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ASSAY OF EPINASTINE IN BULK AND ITS PHARMACEUTICAL FORMULATIONS BY EXTRACTIVE SPECTROPHOTOMETRY

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ABSTRACT

Three simple and sensitive spectrophotometric methods (A – C) for the assay of Epinastine in pure and dosage forms based on the formation of chloroform soluble ion-associates under specified experimental conditions are described. Four acidic dyes, namely, wool fast Blue (WFB BL, method A), Orange II (Tpooo, method B), and Azocarmine (AG, method C) are utilized. The extracts of the ion-associates exhibit absorption maxima at 590, 490, and 540 nm for methods A, B, and C, respectively. Beer's law and the precision and accuracy of the methods are checked by the UV reference method. The results are reproducible with an accuracy of ±1.0%. The methods are found to be suitable for the determination of Epinastine in the presence of the other ingredients that are usually present in dosage forms.

Keywords: Spectroscopy, Epinastine, Azocarmine G, Wool fast Blue, Orange II.

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INTRODUCTION

Epinastine hydrochloride (EPN) is an antihistamine and an inhibitor of histamine release from the mast cell for topical administration to the eyes. It is chemically known as 3-amino-9, 13-b-dihydro-1H-dibenz[c, f] imidazo [1, 5-a] azepine hydrochloride. Literature survey revels only few methods potentiometry[1], HPCL[2-7], UV[8], visible spectrophotometry[9], capillary electrophoresis[10-12] and polarography[13] for its determination in biological fluids and dosage forms. Although spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories, no colorimetric method has seen reported so far for the determination of EPN. Therefore, the need for a fast, low cost and selective method is obvious, especially for routine quality control analysis of pharmaceutical products containing EPN.

As the extraction spectrophotometric procedures are popular for their sensitivity and selectively in the assay of drugs[14,15], the technique was therefore utilized in the present work for the estimation of EPN. A thorough literature survey of the extraction spectrophotometric determination of drugs reveals that many acid dyes belonging to azo, amino anthroquinone, indigoid are studied in determination of compounds exhibiting basic properties (e.g. amines, quaternary ammonium compounds, heterocyclic compounds).

In continuation of these studies, the present paper describes four simple and sensitive extraction spectrophotometric methods for the determination of EPN, based on its tendency to form chloroform extractable ion – association complexes with acidic dyes belonging to different chemical classes, namely, wool Fast Blue (Phenazine dye; method A), Orange II (Azo dye; method B), or Azocarmine (Phenazine dye; method C) under specified experimental conditions by exploiting the basic nature of the drug molecule.

EXPERIMENTAL

Instruments
A systronics UV-vis spectrophotometer 117 with 1 cm matched quartz cells were used for all spectral and absorbance measurements. A systronics digital pH meter 361 was used for pH measurements.

Reagents
All reagents and chemicals used were of analytical or pharmacopoeial grade purity and doubly distilled water was used through out.

Dye solution
Aqueous solutions of WFB BL (0.2% w/v, Flukas), TPooo (0.2% w/v, Fluka), and AG (0.05), w/v, Gurr) where prepared by dissolving the required amount in doubly distilled water. The solutions were washed with chloroform to remove the chloroform soluble impurities and the residual solvent was removed by bubbling with Nitrogen.

Buffer solutions
The glycine-HCl buffer solutions (pH 1.5 for methods A, C and 0.1 M HCl for method B) were prepared[16].

Preparation of standard drug solution
A 1mg/ml stock solution of EPN was prepared by dissolving 100 mg of the drug in 100 ml of water. Working standard solutions were obtained by appropriate dilution of the stock solution with the same solvent (20 μg/ml, for methods A and C and 40 μg/ml, for method B).

Methods A and C
Into a series of 125 ml separating funnels containing aliquots of standard EPN solution [(0.5-2.5ml; 20 μg/ml, method A or 0.5-2.5ml; 20 μg/ml, method C) 6.0ml buffer pH 1.5 (methods A, C) and 2.0 ml of dye solution [WFBBL (method A); AG (method C)] were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0 ml with distilled water and 10.0 ml of chloroform 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 appropriate λ_{max} [(590 nm (method A), or 540 nm (method C)] against the corresponding reagent blank within the stability period (1 min-3 h, method A, C). The amount of EPN was computed from the respective calibration curves.

Method B
Into a series of 125 ml separating funnels containing aliquots of standard EPN solution [(0.5-2.5ml; 40 μg/ml, method B)] 6ml 0.1M HCL and 2.0 ml of dye solution [Tpooo, method B] were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0 ml with distilled water and 10.0 ml of chloroform was added. The contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated organic layer was measured at appropriate λ_{max} [(490 nm (method B)] against the corresponding reagent blank. The amount of EPN was computed from the respective calibration curve.

Analysis of pharmaceutical formulations
A portion of ophthalmic solution equivalent to 100 mg of active ingredient was dissolved in distilled water and subsequently the volume was brought to 100ml with the same solvent to get 1mg/ml. The stock solution was further diluted to provide the working solutions and these were analyzed as described under the procedure for bulk samples.

RESULTS AND DISCUSSION

Conditions under which the reaction of EPN with each dye fulfils the essential analytical requirements were investigated. All the experimental conditions studied were optimized at room temperature (25 $\pm 3^0$C) and were established by varying one parameter at a time[17] and observing its effect on the absorbance of the colored species.

In the preliminary experiments, in view of developing methods of analysis suitable for assaying small quantities of EPN, eight acidic dyes such as Wool Fast Blue, Alizarin Red, suprachen violet 3B, Fast green FCF, Tropacolinooo, Naphthalene Blue 12 BR, Bromocresol green and Bromopyragallol Red were tested at various pH ranges as the colour producing agents by a dye salt partition technique. Different organic solvents such as benzene, chloroform, carbon tetrachloride, ethyl acetate, dichloromethane and methyl isobutyl ketone were tested for the extraction of the ion-association complex formed between the EPN and each dye. The criterion for the best dye was the highest absorbance value of the complex in the organic phase at the wavelength of maximum absorbance[18] .The above studies reveal that four dyes namely WFB BL, TPooo, AG gave better results than the other dyes. These dyes also gave low absorbance for the reagent blank. Chloroform was suggested as the solvent of choice for the extraction of the colored complex with respect to maximum stability.

Figures-1to 3 shows the absorption spectra of the ion-association complexes of EPN with the four dyes, extracted into chloroform and of the reagent blank, obtained as described in the procedure. These ion-association complex spectra show that characteristics λ_{max} (590 nm, method A: 490 nm, method B: and 540 nm, method C) values of the respective dye itself.

In order to establish the optimum pH range (for methods A, C) or acid strength (for method B), the EPN was allowed to react with the respective dye in aqueous solution buffered between pH 1.0-10.0 (methods A, C) or in dilute HCl ranging from 0.05 – 1.5 M (method B) and the complex formed was extracted into chloroform for absorbance measurement. The results show that a quantitative extraction was produced between pH 1.1 – 1.5 (methods A, C), or with an acid strength of 0.08 – 0.12 M HCl (method B). All subsequent studies were carried out at pH 1.5 (for methods A, C) or 0.1 M HCl (for method B). The pH was adjusted using a glycine – HCl buffer solution (this buffer was chosen on account of its elevated complexing ability, which could be of use in overcoming interferences). The volume of this buffer added (4 – 10 ml) had no effect in methods A, and C respectively. A 6.0 ml portion of 0.1 M HCl solution was found to be optimal in method B. The minimum shaking time was determined by varying the shaking time from 1-10 min; although 1 min was sufficient, prolonged shaking had no adverse effect on the extraction and 2 min was selected for this study. A ratio of 2:3 (for methods A, B, and C) of organic to aqueous phases was required for efficient extraction of the colored species and lower reagent blank reading .It was found that better reproducibility and a lower reagent blank were achieved if the dye was purified by extraction with chloroform initially.

Analytical data

The optical characteristics such as the Beer's law limits, molar absorption coefficient, Sandell's sensitivity, regression equation and correlation coefficient obtained by linear least squares treatment[17] of the results for the systems involving EPN with the mentioned dyes are presented in Table-1. Estimating six replicates of EPN within Beer's law limits tested the precision of each method. The percent standard deviation and the percent range of error at 95% confidence limit are given in Table-1.

In order to confirm the utility of the proposed methods, they were applied to the estimation of EPN in various pharmaceutical formulations and the results are presented in Table-2. The results obtained by the proposed and UV reference, which is developed in our laboratory. Methods for the dosage forms were compared statistically by means of F- and t-tests and were found not to differ significantly. As an additional check of accuracy of the proposed methods, recovery experiments were performed by adding a fixed amount of the EPN to the preanalysed formulation and the results are also summarized in Table-2.

Chemistry of the ion-association complex

Epinastine being basic in nature forms an ion-association complex with the acidic dye which is extractable into chloroform. The stoichiometric ratio of the dye to drug was determined by the slope ratio method[18] and found to be 1: 1(for methods A, B and C). The quantitative measure of the effect of complexation on acid-base equilibrium is most likely to be interpretable in terms of electronic, steric and other effects of complexing. The possible structure of the ion-association complex in each instance was established based on the analogy reports for similar types of molecules[9] with acidic dyes and was further confirmed by slope-ratio studies. The protonated nitrogen (positive charge) of the drug molecule in acid medium is expected to attract the oppositely charged part (negative charge) of the dye and behave as a single unit being held together by electrostatic attraction.

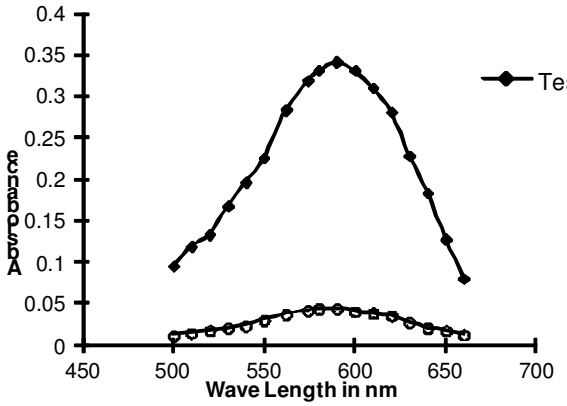

Fig.-1: Absorption spectra of the EPN-WFB BL system (♦ ♦)— (concentration of EPN:1.554x10^{-3}M; WFB BL: 6.52x 10^{-4} M) and reagent blank vs. chloroform (o-o).

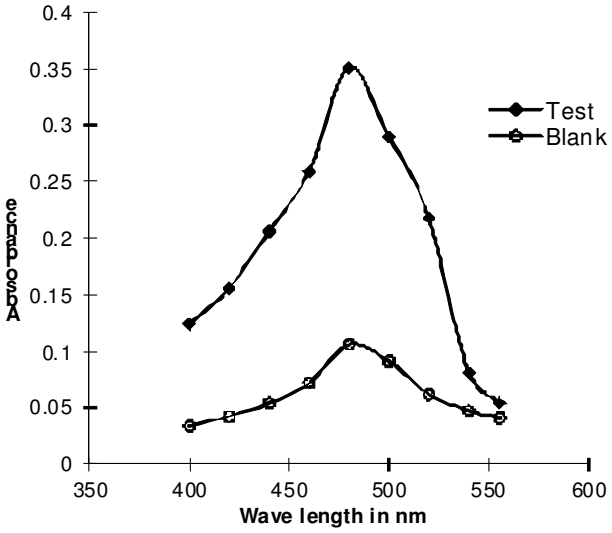

Fig.-2: Absorption spectra of the EPN-TPooo system (♦ ♦) (concentration of EPN:3.886x10^{-3}M; TPooo: 1.14x 10^{-3} M) and reagent blank vs. chloroform (o-o).

Table-1: Optical and Regression Characteristics, Precision, accuracy of the proposed methods

Optical Characteristics	A	B	D
	WFBBL	Tpooo	AG
λ_{max} (nm)	590	480	540
Beer's Law limits (µg/ml)	1 - 6	2 – 12	1 – 6
Molar absorptivity (l $mol^{-1}cm^{-1}$)	3.21 x 10^4	1.736 x 10^4	2.986 x 10^4
Correlation coefficient (r)	0.9999	0.9999	0.9999
Sandell's sensitivity (µg/cm^2/ 0.001 absorbance unit)	0.009	0.016	0.011
Regression Equation (y = a + bc) (i)Slope (b)	0.1123	0.0607	0.1054
(ii) Standard Deviation on slope (S_b)	0.0007	0.0002	0.0011
(iii) Intercept (a)	-0.0007	-0.0001	-0.003
(iv)Standard Deviation on intercept(S_a)	0.0024	0.0015	0.0034
(v) Standard Error of Estimation (S_e)	0.0023	0.0014	0.0033
Relative Standard Deviation *	0.314	0.256	0.403
% Of range error (confidence limit) (i) 0.05 level	0.263	0.215	0.338
(ii) 0.01 level	0.389	0.317	0.499

*Average of six determinations considered.

Table -2: Assay of Epinastine in Pharmaceutical formulations

Pharmaceutical sample (Labeled amount)	% Recovery (mg)			
	Proposed method			Ref. Method
	M_1	M_2	M_3	
Ophthalmic solution I 1 mg	0.99± 0.007 F=1.67 t=0.90	0.99± 0.008 F=1.99 t=1.02	0.99± 0.004 F=1.41 t=1.11	0.99± 0.005
Ophthalmic solution II 1 mg	0.99± 0.005 F=1.33 t=0.97	0.99± 0.008 F=1.92 t=0.93	0.99± 0.005 F=2.42 t=0.57	0.99± 0.005
Ophthalmic solution III 1 mg	1.00± 0.005 F=1.02 t=0.58	1.00± 0.006 F=1.48 t=0.75	1.00± 0.007 F=1.57 t=0.52	1.00± 0.007
Ophthalmic solution IV 1 mg	1.00± 0.007 F=1.56 t=1.61	1.00± 0.005 F=1.85 t=0.22	1.00± 0.012 F=2.05 t=1.34	1.00± 0.007

Average (± RSD) of six determinations; the t and F values refer to comparison of the proposed method with the reference method; theoretical values at 95% confidence limits, t = 2.57, F = 5.05.

CONCLUSION

A significant advantage of an extraction spectrophotometric determination is that it can be applied to the determination of individual compounds in a multicomponent mixture. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibilities in the assay of a particular component in a complex dosage formulation. In the present study, Epinastine was determined successfully as a pure compound as well as a component in representative dosage formulations. The

ingredients usually present in the dosage forms of Epinastine did not interfere in the proposed methods. Thus, the proposed methods are simple, rapid with reasonable precision and accuracy when compared with many of the reported methods and offer advantage in that only a small amount of drug or dosage formulation is enough for analysis.

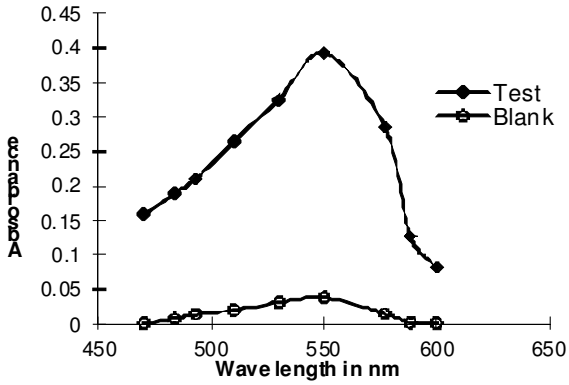

Fig.-3: Absorption spectra of the EPN-AG system (♦ ♦) (concentration of EPN: 7.772×10^{-4} M; AG: 1.75×10^{-4} M) and reagent blank vs. chloroform (o-o).

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