Hindawi Publishing Corporation
Journal of Chemistry
Volume 2013, Article ID 402164, 9 pages
http://dx.doi.org/10.1155/2013/402164

Research Article

Utility of Charge Transfer and Ion-Pair Complexation for Spectrophotometric Determination of Eletriptan Hydrobromide in Pure and Dosage Forms

Ayman A. Gouda,[1,2] Ragaa El Sheikh,[1] and Rham M. El-Azzazy[1]

[1] *Chemistry Department, Faculty of Sciences, Zagazig University, Zagazig 44519, Egypt*
[2] *Makkah Community College, Umm Al-Qura University, Makkah, Saudi Arabia*

Correspondence should be addressed to Ayman A. Gouda; aymangouda77@gmail.com

Received 13 May 2013; Revised 30 July 2013; Accepted 1 August 2013

Academic Editor: David Bongiorno

Three simple, sensitive, and accurate spectrophotometric methods have been developed for the determination of eletriptan hydrobromide (ELT) in pure and dosage forms. The first two methods are based on charge transfer complex formation between ELT and chromogenic reagents quinalizarin (Quinz) and alizarin red S (ARS) producing charge transfer complexes which showed an absorption maximum at 569 and 533 nm for Quinz and ARS, respectively. The third method is based on the formation of ion-pair complex between ELT with molybdenum(V)-thiocyanate inorganic complex in hydrochloric acid medium followed by extraction of the colored ion-pair with dichloromethane and measured at 470 nm. Different variables affecting the reactions were studied and optimized. Beer's law is obeyed in the concentration ranges 2.0–18, 1.0–8.0, and 2.0–32 μg mL^{-1} for Quinz, ARS, and Mo(V)-thiocyanate, respectively. The molar absorptivity, Sandell sensitivity, detection, and quantification limits are also calculated. The correlation coefficients were ≥0.9994 with a relative standard deviation (R.S.D%.) of ≤0.925. The proposed methods were successfully applied for simultaneous determination of ELT in tablets with good accuracy and precision and without interferences from common additives, and the validity is assessed by applying the standard addition technique, which is compared with those obtained using the reported method.

1. Introduction

Eletriptan hydrobromide is a novel, orally active, selective serotonin 5-HT1B/1D receptor agonist and is second gen eration antimigraine drug [1]. Eletriptan hydrobromide is chemically designated as (R)-3-[(1-methyl-2-pyrrolidinyl) methyl]-5-[2-(phenylsulfonyl)ethyl]-1*H*-indole monohydrobromide (Figure 1). Eletriptan hydrobromide is used for the treatment of acute migraine headaches. Its pharmacological effects include the constriction of cerebral blood vessels and neuropeptides secretion blockade which eventually relieves the pain [2]. The pharmacokinetics and metabolism of eletriptan have been investigated in rats, dogs and humans. In all three species, eletriptan was rapidly absorbed and extensively cleared by metabolism. The pathways of eletriptan metabolism are similar in rats, dogs and humans and principal routes include pyrrolidine *N*-demethylation to *N*-desmethyl eletriptan, together with *N*-oxidation, oxidation of the pyrrolidine ring, and formation of tetracyclic quaternary ammonium metabolites [3].

Eletriptan hydrobromide (ELT) is not official in any pharmacopoeia. Literature survey revealed that very few analytical methods have been reported for the determination of eletriptan in pure drug, pharmaceutical dosage forms, and biological samples using HPLC [4–6] and LC-MS [7] techniques. A few methods are found in the literature for the determination of ELT in pharmaceuticals and include spectrophotometry [8–12] and spectrofluorimetry [11, 12].

However, the reported methods, particularly those based on chromatography, are complex, require expensive experimental setup and skilled personnel, and are inaccessible to many laboratories in developing and under developed

Table 1: Comparison between the reported spectrophotometric methods for determination of ELT.

	Reagent	λ_{max} nm	Concentration range (μg mL^{-1})	LOD (μg mL^{-1})	Remarks	Reference
(1)	(a) First-order derivative spectrophotometry	215	5.0–25	*0.119*	Shorter wavelength	[8]
	(b) Second-order derivative spectrophotometry	236	5.0–25	0.347		
(2)	UV-spectrophotometry	221	2.0–10	0.9883	Shorter wavelength	[9]
(3)	UV-spectrophotometry	219	5.0–25	NR	Shorter wavelength	[10]
(4)	7,7,8,8 Tetracyanoquinodimethane (TCNQ)	744	10–70	4.38	Less sensitive, heating required	[11]
(5)	7-Chloro-4-nitrobenzofurazan	480	30–250	0.07	Less sensitive, heating and time required	[12]
(6)	Quinz	569	2.0–18	0.359	Simple, rapid, sensitive, selective and no heating step involved	Proposed methods
	ARS	533	1.0–8.0	0.215		
	Mo(V)-thiocyanate	470	2.0–32	0.332		

NR: not reported.

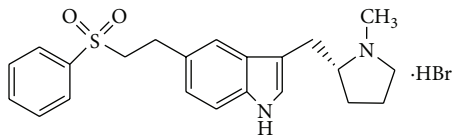


Figure 1: Chemical structure of eletriptan hydrobromide.

nations. In contrast, spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories. All the previously reported spectrophotometric methods are less sensitive, and few methods require a rigid pH control, and some methods have relatively shorter wavelengths and involve heating step as cited in Table 1.

In the present communication, we report the development of three accurate and precise spectrophotometric methods for simultaneous determination of ELT via charge transfer complexation with alizarin derivatives, quinalizarin (Quinz) and alizarin red S (ARS) as chromogenic reagents, and formation of ion-pair complex with Mo(V)-thiocyanate. Different factors affecting these reactions are studied, and then Beer's law is carried out. The methods were applied to the determination of ELT in pure form and its dosage forms (tablets). Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) guidelines [13] for the determination of ELT in bulk sample and in tablet dosage forms.

2. Materials and Methods

2.1. Apparatus. All the absorption spectral measurements were made using Kontron 930 (UV-Visible) spectrophotometer (Germany) with scanning speed 200 nm/min and band width 1.0 nm equipped with 10 mm matched quartz cells. The pH values of different buffer solutions were checked using an Hanna pH-meter instrument (pH 211, Romania).

2.2. Materials and Reagents. All chemicals and reagents were of analytical grade, and water was always bidistilled water.

2.2.1. Materials. Eletriptan HBr (ELT) and its formulation Relpax tablets (40 mg ELT per tablet) were kindly supplied by Pfizer manufacturing Deutschland GmbH, Germany. Its purity was found to be 99.91 ± 0.97 (n = 6) [9].

Stock solutions of ELT (100 μg mL^{-1}) and (1.0×10^{-3} M) were prepared by dissolving the appropriate weight of ELT in 5.0 mL methanol, and the volume was diluted to the mark in a 100 mL calibrated flask with methanol. Working standard solutions were prepared from suitable dilution of the standard stock solution. All solutions are stable for a period of 3.0 days when refrigerated (4°C).

2.2.2. Reagents

(1) Quinalizarin, 1,2,5,8-tetrahydroxyanthraquinone (Quinz), and alizarin red S, 3,4-dihydroxy-9,10-dioxo-2-anthracene sulfonic acid (ARS), were Aldrich products and used without further purification. A stock solution (1.0×10^{-3} M) was prepared by dissolving the appropriate amount of the reagent in approximately 25 mL of methanol. After obtaining a solid-free solution, it was transferred to a 50 mL volumetric flask and completed to the mark with methanol. The solutions were stable for at least one week at 4°C.

(2) A stock solution of ammonium molybdate, (0.2% w/v) aqueous solution, was also prepared by dissolving the accurate weight 0.2 g of ammonium molybdate tetrahydrate in bidistilled water containing a few drops of ammonia and standardized gravimetrically using 8-hydroxyquinoline, Vogel (1989) [14].

(3) Ammonium thiocyanate and ascorbic acid (10% w/v) aqueous solutions were prepared by dissolving the accurate weight 10 g of each substance in 100 mL bidistilled water.

(4) Stock solutions (4.0 M) of HCl, H_2SO_4, and HNO_3 acids were prepared by accurate dilution from concentrated solutions.

3. General Procedures

3.1. Charge Transfer Methods (Using Quinz and ARS Reagents). Aliquots of methanolic solutions containing (0.1–1.8 mL) of 100 μg mL^{-1} ELT are transferred to 10 mL volumetric flasks. To each flask 1.5 and 2.0 mL of (1.0×10^{-3} M) Quinz and ARS solution, respectively, were added. Afterwards, the obtained mixture was shaken in order to promote the reaction, and the volume was completed to the mark with methanol. The absorbance of this final solution was measured at 569 and 533 nm for Quinz and ARS, respectively, against a reagent blank.

3.1.1. Ion-Pair Method (Using Molybdenum(V)-Thiocyanate). In 100 mL separating funnel, 2.0 mL of (0.2% w/v) ammonium molybdate, 3.0 mL of HCl (4.0 M), 3.0 mL of (10% w/v) ammonium thiocyanate, and 2.0 mL (10% w/v) of ascorbic acid were mixed. The mixture was left for 10 min at room temperature (25 ± 2°C). Appropriate volumes of standard solution of ELT in the concentration range (2.0–32 μg mL^{-1}) were added and diluted with bidistilled water to 20 mL, and the reaction mixture was left for another 10 min. the ion-pair was extracted with methylene chloride twice with 5.0 mL portions; the mixture was shaken well for 2.0 min and allowed to separate into two phases. The organic layer was collected in a 10 mL measuring flask, and methylene chloride was dried over anhydrous sodium sulfate, and its absorbance of the extract was measured at 470 nm against a reagent blank prepared similarly without the drug.

3.2. Procedures for Pharmaceutical Formulations (Tablets). The contents of twenty tablets (Relpax, 40 mg per tablet) were crushed, finely powdered, and weighted out, and the average weight of one tablet was determined. An accurate weight equivalent to 20 mg ELT was transferred into a 100 mL calibrated flask, dissolved in 20 mL methanol with shaking for 5.0 min and filtered through a sintered glass crucible (G4). The first 5.0 mL portion of the filtrate was rejected, and the filtrate was diluted to 100 mL with methanol using (Quinz and ARS) or bidistilled water using Mo(V)-thiocyanate in a 100 mL measuring flask to give 200 μg mL^{-1} stock solutions. Aliquot of the cited solution was taken and analyzed as described under the above recommended procedures for construction of calibration curves. For the proposed methods, the content of a tablet was calculated using the corresponding regression equation of the appropriate calibration graph.

3.3. Stoichiometric Relationship. Job's method of continuous variation [15] was employed to establish the stoichiometry of the coloured products. A 1.0×10^{-3} M standard solution of ELT and a 1.0×10^{-3} solution of reagents were used. A series of solutions was prepared in which the total volume of drug and reagent was constant (2.0 mL). The drugs and reagents were mixed in various proportions and diluted in a 10 mL calibrated flask with methanol using (Quinz and ARS) or bidistilled water using Mo(V)-thiocyanate. Measure the absorbance at optimum wavelengths against a reagent blank following the above mentioned procedure.

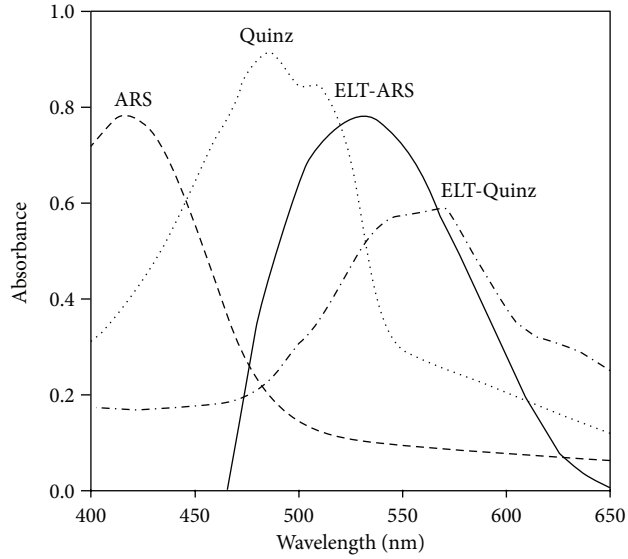


Figure 2: Absorption spectra of reaction products of 20 μg mL^{-1} of ELT with Quinz and ARS, against blank solutions (1.0×10^{-3} M) Quinz or ARS.

4. Results and Discussion

In the present investigation, we investigate the development of simple, rapid, accurate, reproducible, and adequately sensitive spectrophotometric methods for determination of ELT in pure and dosage forms. This work was undertaken based on the formation of charge-transfer complex between ELT as electron-donor when they reacted with selected alizarin derivatives (Quinz and ARS) in methanolic medium and the formation of ion-pair between ELT and molybdenum(V) thiocyanate binary complex via the protonated nitrogen atom of the drug in hydrochloric acid medium. They produce a new band of absorption intensity at a suitable λ_{max} which was characteristic for each complex (Table 2).

4.1. Absorption Spectra. At optimum conditions, the radical anion (absorbing species) was formed in the medium immediately after mixing of the reagents and showed maximum absorption at 569 and 533 nm using Quinz and ARS, respectively, in methanol medium (Figure 2). Thus, these wavelengths were chosen for all further measurements in order to obtain highest sensitivity for the proposed methods. It is important to point out that the Quinz and ARS alone, in methanol medium, exhibit maximum absorption at 489 and 418 nm, respectively. The high difference between maxima of the reagent and the product absorption bands ~80 or 115 nm for Quiz and ARS, respectively, allowed the measurement of

Table 2: Statistical analysis for determination of ELT by the proposed methods.

Parameters	QUIZ	ARS	Mo(V)-thiocyanate
Wavelengths λ_{max} (nm)	569	533	470
Beer's law limits (μg mL^{-1})	2.0–18	1.0–8.0	2.0–32
Molar absorptivity ε, (L/mol^{-1} cm^{-1}) $\times 10^4$	1.1924	3.307	1.1886
Sandell's sensitivity (ng cm^{-2})	38.86	14..01	38.99
Regression equation[a]			
Slope (b)	0.0264	0.0705	0.0262
Intercept (a)	−0.0041	0.0004	−0.0033
Correlation coefficient (r)	0.9994	0.9998	0.9998
Mean recovery % ± SD	100.21 ± 0.927	99.74 ± 0.836	99.79 ± 0.862
RSD %	0.925	0.838	0.861
RE %	0.971	0.982	0.904
Variance	0.859	0.699	0.743
LOD, (μg mL^{-1})[b]	0.359	0.215	0.332
LOQ, (μg mL^{-1})[b]	1.20	0.717	1.11
Calculated t-value[c]	0.5	0.30	0.207
Calculated F-value[c]	1.09	1.35	1.27

[a] $A = a + bC$, where C is the concentration in μg mL^{-1}, A is the absorbance units, a is the intercept, and b is the slope.
[b] LOD: limit of detection; LOQ: limit of quantification; ε: molar absorptivity.
[c] The theoretical values of t and F are 2.57 and 5.05, respectively, at confidence limit at 95% confidence level and five degrees of freedom ($P = 0.05$).

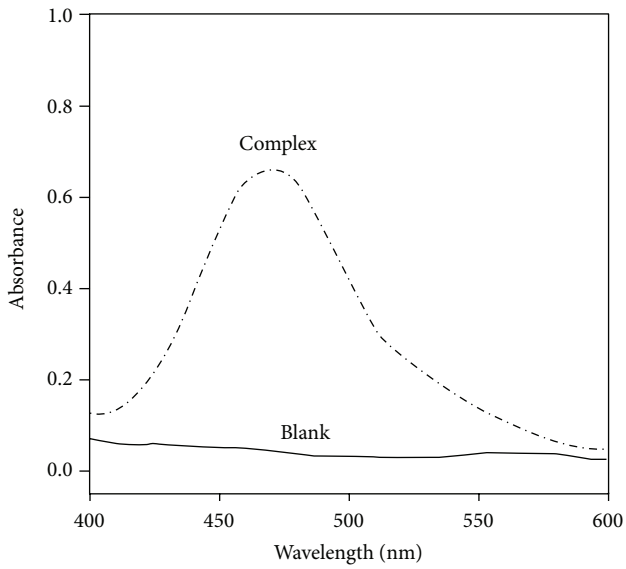


Figure 3: Absorption spectra of ELT-Mo(V)-thiocyanate ion-pair complex in methylene chloride versus reagent blank.

the products with only a small contribution of the reagents that was added in excess in the medium.

Mo(V) formed by the reduction of Mo(VI) with ascorbic acid combines with ammonium thiocyanate to form a red Mo(V)-thiocyanate binary complex in hydrochloric acid solution. On adding ELT solution, orange red ion-pair was formed in the same acid concentration. The ion-pair formed is soluble and extractable in methylene chloride while the Mo(V)-thiocyanate binary complex is insoluble. Double extraction is necessary to extract the ion-pair quantitatively into organic phase. The absorption spectra of the ion pair extracted in methylene chloride show maximum absorption at 470 nm against a reagent blank (Figure 3).

5. Optimization of the Experimental Conditions

5.1. Charge Transfer Methods (Using Quinz and ARS)

5.1.1. Effect of the Solvent Nature. Different solvents such as acetonitrile, acetone, chloroform, methanol, ethanol, 1,4-dioxane, dichloromethane, and 1,2-dichloroethane were examined. Methanol was found to be the best solvent for Quinz and ARS reagents, because of the capacity of this solvent to form stable hydrogen bonds with the radical anion.

5.1.2. Effect of Reagents Concentration. The optimum concentrations that give maximum colour formation, 1.5 and 2.0 mL of (1.0×10^{-3} M) Quinz and ARS reagents solutions, respectively, in methanol were found to be sufficient for the production of maximum and reproducible colour intensity. Higher concentrations of the reagent did not affect the colour intensity (Figure 4).

5.1.3. Effect of Time and Temperature. The optimum reaction time was determined by following the colour intensity at optimum wavelengths at ambient temperature (25 ± 2°C). All measurements were carried out after 5.0 min of mixing of the reagents at laboratory ambient temperature (25 ± 2°C). Stable absorbance values were observed from the beginning of the experiment up to 12 h.

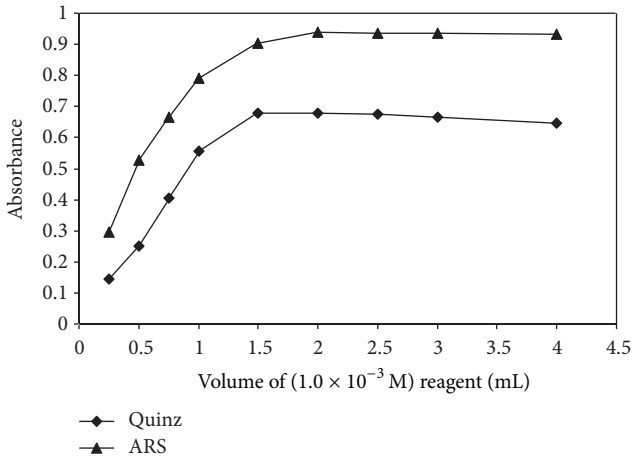


Figure 4: Effect of reagent volume on the formation of ELT complexes with (1.0×10^{-3} M) Quiz and ARS.

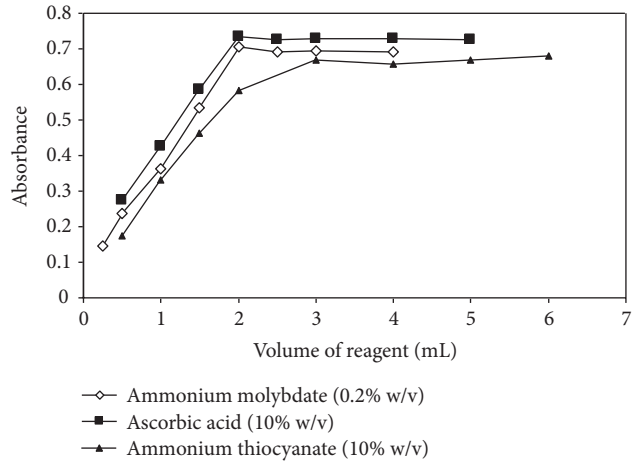


Figure 5: Effect of ammonium molybdate (0.2% w/v), ascorbic acid (10% w/v), and ammonium thiocyanate (10% w/v) volume on the development of the ion-associates of 20 μg mL^{-1} of ELT at λ_{max} = 470 nm.

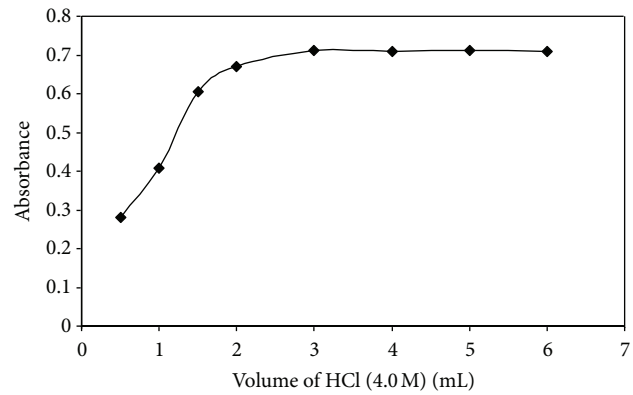


Figure 6: Effect of HCl (4.0 M) on the development of the ion-associate of 20 μg mL^{-1} of ELT at λ_{max} = 470 nm.

5.2. Ion-Pair Method (Using Molybdenum(V)-Thiocyanate)

5.2.1. Effect of Ammonium Molybdate Concentration. The effect of varying ammonium molybdate on the ion-pair formation and its extraction in methylene chloride was optimized (Figure 5). The data showed that 2.0 mL of (0.2% w/v) of ammonium molybdate is required for maximum absorbance in a final volume of 20 mL aqueous solution and in presence of 20 μg mL^{-1} of ELT; after this, the absorbance was nearly constant.

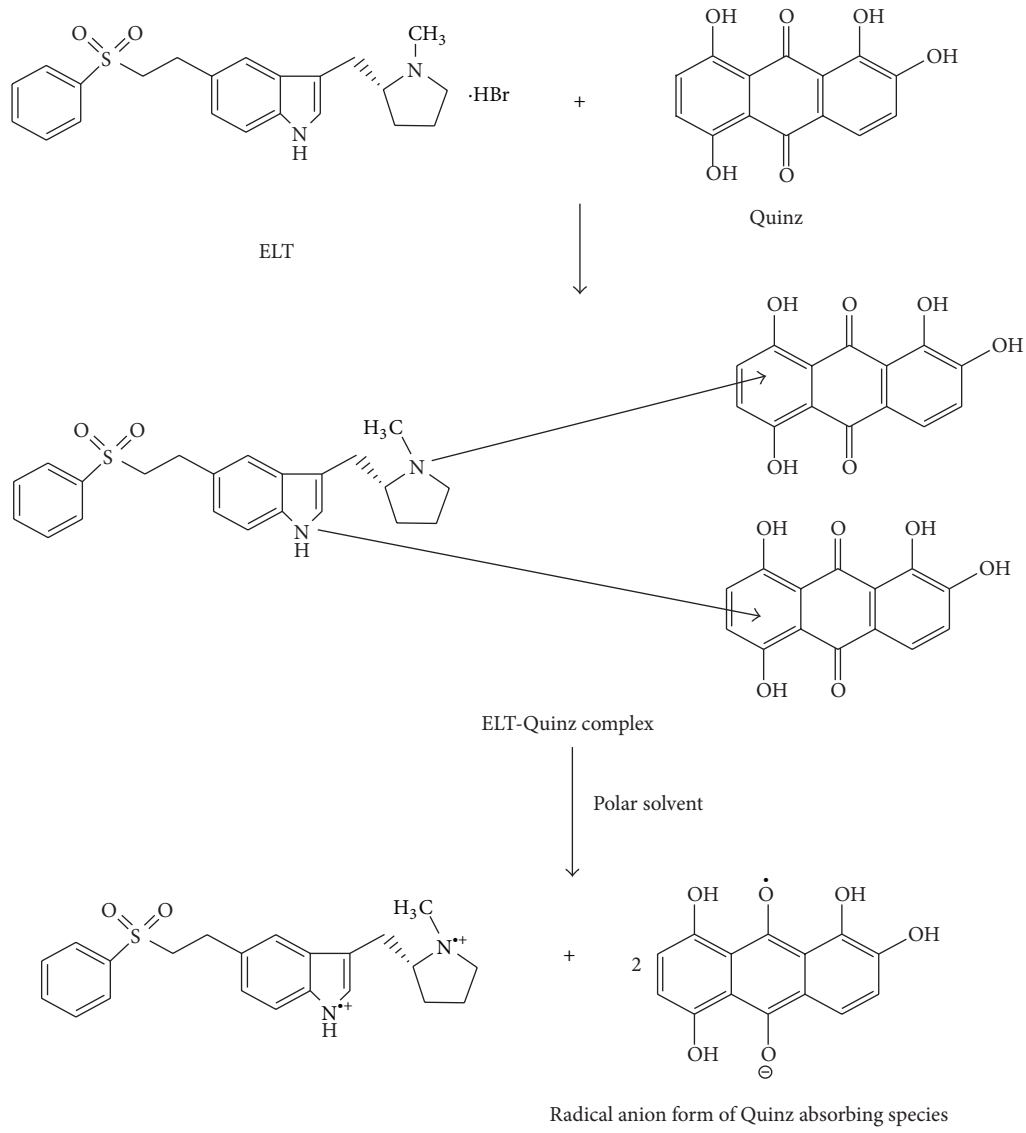
5.2.2. Effect of Ascorbic Acid. It was found that the reduction probability of Mo(VI) to Mo(V) may occur by ascorbic acid or by SCN^- in acidic medium. The rapidity, sensitivity, and stability of Mo(V)-thiocyanate binary complex are enhanced considerably by using ascorbic acid, as ascorbic acid gives reproducible values and masks many interfering ions. From the data obtained, it was found that 2.0 mL of (10%, w/v) ascorbic acid is sufficient for complete conversion of Mo(VI) to Mo(V) (Figure 5). Further addition of an excess amount of ascorbic acid has no effect on the absorbance of the formed ion-pair.

5.2.3. Effect of Ammonium Thiocyanate. It was found that 3.0 mL of (10% w/v) ammonium thiocyanate in a final solution of 20 mL gave the maximum pronounced effect on the absorbance of the ion-pair used in the determination of ELT (Figure 5). From the above results, an equation representing the reaction of Mo(VI) with ammonium thiocyanate in 4.0 M HCl and in the presence of ascorbic acid can be given as

$$\mathrm{Mo(VI)} \xrightarrow[4.0\,\mathrm{M\ HCl}]{\text{Ascorbic acid}} \mathrm{Mo\,(V)} \xrightarrow{6\mathrm{SCN}^-} \mathrm{Mo\,(SCN)_6}^- \quad (1)$$

5.2.4. Effect of Acidity. The effect of acids (HCl, HNO_3, and H_2SO_4) on the formation and extraction of the formed ion-pairs via the reaction of Mo(V)-thiocyanate and ELT in dichloromethane was investigated. The ion-pair was formed only in hydrochloric or sulphuric acid media. The maximum absorbance and high molar absorptivity (ε) values of the dichloromethane extract using (1.0–5.0 M) hydrochloric acid were obtained. The effect of adding different concentrations of 4.0 M hydrochloric acid on the formation of the ion-pairs in the presence of 20 μg mL^{-1} of ELT showed that 3.0 mL of 4.0 M HCl was sufficient for maximum absorbance and the formation of Mo(V)-thiocyanate-ELT ion-pair (Figure 6).

5.2.5. Effect of Solvent. The common organic solvents such as methylene chloride, ethanol, methanol, chloroform, and benzene were examined. Methanol and other oxygenated solvents were found to extract both binary and ternary complex. Using slightly polar or nonpolar solvents, such as dichloromethane and chloroform, the ternary complex could be extracted. Moreover, dichloromethane has high solubility of the ternary complex in this solvent. Reproducible absorbance readings were obtained after double extraction of the complex into the organic phase with 10 mL of methylene chloride (5.0 mL for each) and 2.0 min shaking time.



Scheme 1: Possible mechanism of radical anion formation from Quinz and ELT reaction.

An organic-aqueous ratio of 1 : 1 was suitable for complex extraction of the ternary complex. The intensity of the colour formed after extraction by methylene chloride is stable for at least 24 hours.

5.2.6. Effect of Time and Temperature. The effect of time and temperature on the formation of the ion-pair was studied. In this method, the complete formation of the ion-pair needs 10 min before extraction with methylene chloride at room temperature (25 ± 2°C). The absorbance of Mo(V)-thiocyanate binary complex is stable after 10 min, while Mo(V)-thiocyanate-ELT ion-pair needs another 10 min for their complete formation.

5.2.7. Stoichiometric Ratio. The stoichiometric ratio of the reactants was determined by Job's method of continuous variation [15]. Job's continuous variation graph for the reaction between ELT and (Quinz or ARS) reagents shows that the interaction occurs between an equimolar solution of the drug and the reagents. The result indicated that the complex was formed in the ratio of 1 : 2 (ELT : Quinz or ARS) (Figure 7). This finding supports that the interaction of ELT and (Quinz or ARS) reagents used takes place at two sites, which were the more sterically free terminal basic amino groups. In view of this result a reaction mechanism was proposed considering the transfer of free electron of the two nitrogen atoms present in one molecule of drug to the charge-deficient center of reagent molecule from the total transfer of charge. On the basis of the literature data [16] and our experimental results, tentative reaction mechanisms for ELT-Quinz complexes are proposed and given in Scheme 1, respectively. The composition of the ion-pair was (1 : 1) (ELT : Mo(V) thiocyanate) and is formed through the electrostatic attraction between the positive protonated drug, $[ELT]^+$ and thiocyanate negative complexes of $[Mo(SCN)_6]^-$ (Figure 7) [17]. The structures of the ion-pair is given in Scheme 2.

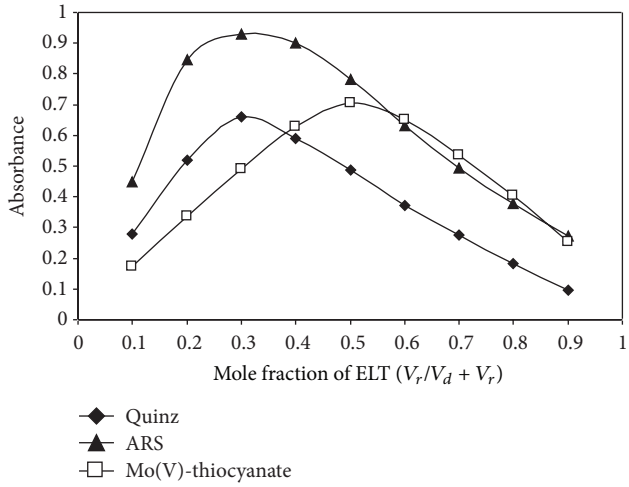


Figure 7: Job's method of continuous variation graph for the complexes formed by reaction of ELT with reagents, respectively. Total molar concentration = 1.0×10^{-4} M, where V_d and V_r are the volumes of added drug and reagent, respectively; $(V_d + V_r) = 1$ mL.

6. Validation of the Proposed Method

6.1. Linearity, Detection, and Quantitation Limits. Following the proposed experimental conditions, the relationship between the absorbance and concentration was quite linear in the concentration ranges given in Table 2. The regression equations were derived using the least-squares method [18]. The intercept (a), slope (b), correlation coefficient (r), molar absorptivities (ε), and Sandell sensitivity values are summarized in Table 2. The percentage recoveries of the pure drugs using the proposed methods compared with that given by the reported methods are illustrated in Table 2. The validity of the proposed methods was evaluated by statistical analysis between the results achieved from the proposed methods and that of the reported methods [19]. Regarding the calculated Student's t-test and variance ratio F-test (Table 2), there is no significant difference between the proposed and reported methods regarding accuracy and precision.

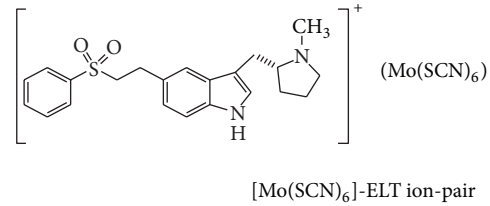
The detection limit (LOD) is defined as the minimum level at which the analyte can be reliably detected for the 3 drugs being calculated using the following equation, [13, 19], and listed in Table 2:

$$\text{LOD} = \frac{3s}{k}, \tag{2}$$

where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, namely, the slope of the calibration graph. In accordance with the formula, the detection limits were found to be 0.359, 0.215, and 0.332 μg mL^{-1} using Quinz, ARS, and Mo(V)-thiocyanate, respectively.

The limit of quantization, LOQ, is defined as the lowest concentration that can be measured with acceptable accuracy and precision [13, 19]

$$\text{LOQ} = \frac{10s}{k}. \tag{3}$$



Scheme 2: Suggested structures of ion-pair complex formed between ELT and Mo(V)-thiocyanate.

According to this equation, the limit of quantization was found to be 1.20, 0.717, and 1.11 μg mL^{-1} using Quinz, ARS, and Mo(V)-thiocyanate, respectively.

6.2. Accuracy and Precision. The accuracy and precision of the methods were evaluated by performing six replicate analyses on pure drug solution at four different concentration levels (within the working range). Percentage relative standard deviation (RSD%) as precision and percentage relative error (RE %) as accuracy of the proposed spectrophotometric methods were calculated. The relative standard deviation (RSD) values were less than 2% in all cases, indicating good repeatability of the suggested methods.

The percentage relative error, calculated using the following equation:

$$\text{RE\%} = \left[\frac{(\text{founded} - \text{added})}{\text{added}}\right] \times 100. \tag{4}$$

The intraday and interday precision and accuracy results show that the proposed methods have good repeatability and reproducibility (Table 3).

6.3. Ruggedness and Robustness. The ruggedness of the proposed methods was assessed by applying the procedures using two different instruments in two different laboratories at different times and two different analytcs. Results obtained from laboratory-to-laboratory and analyst-to-analyst variation were found to be reproducible because the RSD did not exceed 2.0%. Robustness of the procedures was assessed by evaluating the influence of small variation of experimental variables, that is, concentrations of reagent and reaction time, on the analytical performance of the methods. In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the recovery percentage was calculated each time. The small variations in any of the variables did not significantly affect the results. This provided an indication of the reliability of the proposed method during routine work.

6.4. Recovery Studies. The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Preanalyzed tablet powder was spiked with pure ELT at different levels and the total was determined by the proposed methods using standard addition technique. The percent recovery ± relative standard deviation of pure ELT added was in the range 99.62–99.91 ± 0.602–0.652%

Table 3: Evaluation of intraday and interday precision and accuracy obtained by the proposed methods.

Methods	Added (μg mL^{-1})	Intra-day				Inter-day			
		Recovery %	Precision RSD %[a]	Accuracy RE %	Confidence limit[b]	Recovery %	Precision RSD %[a]	Accuracy RE %	Confidence Limit[b]
Quinz	4.0	99.50	0.48	−0.50	19.88 ± 0.089	100.25	0.70	0.25	4.01 ± 0.029
	8.0	99.00	1.09	−1.00	59.52 ± 0.44	99.15	1.30	−0.85	7.932 ± 0.108
	12	99.60	1.32	−0.40	99.70 ± 0.56	99.30	1.70	−0.70	11.916 ± 0.213
	16	99.80	0.88	−0.20	120.6 ± 1.06	99.40	0.97	−0.60	15.904 ± 0.162
ARS	2.0	99.50	0.56	−0.50	1.99 ± 0.012	99.70	0.69	−0.30	1.994 ± 0.014
	4.0	99.25	1.15	−0.75	3.97 ± 0.048	99.90	0.75	−0.10	3.996 ± 0.031
	6.0	99.80	1.50	−0.20	5.988 ± 0.094	99.30	0.83	−0.70	5.958 ± 0.052
	8.0	99.40	0.84	−0.60	7.952 ± 0.07	99.40	1.10	−0.60	7.952 ± 0.092
Mo(V)-thiocyanate	5.0	99.40	0.58	−0.60	4.97 ± 0.03	99.80	0.75	−0.20	4.99 ± 0.039
	10	99.10	1.03	−0.90	9.91 ± 0.107	99.50	1.46	−0.50	9.95 ± 0.152
	20	99.70	1.52	−0.30	19.94 ± 0.318	99.20	1.15	−0.80	19.84 ± 0.239
	30	99.50	0.98	−0.50	29.85 ± 0.307	99.60	0.80	−0.40	29.88 ± 0.251

[a]Mean of six determinations. RSD %: percentage relative standard deviation; RE %: percentage relative error.
[b]Mean ± standard error.

Table 4: Application of the standard addition technique for the determination of ELT in Relpax tablets (40 mg ELT per tab) using the proposed methods.

	Quinz			ARS			Mo(V)-thiocyanate		
	Taken (μg mL^{-1})	Added (μg mL^{-1})	Recovery[a] (%)	Taken (μg mL^{-1})	Added (μg mL^{-1})	Recovery[a] (%)	Taken (μg mL^{-1})	Added (μg mL^{-1})	Recovery[a] (%)
	4.0	—	100.30	2.0	—	99.90	5.0	—	99.75
		2.0	100.40		2.0	99.10		5.0	100.45
		4.0	99.40		3.0	99.70		10	99.90
		8.0	98.70		4.0	100.70		15	100.20
		10	99.30		5.0	100.40		20	99.50
		12	99.60		6.0	99.40		25	99.30
Mean ± SD			99.62 ± 0.643			99.87 ± 0.602			99.91 ± 0.652
V			0.413			0.362			0.652
R.S.D %			0. 645			0.603			0.425
S.E			0.263			0.246			0.266

[a]Average of six determinations.

(Table 4) indicating that the recoveries were good and that the coformulated substance and common excipients did not interfere in the determination.

6.5. Interference Studies. A study was performed in order to evaluate the effect of possible interfering species on the drug reaction with the reagents. The selectivity of the proposed spectrophotometric methods was investigated by observing any interference encountered from some common excipients of the pharmaceutical formulations such as starch, lactose, sucrose, glucose, gum acacia, and magnesium stearate. It was shown that these excipients did not interfere with the proposed methods. So, the proposed methods are able to determine the analyte in the presence of common excipients.

6.6. Application of the Proposed Methods to Analysis of Dosage Forms. The proposed method was successfully applied to the determination of ELT in its pharmaceutical formulations (tablets) (Table 5). The results were compared statistically, by applying the t- and F-tests [19], with the results being obtained by the reference method [12]. The results obtained by the proposed methods revealed that no significant differences were found between the calculated and theoretical values of the proposed and reference methods at 95% confidence level. This indicated similar accuracy and precision in the analysis by the proposed and reported methods. It is evident from these results that the proposed methods are applicable to the analysis of ELT in its dosage forms with comparable analytical performance.

Table 5: Determination of the studied drugs in Relpax tablets (40 mg ELT per tablet).

	Reported methods [12]	Recovery[a] ± RSD %		
		Quinz	ARS	Mo(V)-thiocyanate
$X \pm SD$	100.03 ± 0.92	99.62 ± 0.643	99.87 ± 0.602	99.91 ± 0.652
t[b]		0.82	0.33	2.22
F[b]		2.05	2.34	1.99

[a]Average of six determinations.

[b]The theoretical values of t and F are 2.57 and 5.05, respectively, at confidence limit at 95% confidence level and five degrees of freedom ($P = 0.05$).

7. Conclusions

The present study described the successful evaluation of some alizarin derivatives (Quinz or ARS) and Mo(V)-thiocyanate as an analytical reagents in the development of simple, sensitive, selective, and rapid spectrophotometric methods for the accurate determination of ELT in its pharmaceutical formulations. Also, the developed methods presented some advantages such as it does not need expensive sophisticated apparatus, it is simple and rapid, and it has high sensitivity and low operational cost. In the practical point of view, the methods required minimum sample treatment, which allowed us to achieve a high analytical productivity.

References

[1] S. Sweetman, *Martindale, the Extra Pharmacopeia—the Complete Drug Reference*, Electronic Version, Pharmaceutical Press, Royal Pharmaceutical Society, London, UK, 36th edition, 2007.

[2] D. C. Evans, D. O'Connor, B. G. Lake, R. Evers, C. Allen, and R. Hargreaves, "Eletriptan metabolism by human hepatic CYP450 enzymes and transport by human P-glycoprotein," *Drug Metabolism and Disposition*, vol. 31, no. 7, pp. 861–869, 2003.

[3] P. Morgan, D. Rance, C. G. James, and K. A. Milton, "An in vitro-in vivo correlation of eletriptan pharmacokinetics in rat, dog and human," *Headache*, vol. 37, no. 6, p. 324, 1997.

[4] M. Zecevic, B. Jocic, S. Agatovonic-Kustrin, and L. J. Zivanovic, "Validation of an HPLC method for the simultaneous determination of eletriptan and UK 120.413," *Journal of the Serbian Chemical Society*, vol. 71, pp. 1195–1206, 2006.

[5] J. D. H. Cooper, D. C. Muirhead, and J. E. Taylor, "Determination of eletriptan in plasma and saliva using automated sequential trace enrichment of dialysate and high-performance liquid chromatography," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 21, no. 4, pp. 787–796, 1999.

[6] D. Suneetha and A. L. Rao, "RPHPLC method for the estimation of eletriptan in pharmaceutical dosage forms," *International Journal of Chemical, Environmental and Pharmaceutical Research*, vol. 1, no. 2, pp. 95–99, 2010.

[7] B. Jocić, M. Zečević, L. Živanović, A. Protić, M. Jadranin, and V. Vajs, "Study of forced degradation behavior of eletriptan hydrobromide by LC and LC-MS and development of stability-indicating method," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 50, no. 4, pp. 622–629, 2009.

[8] L. Rajasekhar, R. Venkatamahesh, and P. S. Satyanarayana, "Development and validation of derivative spectrophotometric method for quantitative estimation of eletriptan hydrobromide in bulk and pharmaceutical dosage forms," *International Journal of Research in Pharmaceutical and Biomedical sciences*, vol. 2, pp. 1206–1209, 2011.

[9] K. G. Swamy, J. M. R. Kumar, J. V. L. N. S. Rao, U. A. Kumar, and E. V. Snehalatha, "Spectrophotometric method for the estimation of eletriptan hydrobromide in pure and tablet dosage forms," *International Journal of Chemical and Analytical Science*, vol. 2, pp. 123–125, 2011.

[10] R. Venkatamahesh, N. Amith, K. Sireesha, M. Balaji, and S. Raja, "Development and validation of spectrophotometric method for quantitative estimation of eletriptan hydrobromide in bulk and pharmaceutical dosage forms," *Asian Journal of Research in Chemistry*, vol. 4, pp. 1278–1280, 2011.

[11] R. I. El-Bagary, N. G. Mohammed, and H. A. Nasr, "Fluorimetric and colorimetric methods for the determination of some antimigraine drugs," *Journal of Chemical and Pharmaceutical Research*, vol. 3, no. 4, pp. 304–314, 2011.

[12] A. Önal, "Spectrophotometric and spectrofluorimetric determination of some drugs containing secondary amino group in bulk drug and dosage forms via derivatization with 7-chloro-4-nitrobenzofurazon," *Quimica Nova*, vol. 34, no. 4, pp. 677–682, 2011.

[13] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2(R 1), Complementary Guideline on Methodology 1996, ICH, London, UK, 2005.

[14] A. I. Vogel, *Text Book of Quantitative Chemical Analysis*, The Elbs Longman, London, UK, 5th edition, 1989.

[15] P. Job, *Advanced Physicochemical Experiments*, Oliner and Boyd, Edinburgh, UK, 2nd edition, 1964.

[16] A. A. Gouda and Z. Al Mallah, "Development and validation of sensitive spectrophotometric method for determination of two antiepileptics in pharmaceutical formulations," *Spectrochimica Acta A*, vol. 105, pp. 488–496, 2013.

[17] R. El-Shiekh, F. Zahran, and A. A. F. Gouda, "Spectrophotometric determination of some anti-tussive and anti-spasmodic drugs through ion-pair complex formation with thiocyanate and cobalt(II) or molybdenum(V)," *Spectrochimica Acta A*, vol. 66, no. 4-5, pp. 1279–1287, 2007.

[18] J. Mendham, R. C. Denney, J. D. Barnes, and M. Thomas, "Statistics: introduction to chemometrics," in *Vogel's Textbook of Quantitative Chemical Analysis*, p. 137, Pearson Education, Singapore, 6th edition, 2002.

[19] J. C. Miller and J. N. Miller, *Statistics and Chemometrics for Analytical Chemistry*, Prentice Hall, England, UK, 5th edition, 2005.