Spectrophotometric determination of Epinastin hydrochloride in bulk and in Pharmaceutical dosage form

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Abstract: *Three rapid, selective and sensitive spectrophotometric methods have been proposed for the quantitative determination of Epinastin hydrochloride (EPH) in bulk and ophthalmic solutions. The methods are based on the charge transfer complexation of EPH as n-electron donor with Iodine as σ-acceptor (method-A), and also with 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinon (DDQ) (method-B) and p-chloranilic acid (p-CA) (method-C) as π -acceptors. The CT complexes exhibited absorption bands at 366nm, 585nm and 514 nm for method-A, method-B and method-C respectively. Under the optimized experimental conditions, Beer's law is obeyed over the concentration ranges of 20-120 µg/ml for method-A, 15-75 µg/ml for method-B and 25-125 µg/ml for method-C.The coefficient of correlation was found to be 0.998 for three methods. The effect of reagent concentrations, polarity of solvent and effect of reaction time have been studied and optimized. The stoichiometric relationship was determined by Job's continuous method and is found to be 1:1 in each case. The methods have been validated in terms of ICH guidelines and applied to the quantification of EPH in pure form and ophthalmic solutions.*

Key words: *Charge transfer complexes, spectrophotometry, Epinastine hydrochloride, σ and π acceptors, validation.*

I.   Introduction

Epinastine (EPH Fig.1) is chemically 3-amino-9, 13b-dihydro-1H-dibenz(c,f) imidazo(1,5-a)azepine hydrochloride. It is a selective H1-receptor antagonist and also has an antiallergic effect by inhibiting the release of allergy-inducing substances such as histamine [1].
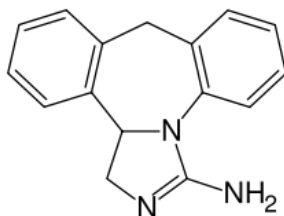

Fig.1Epinastine

A thorough survey literature reveals that a few analytical methods have been reported for estimation of Epinastine*viz.*potentiometry [2], different liquid chromatographic techniques including electrophoresis and triple stage mass spectrometry [3-6].Colourimetry, UV Spectrophotometry and Ultraviolet Derivative spectrometric methods have also reported. [7, 8].

 Charge transfer complexation is an important phenomenon in biochemical and bio electrochemical energy transfer process [9]. The electron donor-acceptor interactions have been widely studied spectrophotometrically in the determination of the drug based on the formation of CT complexes with some π-acceptors [10-11] and σ-acceptors. Literature survey reveals that there is no charge transfer complexation method for determination of Epinastine hydrochloride inspite of the importance of method.

This paper reports simple, direct, and sensitive spectrophotometric method for determination of Epinastine with Iodine, a σ –acceptor and DDQ &*p*-CA as π-acceptors based on the formation of charge transfer complexes.

II.   Experimental

2.1. Instrument

Shimadzu 2600 double beam UV-Visible spectrophotometer is used to record the spectra of individual components as well as the charge transfer complexes, using matched pair of Quartz cells of 10mm path length.

2.2. Materials

The Iodine, *p* -Chloranilic acid and DDQ weresupplied by sigma Aldrich. The AR grade solvents *viz.*, acetonitrile, 1, 2-dichloroethane and chloroform are supplied by SD Fine chem. Ltd. Mumbai, India. The Epinastine.

Hydrochloride used in study was procured from Hetero drugs pvt.Ltd. Hyderabad as gift sample. The Relestat eye drops (0.5mg/ml) (Batch No. E54531), manufactured by Allergen company was purchased from the local market.

III.   Preparation of standard stock solutions

An accurate weight of EPH (100mg) was dissolved in distilled water in a 50ml measuring flask and transferred into a 125ml separating funnel ,where 10 ml of 0.1N NaOH solution were added .The mixture was mixed and extracted three times with 15ml $CHCl_3$ each, then the chloroform layer was evaporated to dryness where the obtained residue was dissolved quantitatively in 100ml 1,2dichloroethane(final conc. 1mg/ml) for method –A and in 100 ml of acetonitrile (final conc. 1mg/ml) for method –B and method-C

IV.   Results and Discussion

4.1General procedures
4.1.1. Method –A (using I_2)

The I_2solution of 2mg/ml in 1, 2-dichloroethane was freshly prepared. Aliquots of EPH (0.5-2.5ml; 400 μg/ml) were transferred into a series of 10 ml calibrated flasks, to each flask, 1ml of I_2 solution in 1, 2-dichloroethane was added and remaining volume was made up by solvent .The absorbance of colored solution was recorded after 10 min of mixing against blank at 365nm and plotted absorbance versus the corresponding concentrations (μg/ml) of the drug to construct the calibration curve and to obtain regression equation.

4 .1.2. Method –B (using DDQ)

The DDQ solution of 2mg/ml in acetonitrile was freshly prepared. Aliquots of EPH (0.5-2.5 ml; 300 μg/ml) were transferred into a series of 10 ml calibrated flasks, to each flask, 1ml of DDQ solution in acetonitrile was added and remaining volume was made up by solvent .The absorbance of colored solution was recorded after 15 min of mixing against blank at 585nm and plotted absorbance versus the corresponding concentrations (μg/ml) of the drug to construct the calibration curve and to obtain regression equation.

4.1.3.. Method-C (using p-CA)

The *p*-CA solution of 2mg/ml in acetonitrile was freshly prepared. Aliquots of EPH (0.5-2.5ml; 500 μg/ml) were transferred into a series of 10 ml calibrated flasks, to each flask ,1ml of p-CA solution in acetonitrile was added and remaining volume was made up by solvent .The absorbance of colored solution wasrecorded after 5 min of mixing against blank at 514nm and plotted absorbance versus the corresponding concentrations (μg/ml) of the drug to construct the calibration curve and to obtain regression equation .
Calibration curves were linear for three methods whose limits are mentioned in Table 1. Slope, intercept Limit of detection, limit of quantification, Molar absorptivity, and correlation coefficient of the calibration curves are calculated and tabulate (Table 1).

V.   Determination of EPH in Pharmaceutical form

Twenty ml of aliquot of the" Relestat" eye drops was shaken in a 125ml separating funnel with 10ml of 0.1N NaOH solution fallowed by extracted three times each with 15ml of chloroform. The collected chloroformic extracts were evaporated and the residue was dissolved in 10 ml1,2-dichloroethane for method –A and in 10ml of acetonitrile for method- B and method –C .Different aliquots were treated as bulk mentioned under general procedure.

VI.   Effect of concentration of acceptor

To establish the optimum concentration of reagent, Epinastin 30 μg/ml was react with different volumes of DDQ ($8.81X10^{-3}$), p-CA ($9.57X10^{-3}$) and I_2 ($7.88X10^{-3}$). *viz.*, 0.5, 1, 1.5, 2, 2.5ml. The results showed that the highest absorbance was obtained with 1ml. Hence 1ml of the reagent was used for the determination of drug**.**

VII.   Effect of solvent

Both polar and non-polar solvents such as methanol, acetone, chloroform, 1, 2-dichloroethane and acetonitrile were used to select elegant solvent for the analysis of drug. Acetonitrile is found to be suitable solvent for p-CA and DDQ for Iodine 1, 2-dicloroethane is suitable solvent it produces maximum absorbance

with a fixed concentration of drug,   while other solvents produced lower absorbance due to incomplete dissociation of complex.

VIII.   Effect of reaction time

The interaction of epinastine with p-CA, Iodine and DDQ resulted in the formation of colored complexes   which stabilized   5min,10min and 15 min for p-CA, Iodine and DDQ respectively.   The developed color remained stable at room temperature for about an hour. After a day all solutions decolorized.
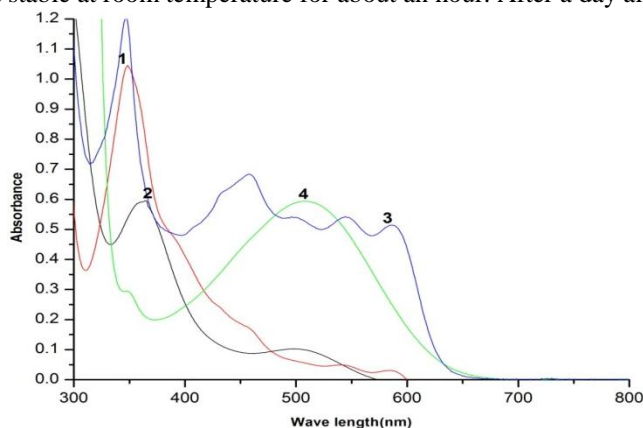

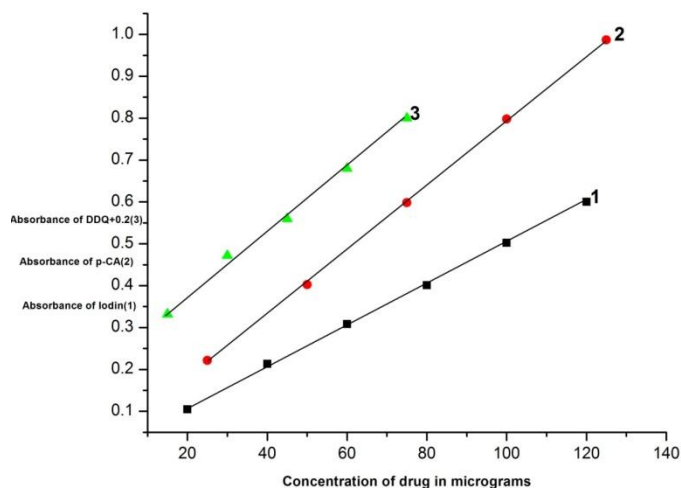Fig (2) :(1) Epinastine and charge transfer complexes of Epinastine with (2) Iodine, (3) DDQ and (4) p-CA

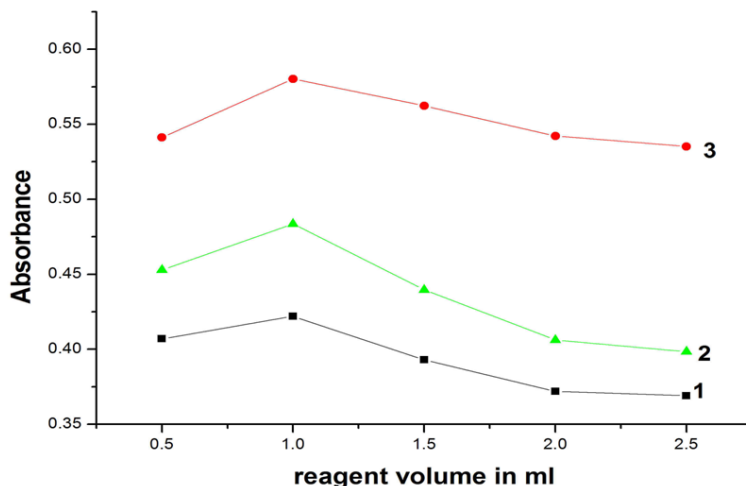Fig (3): Calibration curves of epinastine with (1) Iodine, (2) p-CAand (3) DDQ

Fig (4): Effect of volume of reagent on the optical density of the Ion-pair complex of Epinastine with (1) DDQ, (2) Iodine and (3) p-CA
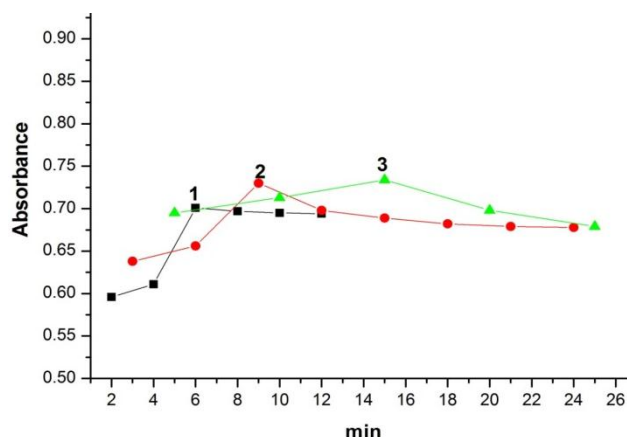
Fig (5): Effect of reaction time on formation of charge transfer complexes of Epinastine with (1) I₂, (2) p-CA, and (3) DDQ.

IX. Validation of the proposed methods

The methods developed have been validated in terms of guidelines of international conference of harmonization*viz.*, selectivity, sensitivity, precision, accuracy, linearity, LOD, LOQ. Sandell's sensitivity androbustness. The precision is tested by repeating each experiment atleast 6 times while the accuracy has been tested by taking known weight of sample and performing recovery experiments. Therobustness of themethodswere examinedby performing he experiments on three different spectrophotometers with excellent tally of absorbance values.

The methods developed have also been applied for the analysis of pharmaceuticals .The recovery experiments performed show high accuracy and precision and the results are compared with the available validated reported methods on this drug .The values % RSD and t-and F tests are in the permissible range of experimental errors (Table 2).And show that the methods can be used in both pharmaceutical and drug industries.

X. Stability constants of Ion-pair complexes

Benesi - Hildebrand method (BH)   is used for determination of stability constant K and molar absorption coefficient of the charge transfer complexes

$A_0/d = 1/K (D_0) € + 1/€$

Where A_0 = conc. of acceptor, d= optical density, D_0 = conc. of drug, €=Molar absorption coefficient and K= stability constant.

A plot of A_0/d Vs $1/D_0$ is a straight   line from whose slope and intercept the K and € are determined (Fig-6)

Table (1). Spectral, analytical and statistical parameters of charge transfer complexes of Epinastine with DDQ, p-CA and I₂.

Parameters	DDQ	p-CA	IODIN
λ max, nm	585	514	365
Beer's law limit (μg/ml)	15-75	25-125	20-120
Molar absorptivity (L mol⁻¹ cm⁻¹)	5250	8050	7990
Slope(specific absorptivity),b	0.0047	0.006	0.007
Intercept , a	0.0164	0.0802	0.0571
Correlation coefficient, r	0.9986	0.9981	0.9983
Sandell's sensitivity (μg cm⁻²)	0.16	0.14	0.21
Formation constant , K(M⁻¹)	581	500	416
Standard deviation of intercepts (n=6)	0.0024	0.0026	0.002
Limit of detection (μg/ml)	1.65	1.43	1.22
Limit of quantification (μg/ml)	5	4.33	3.7

Regression equation Y=a+bx ; x=conc. of drug((µg/ml)	0.005x	0.008x	0.0078x

Scheme 1 Charge transfer complex of Epinastine with p-Chloranilic acid (p-CA)

Acceptor   Charge transfer complex   anion   cation

(Donor) (p-Chloranilic acid)

Drug cation
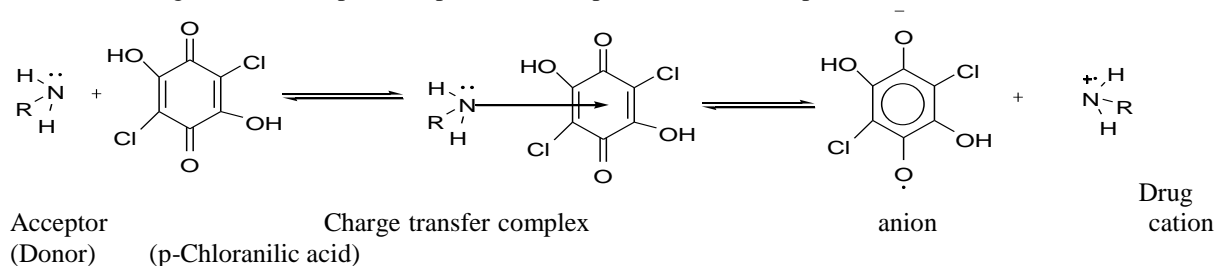
Scheme 2 Charge transfer complex of Epinastine with 2, 3-dichloro-5, 6-dicyano-1, 4-dibenzoquinone (DDQ)

Drug   Acceptor   Charge transfer complex   anion

cation (Donor)   (DDQ)
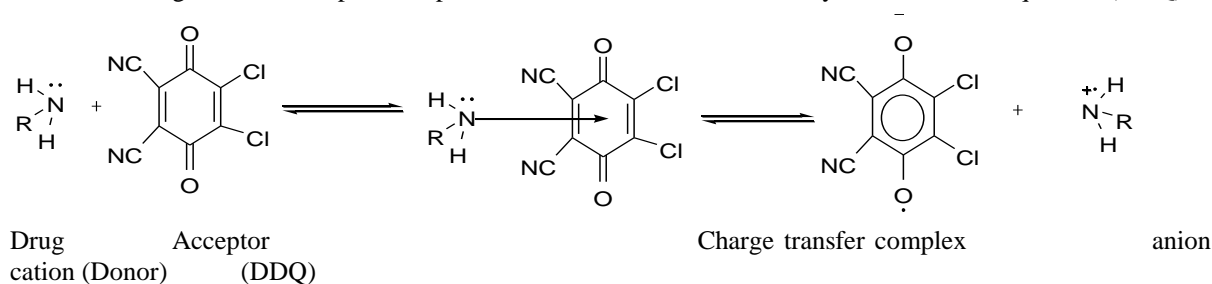
Scheme 3 Charge transfer complex of Epinastine with Iodine (I_2)
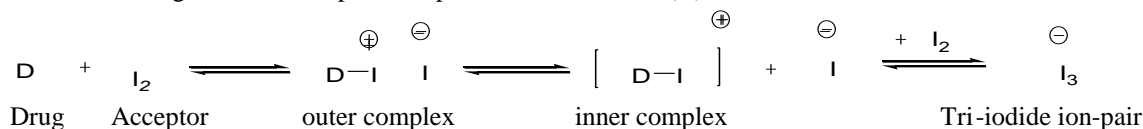
Drug Acceptor   outer complex   inner complex   Tri-iodide ion-pair

	DDQ	p-CA	Iodine
Amount Taken (µg/ml)	15 30 45 60	25 50 75 100	20 40 60 80
Amount Found (µg/ml)	14.9 29.97 44.92 59.87	24.7 49.9 74.98 98.76	19.86 39.57 59.8 80.01
% Recovery	99.33 99.9 99.82 99.78	98.8 99.81 99.97 98.76	99.3 98.9 99.6 100
% RSD	0.232 0.12 0.1 0.2	0.53 0.1 016 0.25	0.4 0.11 0.07 0.6
Proposed Mean ± SD	99.7±0.2327	99.335±0.5334	99.425±0.4031
Ref Mean ± SD	100.1±0.68	99.85±0.86	100.02±0.8
t-test	1.39 (1.83)	0.72 (1.83)	0.09 (1.83)
F-test	0.11 (5.19)	0.38 (5.19)	0.25 (5.19)

Table (2): Application of proposed methods for the analysis of the studied drug in their pure form.

	DDQ	p-CA	Iodine
Amount Taken (µg/ml)	15 30 45 60	25 50 75 100	20 40 60 80
Amount Found (µg/ml)	14.98 29.89 39.99	24.98 49.89 74.96	19.96 39.78 59.99

	59.76	99.99	80
% Recovery	99.86	99.92	99.8
	99.63	99.78	99.45
	99.97	99.94	99.98
	99.6	99.99	100
Proposed Mean ± SD	99.76±0.155	99.9±0.078	99.81±0.222
Ref Mean ± SD	100.12± 0.74	100.91±1.09	100.37±0.86
t-test	1.74(1.86)*		1.59 (1.86)*
F-test	0.04(6.39)*	0.005 (6.39)*	0.06 (6.39)*

Table (3): application of proposed methods for the analysis of studied drug in their pharmaceutical form

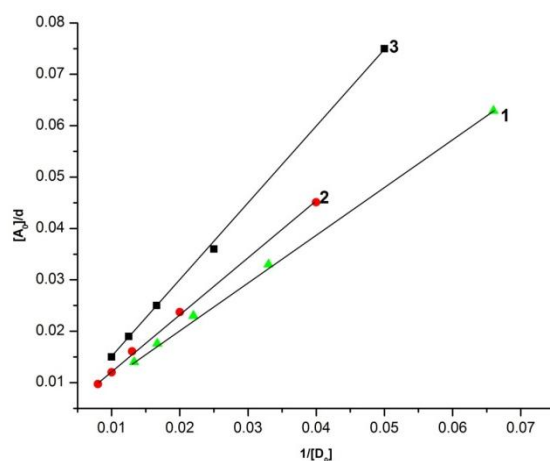Fig (6): Benesi-Hildebrand plot of Epinastine with (1) DDQ, (2) p-CA, and (3) Iodine

XI.   Stoichiometry

The stoichiometry of each of the complex has been determined from Job's continuous variation method and found to be 1:1 in each case .A typical Job's plot of Epinastine with IODINE,DDQand p-CA is presented in (Fig 7).

XII.   Conclusion

I_2,DDQ and p-CAforms ion-pair complexes with Epinastine drug and exhibits an absorption band at 365nm, 585nm and 514nm respectively. The interaction enabled the quantitative determination of this drug. The methods are validated; conditions are optimized and applied to the analysis of drugs and pharmaceuticals.
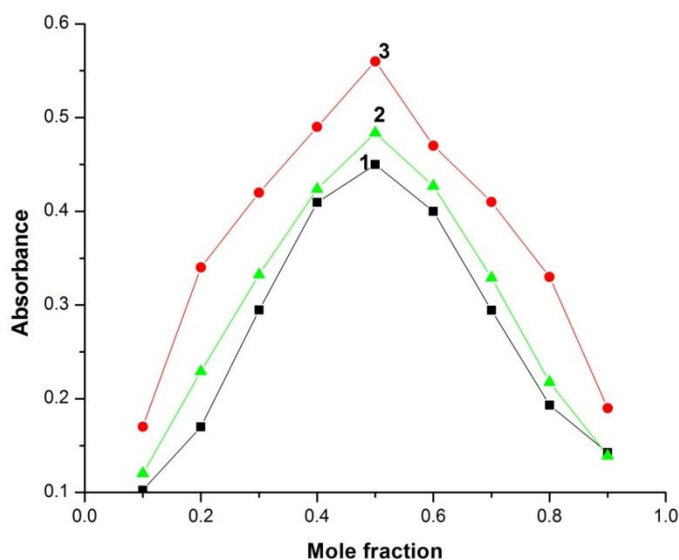

Fig (7): Job's continuous variation plot of Epinatine with (1) Iodine, (2) DDQ and (3) p-CA

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