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A Simple Spectrophotometric Approach for the Assessment of Etidronate in Pharmaceutical Dosage Forms

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Abstract: Etidronate belongs to the bisphosphonates class of drugs. It is well known that the quantitative analysis of bisphosphonates is challenging because they don't exhibit significant absorption spectrum or fluorescence intensity due to the absence of the chromophoric function groups in their chemical structures. In the current study, a simple and economical spectrophotometric approach has been developed and validated for the estimation of etidronate. The suggested technique depends on the complex formation between etidronate and ferric chloride, where the formed complex was measured at 300 nm. This approach exhibited good linearity over the range of 5.0–50.0 µg/mL with a detection limit (LOD) of 0.454 µg/mL. The approach was successfully applied for the determination of the cited drug in its tablet formulation with high % recoveries (99.82 % - 100.09%) and low percentage RSD readings (less than 2%). The developed method solved the problem of the absence of chromophoric groups in the cited drug using a simple, rapid, accurate, and precise procedure. According to ICHQ2 (R1) guidelines, the proposed method was validated.

Keywords: Etidronate; Spectrophotometry; Complex formation; Bisphosphonates; Tablets

1. INTRODUCTION

Etidronate (ETD) is chemically known as disodium;hydroxy-[1-hydroxy-1-[hydroxy(oxido)phosphoryl]ethyl]phosphinate [1] (**Fig. 1**). ETD is a member of the bisphosphonate medication class. It is prescribed in cases of Paget's disease of the bone and osteoporosis, both of which involve significant bone resorption. Because ETD can prevent bone resorption, it is useful in clinical utility [2, 3]. Patients with metastatic prostate cancer who use etidronate were found to have a significant reduction in the bone stiffness [4]. ETD is a member of the bisphosphonate medication class.

It is well known that the quantitative analysis of bisphosphonates is challenging because they don't exhibit significant spectrum of absorbance or fluorescence intensity due to the absence of the chromophoric function groups in their chemical structures [5]. It has been documented that indirect Ultraviolet or fluorescence detection of bisphosphonate can be achieved by inserting a UV-absorbing compound or a fluorescent substance [6]. Through the addition of chromophores and fluorophores *via* derivatization, their estimation was performed using UV or fluorescence detectors [7]. From the literature, oxidation and metal complexation were reported to be effective methods for the determination of such drugs [8-10].

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A literature review showed various techniques for the determination of ETD, including spectrophotometry [8, 9, 11], spectrofluorimetry [12, 13], chromatography [7, 14-18], potentiometry [19, 20], and ion exchange chromatography [21-24]. Only a few spectrophotometric approaches were described for the assessment of ETD, and the majority of reported analytical methods utilized for its determination were HPLC with fluorimetric detection [7], HPLC with a charged aerosol detector [14], ion pair RP-HPLC [25], isocratic HPLC method with evaporative light-scattering detection [16], electrospray ionization mass spectrometry [17], ion chromatography with indirect UV detection [26-28], ion chromatography with suppressed conductivity detection [22]. All of these techniques were expensive, tedious [7], time-consuming [7, 14], and need large amounts of organic solvents [26, 28], which decreases the method greenness.

Spectrophotometry is a good analytical approach for routine analysis of ETD because it is widely available in most laboratories, simple and inexpensive technique. ETD lacks of chromophores, therefore, its determination is quite problematic. Accordingly, the derivatization of ETD and measuring the produced reaction product spectrophotometrically can be adopted for designing a simple and affordable method for its estimation in pure form and pharmaceutical dosage forms. The developed spectrophotometric method is superior to the previously published ones [8, 10, 29], as it has a simpler process and wider linearity range, as well as being rapid and more convenient, so that it can be applied in quality control laboratories for the routine analysis of ETD.

2. METHODS

2.1. Instrumentation

- Shimadzu,1900i spectrophotometer (double-beam UV-visible) was used to detect the absorption spectra.

- Sonicator, model SS 101 174 H 230 (USA).

- pH-meter Jenway model 3510 was used for adjustment of the pH.

2.2. Materials and reagents

- Etidronate (98.3%) was obtained from Memphis Co. for Pharmaceutical and Chemical Industries, Cairo, Egypt.

- All of the reagents and chemicals used were with high purity, and the water used in this technique was distilled. Ferric chloride, phosphoric acid and potassium dihydrogen phosphate; were obtained from El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt.

2.3. General procedure

2.3.1. Preparation of stock solution of pure drug, reagent, and buffer

-Stock solution of ETD (100.0 µg/mL) was prepared by dissolving 10.0 mg of ETD in distilled water then completing to 100 mL with the same solvent to get the desired concentration. The working solutions were obtained by further dilution with the same solvent. When stored in the refrigerator, the stock solution remained stable for at least two weeks.

-Stock of Ferric chloride (0.0811 g) was dissolved in deionized distilled water to produce this solution, then completed to 100 mL to get the desired concentration (5.0×10^{-3} M).

- Phosphate buffer (0.05 M) pH (5) was prepared by using of potassium dihydrogen phosphate and its pH was adjusted with 0.04 M Phosphoric acid.

2.3.2. Calibration curve

2.3.2.1. Complex formation with ferric chloride

Into several sets of volumetric flasks of 10 mL, working solutions of ETD (5.0 -50.0 µg/mL), 1.0 mL (pH 5) of phosphate buffer solution, and 40.0 µl (5.0×10^{-3} M) ferric chloride solution were added. These flasks were then diluted to 10 mL with distilled water, and the mixtures were gently shaken. The wavelength used to estimate the absorbance intensity was 300 nm. By graphing the absorbance versus drug concentration, the calibration curve was generated. The regression equation was then established.

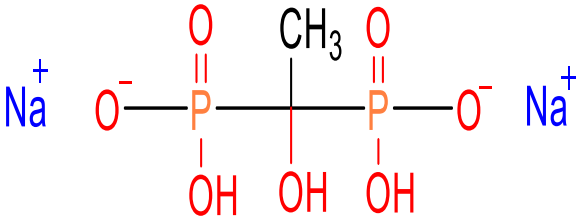
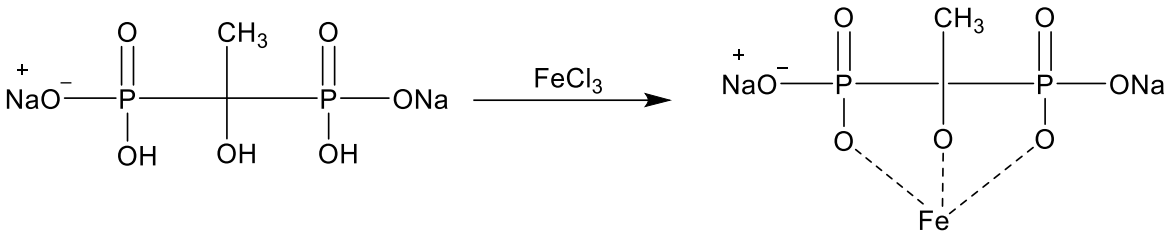


Figure 1. Chemical structure of etidronate

2.3.3. Application for determination of ETD in prepared tablets

Since ETD (Bonatronil®) tablets are not available in Egyptian market, they were prepared in our lab by mixing talc (20 mg), maize starch (15 mg),



Scheme 1. Complexation of ETD with ferric chloride.

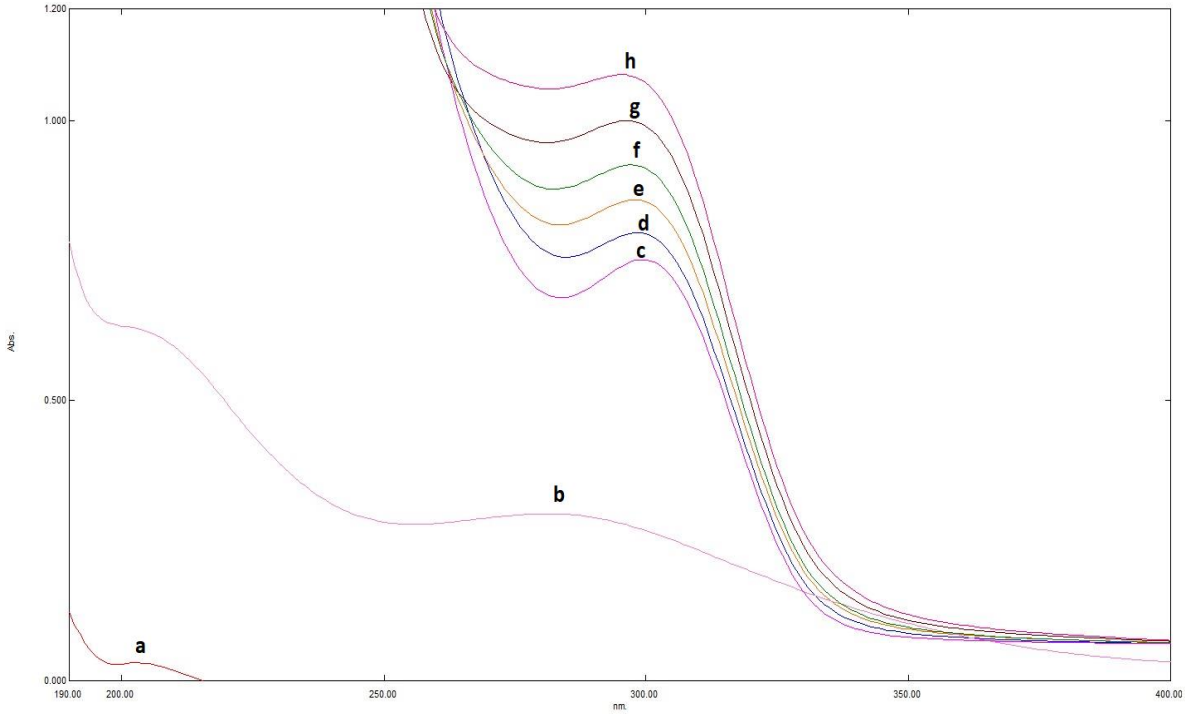


Figure 2. Absorbance spectra of ETD and $FeCl_3$ complex (5.0×10^{-3} M) at 300 nm using different concentrations of ETD (5.0-50.0 µg/mL) where, ETD (a), ferric chloride (b), concentrations of ETD- $FeCl_3$ complex (c, d, e, f, g, h).

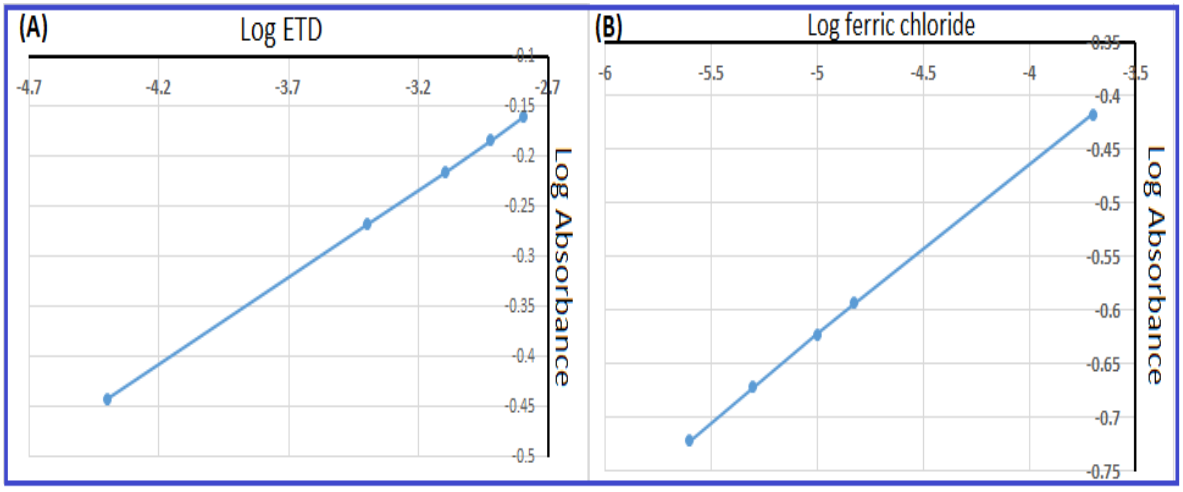


Figure 3. Limiting logarithmic plots for the molar reactivity of ETD with $FeCl_3$

magnesium stearate (10 mg), and lactose (15 mg) for each tablet keeping the drug in its pharmaceutical concentration. Then, a weighed quantity of the powder corresponding to 10 mg ETD was transferred to a 100-mL flask, then 40 mL of distilled water were added, and the mixture was sonicated for 20 minutes. The solution was filtered before dilution with distilled water to a final volume of 100 mL. The filtrate was serially diluted to produce various concentrations. After that, the steps for construction of calibration curves described in sections "2.3.2.1" for developing calibration curve for the suggested approach, respectively was carried out. The regression equation was utilized for calculation the ETD content in the prepared tablet.

3. RESULTS AND DISCUSSION

3.1. Complex formation with ferric chloride

The formation of binary complexes of ETD with metal ions was the base for the development of this approach. This study provides a simple, specific, and economic spectrophotometric approach for estimation of ETD through complex formation reaction with ferric chloride **(Scheme 1)**. The maximum absorbance was measured at 300 nm after complexation with ferric ions **(Fig. 2)**.

3.1.1. Optimization of experimental conditions

3.1.1.1. Effect of pH and volume of the buffer

Using phosphate buffer, the effect of pH of the complex formation was evaluated. It was observed that pH 5 had the highest absorbance for the ETD in this technique (**Fig. S1**). As a result, several buffer volumes ranging from 0.5 to 2.5 mL were investigated. It was revealed that 1 mL of phosphate buffer produced the optimal absorbance ETD (**Fig. S2**). Therefore, 1 mL of phosphate buffer with pH 5 was used throughout the investigation.

3.1.1.2. Metal ion concentration

Increasing the amounts of ferric ion solution ranging from 20 to 80 µl was used to study the effect of ferric ion concentration. It was observed that 40 µl of 5.0×10^{-3} M ferric chloride was the ideal concentration to obtain the maximum absorbance of ETD-ferric iron complex (**Fig. S3**).

3.1.1.3. Effect of temperature

Evaluation of the influence of temperature on the formation of the complex in the range of 25-70°C was performed. The optimum response was carried out at room temperature (25 °C) and the absorbance decreased when the temperature was increased. (**Fig. S4**).

3.2. The reaction stoichiometry

The reaction stoichiometry between ETD and $FeCl_3$ was investigated using the limiting logarithmic technique. The curves of log absorbance versus log [ETD] produced straight lines with slopes of 0.174 / 0.160 in the suggested technique. **(Fig. 3)**. As a result, it is shown that the reaction occurs in a 1:1 ratio. **Scheme 1** illustrates a proposal of the reaction mechanism based on the molar reactivity of $FeCl_3$ and ETD.

3.3. Method's Validation

In conformity with the International Conference on Harmonization's (ICH) guidelines [30], full validation was carried out for the suggested technique.

3.3.1. Linearity and range

The calibration charts were constructed by plotting the concentrations of ETD (µg/mL) versus the obtained absorbance values. The analysis of data using the following equations can be applied to represent linear regression:

$$y = 0.0070x + 0.721 \qquad r = 0.9999$$

The linearity range for the developed technique is 5.0-50.0 µg/mL (**Fig. 4**). The correlation coefficient values (r) were 0.9999, demonstrating the good linearity of the calibration curve for the developed method (**Table 1**).

Table 1: Performance data for etidronate by the proposed spectrophotometric method

3.3.2. Accuracy and precision

Parameters	Result
Concentration range (µg/mL)	5.0-50.0
LOD (µg/mL)	0.454
LOQ (µg/mL)	1.37
Correlation coefficient (r)	0.9999
Slope	0.0070
Intercept	0.721
Standard deviation of the residuals ($S_{y/x}$)	0.0012
Standard deviation of the intercept (S_a)	0.0009
Standard deviation of the slope (S_b)	0.00003
% Error	0.047
%RSD	0.114

The proposed technique was statistically evaluated using the variance ratio F-test and student t-test [31] which indicated no difference in the results between the proposed approach and the comparison method for determination of ETD [8] in terms of accuracy and precision, respectively (**Table 2**).

Evaluating three concentrations of ETD within one day for the intra-day precision; while analysis of triplet concentrations in three different days was performed to estimate the inter-day precision. Intra-day and inter-day precision values were demonstrated in **Table 3**. low percent of RSD values (less than 2%) indicated the high precision of the suggested technique.

Table 2. Application of the proposed spectrophotometric method for the determination of etidronate in pure form and dosage forms

Parameters	**Method**		**Comparison method [8]**	
	Amount taken (µg/mL)	Found (%)	Amount taken (µg/ml)	Found (%)
ETD (Pure form)	5.0	99.82	1.0	99.57
	10.0	100.09	5.0	100.09
	20.0	99.97	8.0	99.87
	30.0	100.08		
	40.0	99.87		
	50.0	99.88		
X- ± SD		99.95 ± 0.114		99.54± 0.53
Prepared ETD tablets	5.0	99.37	1.0	100.0
	10.0	100. 87	5.0	101.08
	20.0	98.86	8.0	100.34
	30.0	100.03		
X- ± SD		99.70± 1.05		100.58± 0.54
Student's t-test		1.35 (2.57)		
Variance ratio F-test		3.78 (19.16)		

Each reading is the average of three separate determinations.

3.3.3. LOQ and LOD

The following formulas were used to determine LQ and LD, according to ICH:

$$\mathbf{LOD = 3.3\ Sa / slope}$$
$$\mathbf{LOQ = 10\ Sa / slope}$$

Where LOD refers to the limit of detection, LOQ is the limit of quantitation, and S_a is the standard deviation of intercept. Low values of LOQ and LOD were obtained for the proposed approach, indicating the high sensitivity of the proposed method (**Table 1**).

Table 3. Intra-day and inter-day precision data for the determination of etidronate by the proposed method

Sample concentration	**Intra-day**	**Inter-day**
10.0 µg/mL		
Mean found (%) **X`**	98.77	98.81
± SD	0.288	0.448
%RSD	0.291	0.453
% Error	0.168	0.261
20 .0 µg/mL		
Mean found (%) **X`**	99.57	99.41
± SD	0.521	0.697
%RSD	0.523	0.701
% Error	0.302	0.404
30.0 µg/mL		
Mean found (%) **X`**	99.17	100.35
± SD	0.271	0.711
%RSD	0.273	0.708
% Error	0.157	0.408

3.3.4. Robustness

The robustness of the proposed approach was verified through an estimation of the effect of minor changes in the experimental conditions affecting the absorbance of the formed complex including pH (5 ± 0.1), volume of buffer (1 mL ± 0.1), and volume of reagent (40.0 µl ± 0.1) for the developed technique. No obvious effects were found on the values of the absorbance, demonstrating the proposed technique's robustness (**Table 4**).

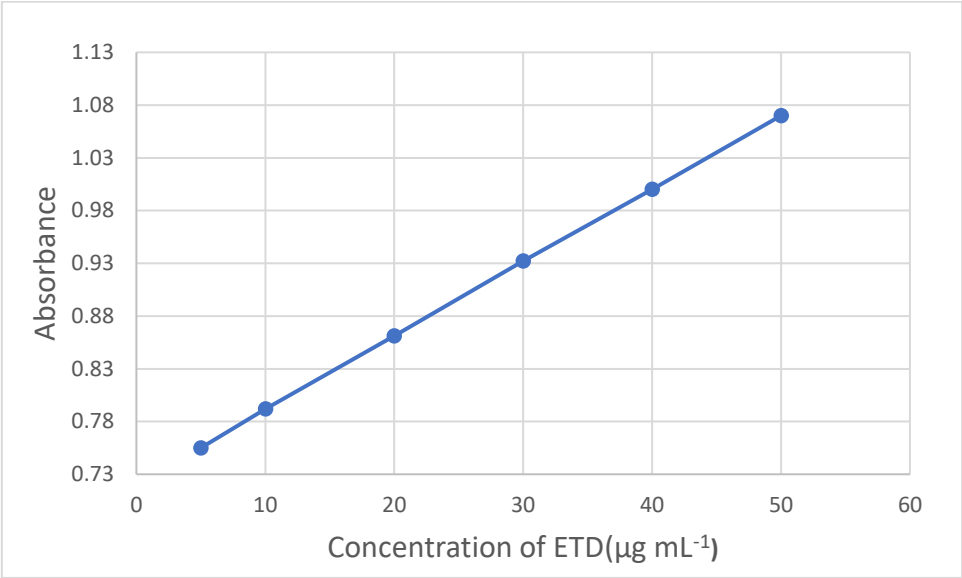


Figure 4. Calibration curve of ETD and $FeCl_3$ complex

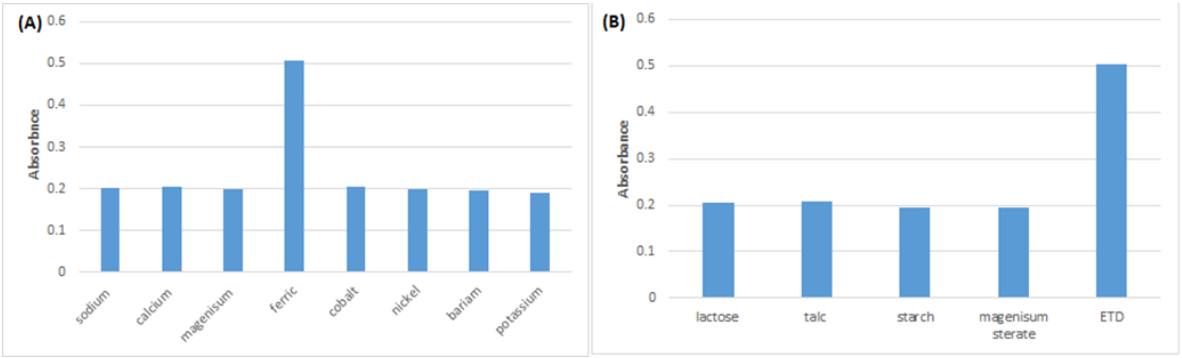


Figure 5. Evaluation of the specificity: response towards various metal ions (A), and response towards possible tablet interferents (B).

Table 4. Investigation of the robustness of the proposed spectrophotometric method

Parameters	%Recovery	% RSD
1- pH (5 ± 0.1)		
4.9	**100.42**	**0.176**
5.0	**100.53**	**0.475**
5.1	**100.11**	**0.812**
2-Volume of buffer (1 mL ± 0.1)		
0.9	**99.84**	**0.746**
1.0	**100.54**	**0.290**
1.1	**99.18**	**1.22**
3-Volume of $FeCl_3$ (40µl ±1.0)		
39.0	**99.22**	**0.414**
40.0	**100.57**	**0.304**
41.0	**99.60**	**0.970**

3.3.5. Specificity

Investigation of the specificity was performed by examining the presence of any influence from additives of the prepared tablets such as talc, starch, magnesium stearate, and lactose (**Fig. 5A)**. Moreover, to examine the specificity of the ferric chloride to ETD, other metal ions, such as Na+, Ba^{+2}, Co^{+2}, K^{+}, Ni^{+}, Fe^{+3}, Mg^{+2}, and Ca^{+2} were tested. Stocks of sodium chloride, barium chloride, cobalt sulfate, potassium chloride, nickel sulfate, ferric chloride, magnesium sulfate, calcium chloride, (0.0292), (0.1041), (0.0774), (0.0372), (0.0773), (0.0811), (0.0601), (0.0554) were dissolved in deionize distilled water to produce these solutions respectively, then completed to 100 mL to get the desired concentration at a concentration of (5.0×10^{-3} M). Into several sets of volumetric flasks of 10 mL, working solution of ETD (10.0 µg/mL), 1.0 mL (pH 5) of phosphate buffer, and 40.0 µl (5.0×10^{-3} M) of the solutions of several metals were added. It was found that the absorbance was unaffected by these metal ions, and only ferric chloride had an impact (**Fig. 5B)** illustrating the proposed spectrophotometric method could determine ETD with high % recoveries (99.82% - 100.09%) and low percentage RSD readings (less than 2%), proving the high level of specificity of the suggested approach (**Table 2**).

3.4. Application in dosage form

The approach mentioned was used successfully for the estimation of ETD in prepared tablets. The result of the proposed technique introduced high reproducibility and specificity. This approach was employed for the estimation of the content of ETD in prepared tablets with low percent of RSD readings (less than 2%) and a high percent of recovery (98.86%- 100.87%). It was observed that no major variation was detected between the performance of the proposed technique and the comparison approach utilizing (Variance ratio F-test) and (student t-test) [8] for precision and accuracy, respectively.

4. CONCLUSIONS

Absence of chromophoric function groups in the structure of ETD makes its direct determination challenging. This work introduces simple and economic technique of spectrophotometry for the estimation of ETD in pure form and tablet form. The suggested approach based on the creation of the complex between ETD and ferric chloride, where the formed complex had absorbance at a wavelength of 300 nm. The developed approach didn't require any dangerous chemicals or complicated requirements as in the chromatographic techniques. According to ICH criteria, the suggested technique was fully validated.

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