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Analytical Applications of Tropaeolineooo and Azocarmine-G In Visible Spectrophotometric Determination of Eletriptan Hydrobomide In Pure And Pharmaceutical Formulations

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ABSTRACT

Two simple and sensitive extractive visible spectrophotometric methods (A and B) for the assay of Eletriptan Hydrobomide (EHB) in pure and pharmaceutical formulations based on the formation of colored chloroform soluble ion-association associates under specified experimental conditions are described. Two dyes namely acidic dye Tropaeolineooo (TPOOO, method A), Azocarmine-G (ACG, method B) are utilized. The extracts of the ion-associates exhibit absorption λ_{max} at 486 nm and 545 nm for methods A and B respectively. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges (4-24) µg/ml for method A, (20-120) µg/ml for method B and correlation co-efficients are 0.9907(A), 0.9984(B) respectively. The proposed methods are applied to commercial available formulations and the results are statistically compared with those obtained by the UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. These methods are applied successfully for the estimation of the Eletriptan Hydrobomide (EHB) in the presence of other ingredients that are usually present in formulations. These methods offer the advantages of rapidity, simplicity and sensitivity and low cost without the need for expensive instrumentation and reagents.

Keywords: Azocarmine-G(ACG), Assay, Ion-association methods, Tropaeolineooo (TPOOO).

INTRODUCTION

Eletriptan Hydrobromide, IUPAC name is 3-(1-methyl-2-pyrrolidinylmethyl)-5-((phenylsulfonyl) ethyl-1H-indole hydrobromide**.** Eletriptan Hydrobromide is selective serotonin receptors agonist used to treat migraine headache EHB will only treat a headache that has already begun. It will not prevent headache or reduce the number of attacks. Eletriptan Hydrobromide is a second generation triptan. The volume of distribution of eletriptan following IV administration is 138L plasma protein binding is moderate and approximately 85%.It is white crystalline powder firmly soluble in water and methanol.

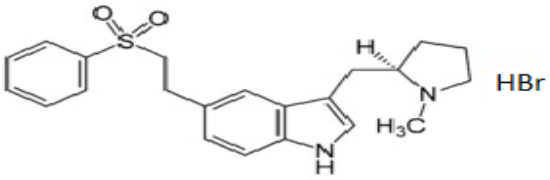

Fig.1 Chemical Structure of Eletriptan Hydro bromide

Literature Survey on the analytical methods for EHB: Eletriptan hydrobromide is a second generation triptan drug and it intended for treatment of migraine headache[1-3].A very few physico-chemical methods appeared for EHB in biological fluids and pharmaceutical formulations and most of them are based on HPLC[4-9] and visible spectrophotometric methods[10,11], plasma and saliva using automated sequential trace enrichment [12], few chromatographic methods i.e HPLC [13,14,15] and TLC to determine anti-migraine drugs[16,17,18], determination of EHB in plasma using liquid chromatography coupled with Tandem mass spectroscopy[19],forced degradation studies and development of stability indicating method[20], Spectroscopic method for the same[21,22],determination of process related impurities in Eletriptan using UPLC method[23],TLC and densitometric method[24] Fluorimetric and colorimetric method [25], Capillary Electrophoresis[26], Thermal Diffractometric and spectrometric studies[27] were observed. The analytical useful functional groups in EHB have not been fully exploited for designing suitable visible spectrophotometric methods and so still offer a scope to develop more visible spectrophotometric methods with better sensitivity, precision and accuracy. The author has made some attempts in this direction and succeeded in developing these methods. All these methods have extended pharmaceutical formulations as well. The RP-HPLC method reported in this study was validated in accordance with the International Conference on Harmonization (ICH) guidelines [28-30]. Upon thorough survey of literature there is no single method available for the estimation by visible spectrophotometry which is far simpler and economical and less time consuming as compared to above mentioned methods. So, the author has made some attempts in developing visible spectrophotometric methods and succeeded in developing two methods based on the reaction between the drug and acidic dyes namely TPOOO and ACG under specified experimental conditions. As the extraction spectrophotometric procedures are popular for their sensitivity and selectivity in the assay of drugs, the extractive spectrophotometric acid-dye technique was therefore, utilized in the present work for the estimation of EHB. The present paper describes two simple and sensitive extraction visible spectrophotometric methods for the determination of EHB, based on its tendency to form chloroform extractable ion-associates with acidic dyes Azocarmine G [31,32] belonging to Phenazine category dye (method B),TPOOO belonging to Azo category dye (method A) [33-37] or under experimental conditions by exploiting the basic nature of Nitrogen in tertiary amine of the drug molecule. According to the literature, it is the first time for EHB determination in bulk as well as formulations by visible spectrophotometry.

MATERIALS AND METHODS

Apparatus and chemicals: A Shimadzu UV-Visible spectrophotometer 1601 with1cm matched quartz cells was used for all spectral measurements. A Systronics digital pH meter mode-361was used for pH measurements. All the chemicals used were of analytical grade. EHB Pure drug was obtained as a gift sample from Gemmed, Pfizer Canada Inc, Gd Eletriptan-40mg-(Tablet-1), and Relpax 40mg-(Tablet-2) were purchased from local market.Tropaeolin000(Fluka,0.2%,$5.7x10^{-3}$M prepared by dissolving 200mg of Tropaeolin000 in 100mL distilled water and subsequently washed with chloroform to remove chloroform soluble impurities), 0.1M HCl (prepared by diluting 8.6ml of Conc. hydrochloric acid to1000mL with distilled water and standardized), ACG solution(Gurr,0.05%,$8.75x10^{-4}$M) prepared by dissolving 50mg of Azocarmine-G in 100mL of distilled water containing traces of sodium hydroxide and subsequently washed with chloroform to remove chloroform soluble impurities, pH 1.5 buffer solution (prepared by

mixing 28.9mL of 0.1M glycine solution (7.507g of glycine and 5.85g NaCl was dissolved in 100mL of distilled water) with 71.1mL of 0.1M HCl and the pH of the solution was adjusted to1.5. Chloroform A.R. grade was used as it is.

Preparation of Standard stock solution: The stock solution (1 mg mL^{-1}) of Eletriptan Hydrobromide (EHB) was prepared by dissolving 100 mg of it in 100 mL of millipore- distilled water. A portion of this stock solution was diluted stepwise with the distilled water to obtain the working standard EHB solution of concentrations 04-500 µg mL^{-1}.

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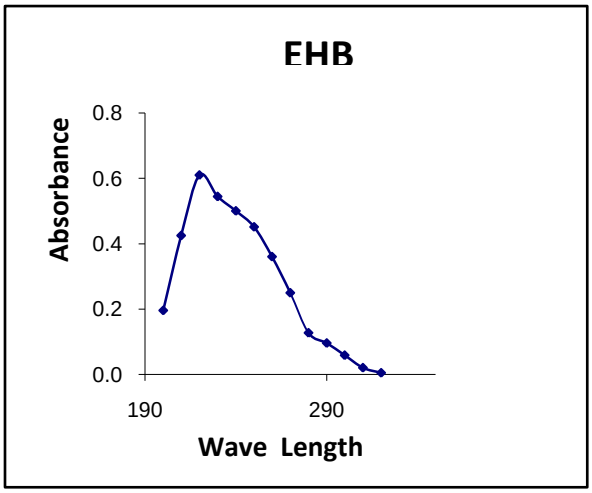


Fig.2. Absorption spectra of EHB

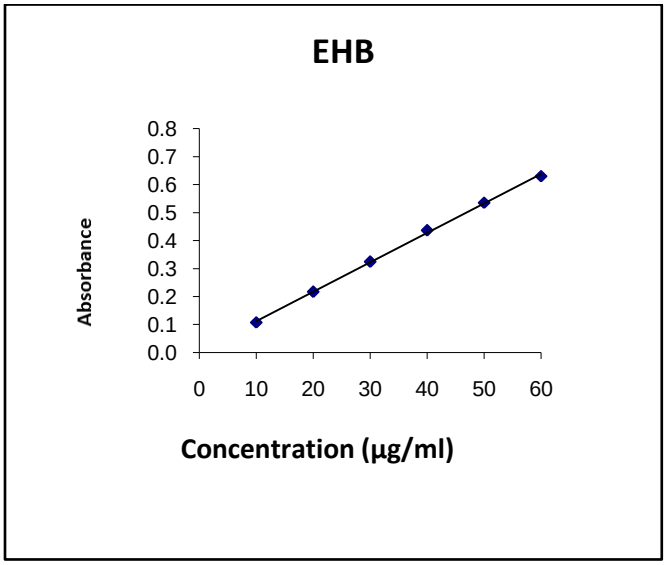


Fig.3. Beer's Law plot of EHB in Methanol (UV reference)

Preparation of Sample solution: About 2 tablets of 20mg were pulverized and the powder equivalent to 40mg of EHB was weighed, dispersed in 25mL of alcohol, shaken well and filtered. The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation.

Recommended Procedures: After systematic and detailed study of the various parameters involved, as described under results and discussion in this chapter, the following procedures were recommended for the determination of EHB in bulk samples.

Method A (TPOOO): Into a series of 125 mL separating funnels containing aliquots of standard EHB solution 0.1-0.6 mL(24 µg mL^{-1}), 6.0 mL of 0.1M HCl and 2.0 mL of dye solution (TPOOO) were added. The total volume of aqueous phase in each separating funnel was adjusted to 15 mL with distilled water and then10 mL of $CHCl_3$ was added. The contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated organic layer were measured at λmax 486nm (Fig.4) against a similar reagent blank. The colored species was stable for 1 h. The amount of EHB in sample solution was obtained from the Beers-Lambert's plot (Fig.5).

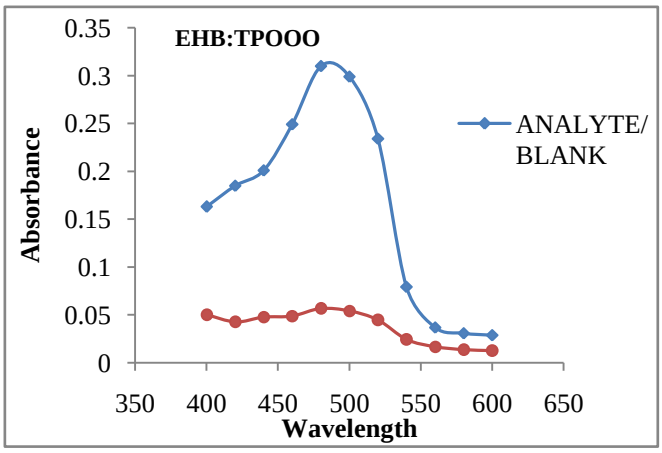


Fig.4. Absorption spectra of TPOOO

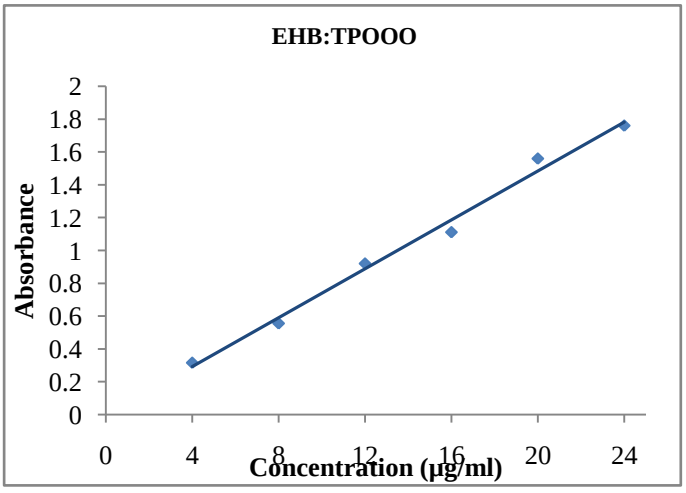


Fig.5. Beer's law plot of TPOOO

Method B (ACG): Aliquots of standard EHB solution (1.0 – 6.0 mL, 120 µg mL^{-1}) were placed in a series of 125 mL separating funnels. A volume of 5.0 mL of buffer (pH 1.5) and 2.0 mL of ACG were added respectively. The total volume of aqueous phase in each separating funnel was adjusted to 15.0 mL with distilled water. Then 10 mL of $CHCl_3$ was added to each separating funnel and the contents were shaken for two minutes and allowed to separate. The organic layer was collected through cotton plug and the absorbance was measured immediately at λmax 545nm (Fig.6) against a reagent blank. The colored species was stable for 1 h. The amount of EHB in sample solution was obtained from the Beers-Lambert's plot (Fig.7).

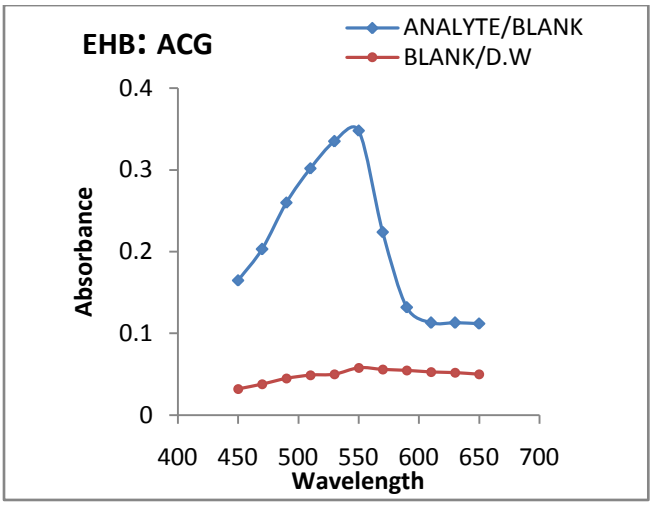


Fig.6. Absorption spectra of EHB: ACG

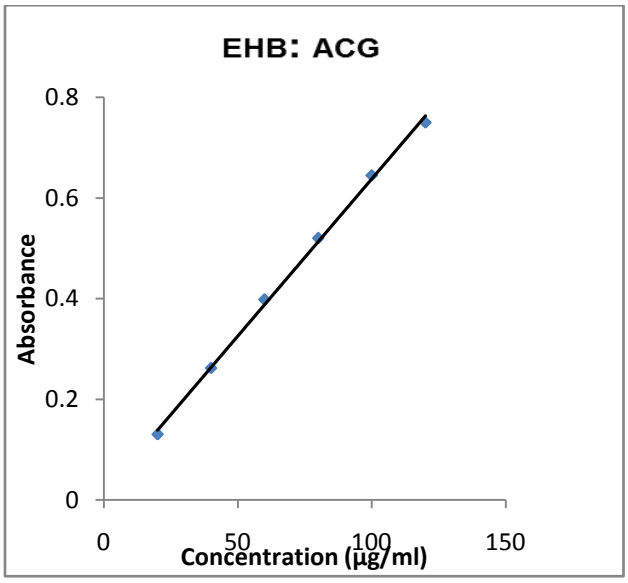
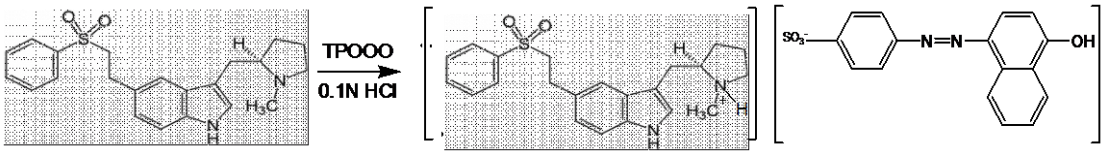


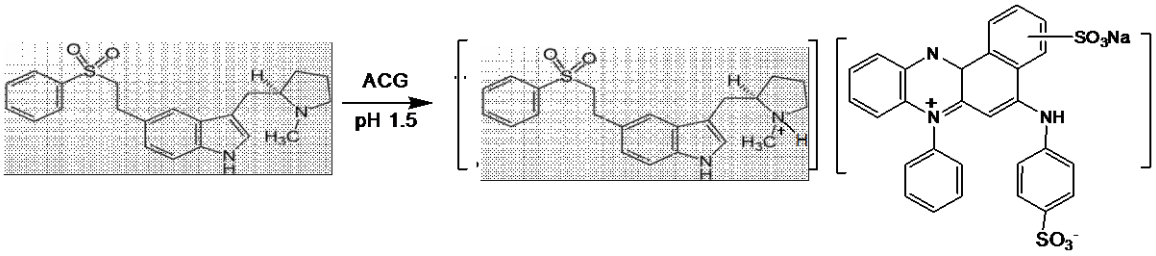
Fig.7: Beer's plot of EHB: ACG

Procedure of Assay of EHB in formulations: An accurately weighed amount of formulation (Tablets-1,2) equivalent to 100 mg of drug was dissolved in 20 mL of distilled water, shaken well and filtered. The filtrate was further diluted to 100 ml with distilled water to get 1 mg m L^{-1} solution of drug in formulations. One mL of this solution was furthered diluted to 25 mL to get 40 µg m L^{-1} solution.

Chemistry of Colored species: EHB under slightly acidic conditions forms ion-association complex with acid dyes EBT which is extractable into chloroform from aqueous phase. The protonated nitrogen (positive charge) of EHB is expected to attract the oppositely charged part of the dye and behave as a single unit being held together by electrostatic attraction as represented in the schemes A and B.



Scheme A



Scheme B

RESULTS AND DISCUSSION

Optimum operating conditions used in the procedure were established adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of TPOOO, ACG reagents, 0.1M HCl, pH of buffer solutions and solvent for final dilution of the colored species were studied. TPOOO and ACG were preferred for this investigation as they yield high molar absorptivity values among six dyes belonging to different chemical classes. The water immiscible solvents tested for the extraction of colored complex into organic phase include chlorobenzene, dichloromethane, carbon tetrachloride, benzene, nitro benzene, n-butanol or chloroform. Chloroform was preferred for its selective extraction of colored drug -dye complex into organic layer from the aqueous phase. The stoichiometric ratio of the dye-drug was determined by the slope ratio method and was found to be 1:1 for methods A and B respectively. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements, Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (Sa), standard error of estimation (S_e) and % range of error (0.05 and 0.01confidence limits) were calculated and the results are summarized in **table-1.**Commercial formulations containing EHB were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-test and F-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pure analyzed formulations at three different concentration levels. These results are summarized in **table-2.**

Table1. Optical and Regression characteristics, precision and accuracyof the proposed methods for EHB

Sl.No	Parameter	Method-A	Method-B
1	Wave length λ_{max} (nm)	486	545
2	Beer's law limits (µg ml^{-1})	4-24	20-120
3	Detection limits (µg ml^{-1})	2.2630	15.9883
4	Molar absorptivity (1 mole cm^{-1})	8.4962×10^5	4.3444×10^4
5	Sandell's sensitivity (µg cm^{-2} / 0.001 absorbance unit)	5.4545×10^{-4}	1.0666×10^{-2}
6	Regressionequation (Y = a + bC) Slope (b)	0.0744	0.0062
7	Standard deviation of slope (S_b)	3.6028×10^{-3}	4.2422×10^{-4}
8	Intercept (a)	-0.0044	0.0137
9	Standard deviation of intercept (S_a)	0.5612×10^{-1}	3.304×10^{-2}
10	Standard error of estimation (S_e)	6.0286×10^{-2}	1.1224×10^{-2}
11	Correlation coefficient (r^2)	0.9907	0.9984
12	Relative standard deviation (%)*	0.4186	0.4439
13	% Range of error(Confidence Limits) 0.05 level*	0.4394	0.4660
14	% Range of error(Confidence Limits)0.01 level	0.6891	0.7308
15	% Error in bulk samples**	0.1	0.188

*: Average of six determinations considered**: Average of three determinations considered

Table 2. Assay and recovery of EHB in Pharmaceutical Formulations

Sample	Amount taken (mg)	Amount found by proposed methods		Reference Methods	Percentage recovery by proposed methods	
		Method-A	Method-B		Method-A	Method-B
Tablet-I	40	39.75 ±0.02 F=1.56 t=0.77	39.82 ±0.018 F=1.26 t=0.85	39.92 ±0.016	99.786 ±0.042	99.749 ±0.045
Tablet-II	40	39.80 ±0.022 F=1.61 t=0.54	39.84 ±0.030 F=1.14 t=0.80	39.88 ±0.028	99.802 ±0.081	99.744 ±0.084

*: Average ± standard deviation of six determinations; the t- and F- values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit t=2.57, F=5.05, **: After adding 2 different amounts of the pure labeled to the pharmaceutical formulations, each value is an average of 3 determinations.

APPLICATIONS

The proposed methods can be applied for the determination of the amounts of drug present in bulk as well as formulations successfully.

CONCLUSIONS

The proposed methods for EHB determination have many advantages over other analytical methods due to its rapidity, lower cost and environmental safety. Unlike HPLC, LC procedures, the instrument is simple and is not costly. Economically, all the analytical reagents are in expensive and available in any analytical

laboratory. The proposed methods report a new for the determination of EHB in pharmaceuticals. These methods can be extended for the routine assay of EHB formulations.

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