fitted full quadratic model equation 1, a response surface regression analysis for each response factor was performed using coded units. Table 2 shows the values calculated for the coefficients and *p*-values (*p*-value is the probability of the null hypothesis). Using a 5% significance level, a factor is considered to affect the response if the coefficients differ significantly from zero and the *p*-value < 0.050.[8]

$$\hat{y} = \hat{\beta}_0 + \sum_{i=1}^{k} \hat{\beta}_i x_i + \sum_{i=1}^{k} \hat{\beta}_{ii} x_i^2 + \sum_{i}\sum_{\substack{j \\ i<j}} \hat{\beta}_{ij} x_i x_j + r_i \quad (1)$$

Table 2. Model estimators for absorbance

Term	Coefficient	Error	*p*-value
Constant	0.532	± 0.004	0.000
[Cu(II)]	0.245	± 0.003	0.000
[HAc/NaAc]	–0.0475	± 0.003	0.001
[Cu(II)] × [Cu(II)]	–0.0629	± 0.005	0.001
[HAc/NaAc] × [HAc/NaAc]	–0.00690	± 0.005	0.277
[Cu(II)] × [HAc/NaAc]	–0.00575	± 0.004	0.268

The results show that only the terms [HAc/NaAc] × [HAc/NaAc] and [Cu(II)] × [HAc/NaAc] were not significant for absorbance, in other words the buffer quadratic parameter and the Cu(II) with buffer interaction were not significant. The fit models were evaluated (ANOVA) and the results found indicated that no evidence of lack of fit was observed in the 95% confidence interval, because $F_{cal} = 2.97$ is lower than $F_{tab(3;3;0.05)} = 9.28$. Thus, the representative surface response for the model is presented in Figure 2.

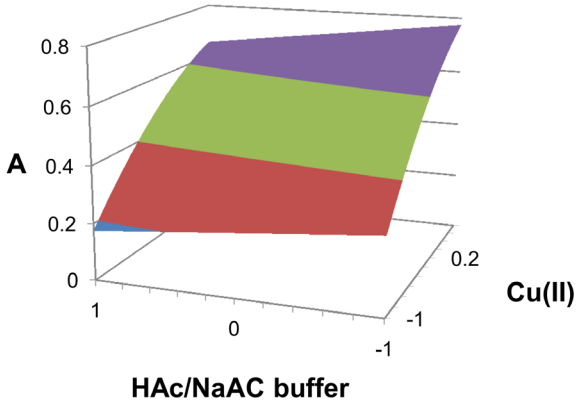

Figure 2. Surface response for fitted model.

Table 3. Experiments for the continuous variation method

Experiment	Volume of ETB 1 mmol L^{-1}	Volume of Cu(II) 1 mmol L^{-1}	Molar ratio Cu:ETB	Mole fraction of Cu(II)	$A_{mean\ corrected}$
1	0.50	4.50	9.00	0.90	0.078
2	1.00	4.00	4.00	0.80	0.175
3	1.25	3.75	3.00	0.75	0.221
4	1.67	3.34	2.00	0.67	0.266
5	2.00	3.00	1.50	0.60	0.314
6	2.50	2.50	1.00	0.50	0.330
7	3.00	2.00	0.67	0.40	0.316
8	3.34	1.67	0.50	0.33	0.293
9	3.75	1.25	0.33	0.25	0.238
10	4.00	1.00	0.25	0.20	0.191
11	4.50	0.50	0.11	0.10	0.086

n = 2.

CuETB spectrophotometric study

Continuous variation method

The continuous variation method or Job's method was used in order to establish the stoichiometry of the predominant complex:[21]

$$a\ Cu^{2+}_{(aq)} + b\ ETB_{(aq)} \rightleftharpoons Cu_bETB_{a(aq)}$$

The procedure consisted of a mixture of equimolar quantities of Cu(II) and ETB as shown in Table 3. The HAc/NaAc buffer concentration was kept constant at 5.0 mmol L^{-1} (according to optimization by 3^2 experimental design) and absorbances were monitored at 262 nm. The corrected absorbance (equation 2) versus Cu(II) molar fraction was plotted.

$$A_{corrected} = A_{measured} - \varepsilon_{ETB}\ b\ c_{ETB} - \varepsilon_{Cu(II)}\ b\ c_{Cu(II)} \quad (2)$$

Figure 3 shows UV-Vis spectra for the 11 experiments and Job's diagram. Job's diagram analysis shows that the maximum absorbance reached in Cu(II) molar fraction was equal to 0.5 corresponding to the stoichiometry 1:1 of the predominant complex.

CuETB formation constant

Rigorously, to obtain the constant CuETB complex formation (K_f) it must be considered the activities of the species involved in the chemical equilibrium. However, for diluted solutions the coefficient of activity is close to the unity and the activity is close to the species concentration in the chemical system. Thus, K_f can be determined according to equation 3.[21]

$$K_f = \frac{[CuETB]}{[Cu^{2+}][ETB]} \quad (3)$$

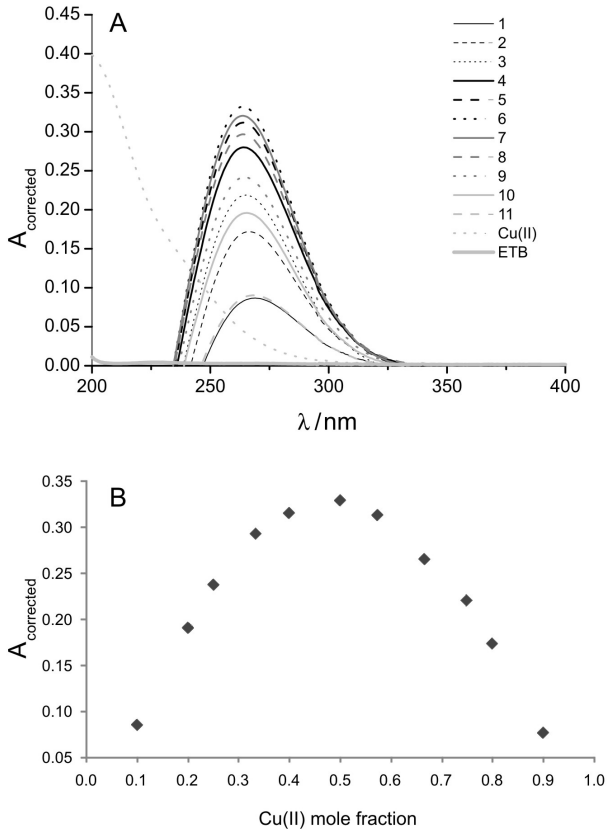

Figure 3. (A) UV-Vis spectra obtained for the continuous variation method; (B) Job's diagram.

A set of solutions containing constant ETB concentration and Cu(II) increments diluted in 5.0 mmol L^{-1} of HAc/NaAc buffer (Table 4) was performed. The total ETB concentration is equal to:

$$[ETB] = [ETB]_{initial} - [CuETB] \quad (4)$$

Replacing equation 4 in equation 3:

$$\frac{[CuETB]}{[Cu]} = K_f([ETB]_{initial} - [CuETB]) \quad (5)$$

The complex concentration formed was calculated by Scatchard approach (equation 6). The molar absorptivities of ETB and complex (ε_{ETB} and ε_{CuETB}) were determined by experiments 1 and 7, respectively (Table 4):

$$[CuETB] = \frac{\Delta A}{\Delta \varepsilon} = \frac{A_n - A_{n-1}}{\varepsilon_{CuETB} - \varepsilon_{ETB}} \quad (6)$$

A plot of [CuETB]/[Cu] *vs.* [Cu] known as Scatchard diagram was fitted (Figure 4) by linear regression model, obtaining a negative slope which corresponds to the K_f.

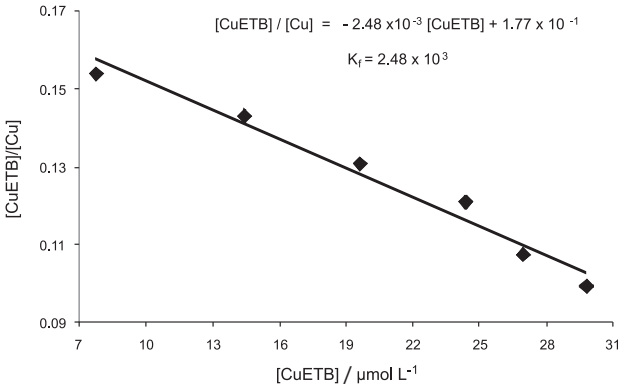

Figure 4. Scatchard diagram for the binding of Cu (II) to ETB.

CuETB X-ray diffraction

Parallel to the CuETB complexation study, a study involving X-ray diffraction method on a single crystal of CuETB complex was performed. The crystal data are listed in Table S1 in supplementary material. It may be inferred from the crystal structure that the complex CuETB is cationic, similar to the crystal obtained and analyzed by liquid chromatography–mass spectrometry (LC–MS) after dissolution in methanol and water solution (1:1, v/v),[10] although the species observed in the crystal is mono-cationic. This complex is mono-cationic and the neutrality is obtained by a nitrate anion present in the lattice. The

Table 4. Experiments and results for the Scatchard diagram

Experiment	[ETB] µmol L^{-1}	[Cu(II)] µmol L^{-1}	A_{mean}	ΔA_{mean}	[CuETB] µmol L^{-1}	[CuETB]/[Cu]
1	300.3	0.0	0.004	-	-	-
2	300.4	50.5	0.115	0.111	7.8	0.154
3	300.2	100.9	0.210	0.206	14.4	0.143
4	300.2	150.1	0.284	0.280	19.6	0.131
5	300.7	201.2	0.352	0.348	24.4	0.121
6	300.7	251.1	0.389	0.385	27.0	0.107
7	300.2	300.3	0.429	0.426	29.8	0.099

n = 2; $\Delta\varepsilon_{CuETB}$ = 14284.4 L mol^{-1} cm^{-1}.

copper is coordinated to five atoms giving rise to a square-pyramidal geometry (the trigonality index $\tau = 0.01$), where the ETB acts as a tetradentate ligand (NH and OH groups) in a *cis* conformation and the geometry is completed with a chloride ion. The basal plane is formed by two N atoms, one O atom and the chloride ion, although the apical position is occupied by the OH group of the ETB molecule (Figure 5). The Cu-O bond distance of apical position is larger than the other copper bond distances of basal plane, indicating the Jahn-Teller distortion in this complex (Table S2). The Cl atom of cationic unit interacts with Cu of another cationic unit with Cl-Cu distance of 2.963(1) Å. This interaction form a one-dimensional network with a wave design which presents two different orientations in the crystal that are parallel to (011) and (001) directions. The Cu-Cu distance and the Cu-Cu-Cu angle in the same 1D chain are 4.968(1) Å and 84.8(1)°, respectively. The 1D chains are involved in hydrogen bonds to nitrate ions which form a three-dimensional arrangement.

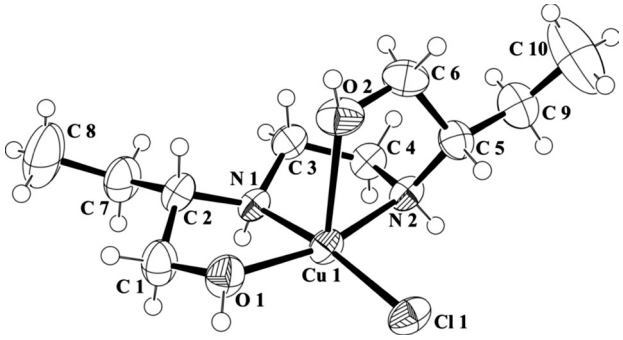

Figure 5. Crystal structure of CuETB complex. Thermal ellipsoids are drawn at the 50% probability level.

Compounds formed by reaction of Cu(II) and ETB are described in the literature. Annapurna *et al.*[22] synthesized the bivalent species $[Cu(ETB)_2]^{2+}$ by reaction of ETB·2HCl and $CuCl_2$ using spectroscopic techniques to propose a structure where the Cu(II) is coordinated to two ETB molecules by NH groups only. Hassan and Shalaby[5] also used spectroscopic information for suggesting the formation of cationic complex with ETB and Cu(II) in 1:1 ratio and that the ETB acts as a tetradentate ligand, similar to the crystal structure here obtained. Some cationic species of ETB unit are described in the literature.[23-26] In these compounds the positive charge is obtained by protonation of NH groups of ETB. In solid state two conformations for quiral ETB cationic molecule (SS and RS) with some counter-ion were observed: nitrate,[23,25] oxalate[24] and sulfate.[26] These species are stabilized by O-H···O and N-H···O hydrogen bond forming two[23,25] and three-dimensional network.[26] The observation of compound (I) in solid state indicates that the ETB residue is in SS conformation. The Flack parameter[27] for this compound is [0.04(2)] indicating that the absolute conformation is correct. This conformation is also observed in therapeutic active form of ethambutol dihydrochloride.[28]

The CuETB and ETB forms I and II crystallize in the same crystal system (orthorhombic) and the most relevant difference among of CuETB and ETB (forms I and II) is in N-C-C-O torsion angles. In ETB, which present a 2-fold axis in the middle of the molecule, this torsion angle is 58.6(6) and 54.2(10)° for forms I and II, respectively.[28] In CuETB, N-C-C-O torsion angles are smaller than that observed to ETB (51.0(4) and 48.5(4)°) due to the fact that the OH groups are coordinated to metal site in the complex. Despite this, the coordination does not change the ETB residue conformation.

The CuETB crystal is stabilized by O-H···O and N-H···O hydrogen bonds where the O-O and N-O distances are around 2.740 and 3.035 Å, respectively. These interactions form a complex three-dimensional arrangement with three distinct graph-set[29] N1 = C(8), N2 = C(17) $R^9_{10}(38)$, N3 = C(6). The O···O interactions give rise to a one-dimensional arrangement with wave design parallel to b axis formed by $CuCl(NH)_2(OH)_2$ unit and NO_3^{2-}. The distance Cu-Cu in this wave design is 10.586(1) Å and the angle Cu-Cu-Cu is 82.3(1)°. On the other hand, the N···O interaction does not form a chain and these interactions are associated to another O-H···O hydrogen bond forming a wave design one-dimensional chain parallel to c axis. These two hydrogen bond wave designs form a two-dimensional net whose graph-set is $C(17)R_{10}(38)$, as it can be seen in Figure S6. The details of the hydrogen-bonding geometry are listed in Table S1. Two weak interactions are also observed in compound (I): N-H···Cl and C-H···O, where the N-Cl distances are 3.333(3) and 3.459(3) Å and C-O distances are 3.253(6) and 3.690(7) Å. These interactions give rise to a bi-dimensional network parallel to (110) direction.

Pharmaceutical formulation analysis

After method optimization, it was applied to the analysis of tablets. The quantification was performed by external calibration under eight concentration levels: 0.08, 0.12, 0.16, 0.20, 0.24, 0.28, 0.32 and 0.36 mmol L^{-1} of ETB·2HCl in genuine triplicate. Once the analysis of regression study using least squares method for linear model presented lack of fit, a polynomial model was fitted: $\hat{Y} = 0.009\ (\pm 0.013) + 2.725\ X\ (\pm 0.136) - 2.298\ X^2\ (\pm 0.305)$. The fitted model was evaluated using an a priori linearity hypothesis test[30] (equation 7) and the results found indicated that no evidence of lack of fit was observed in the 95%

Table 5. Statistical analysis for comparison between methods

Genuine replicates	1	2	3	4	5	mean	s
*Spectrophotometry (mg)	413.8	395.4	421.5	413.8	395.4	408.0	11.9
**CZE (mg)	392.8	382.7	397.6	404.9	400.1	395.6	8.4

s: standard deviation; label claim: 400.0 mg; Normality test (Shapiro-Wilk): 0.075*; 0.703**; Homogeneity of variance p-value (Levene): 0.203; Independent sample t test p-value: 0.095.

confidence interval, because F_{cal} = 2.66 is lower than $F_{tab.(6;16;0.05)}$ = 2.74.

$$F_{calculated} = \frac{s_{yx}^2}{s_y^2} = \frac{\sum_{i=1}^{p} m_i(\bar{y}_i - \hat{y}_i)^2/(p-2)}{\sum_{i=1}^{p}\sum_{j=h}^{m_i}(y_{ij} - \bar{y}_i)^2/(m-p)} \quad (7)$$

Where: m_i is the number of measurement; p is the calibration points; m is the product between p and m_i.

Table 5 shows the results obtained to ETB·2HCl determination in pharmaceutical formulations by spectrophotometry in comparison with CZE.[10] Statistical tests such as normality test (Shapiro-Wilk), homogeneity of variance (Levene) and independent sample t test have shown that no significant difference between methods was evidenced (p-value > 0.05).[31]

Recoveries were evaluated by standard additions method, according to equation 8. The results found are within interval of 100.9 to 104.0%, being considered satisfactory.

$$R\,(\%) = \frac{C_{X+S} - C_X}{C_S} \times 100 \quad (8)$$

Where: C_x is the sample concentration; C_{x+s} is the sample plus standard concentration; C_s is the standard concentration.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated from UV-Vis spectra obtained for pharmaceutical analysis taking into account signal-noise ratio equal to 3 and 10 respectively.[32,33] The values found were 0.8 and 2.8 mg L^{-1} of ETB·2HCl for LOD and LOQ, respectively.

Conclusions

An alternative method for ETB·2HCl determination by UV spectrophotometry using the ETB complexation with Cu(II) was proposed. It is important to stress that the main contributions of the spectrophotometric method here optimized are: low cost for instrumental acquisition and by analysis, lack of necessity of analyst expertise to perform analysis and possibility to perform quality control in remote locals without sophisticated analytical conditions. On the other hand, the crystal structure revealed that the complex CuETB is cationic, similar to that observed in solution where the ETB act as a tetradentate ligand (NH and OH groups) in a *cis* conformation. Finally, the optimized method can be useful to quality control of the active ingredient in pharmaceutical formulations.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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