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A Validated Stability Indicating LC Method for Simultaneous Estimation of Phenylephrine and Ebastine in Combined Pharmaceutical Dosage Form and their Application to Stress Degradation Studies

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ABSTRACT

A simple, sensitive and reproducible stability indicating RP-HPLC method for the simultaneous determination of Phenylephrine and Ebastinein bulk and Pharmaceutical dosage formhas been developed and validated. Chromatographic separation was carried out on kromasil C_{18} (250×4.6mm, 5µparticle size) column using a mobile phase composed of Phosphate buffer (adjusted to pH 5.0 with dilute OPA): acetonitrile: methanol in the ratio of 30:45:25 %v/v/vat a flow rate of 0.8 ml/min. The analyte was monitored using PDA detector wavelength at 211 nm. The retention time was found to be 2.295 min and 4.225 min for Phenylephrine and Ebastine respectively. The proposed method was found to be having linearity in the concentration range of 25-150µg/ml for both Phenylephrine($r^2$0.99996)and Ebastine($r^2$0.99987)respectively. The developed method has been statistically validated according to ICH guidelines. Stress testing which covered acid, alkali, peroxide, photolytic and thermal degradation was performed on under test to prove the specificity of the method and the degradation was achieved. The proposed method can be successfully applied for the stability indicating RP-HPLC simultaneous determination of Phenylephrine and Ebastine in bulk and combined tablet dosage form and in routine quality control analysis.

Keywords: Phenylephrine, Ebastine, RP-HPLC, Forced degradation, Method validation.

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INTRODUCTION

Phenylephrine

Chemically (Figure1), it is 3-[(1R)-1-hydroxy-2-(methylamino)-ethyl] phenol. It has a molecular formula of $C_9H_{13}NO_2$ and molecular weight of 167.21g/mol. Phenylephrine is a nasal decongestant, sympathomimetic and α-adrenergic receptor agonist. Phenylephrine produces its local and systemic actions by acting on α_1-adrenergic receptors peripheral vascular smooth muscle. Stimulation of the α_1-adrenergic receptors results in contraction arteriolar smooth muscle in the periphery. Phenylephrine decreases nasal congestion by acting on α_1-adrenergic receptors in the arterioles of the nasal mucosa to produce constriction; this leads to decreased edema and increased drainage of the sinus cavities.

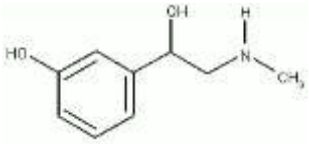

Figure 1: Chemical structure of Phenylephrine

Ebastine

Chemically (Figure 2),it is 4-(4-benzhydryloxy-1-piperidyl)-1-(4-tert-butylphenyl)butan-1-one. It has a molecular formula of$C_{32}H_{39}NO_2$ and molecular weight of 469.658 g/mol. Ebastine is a piperidine derivative, a long-acting, non-sedating, second-generation histamine receptor antagonist. It has antihistaminic and anti-allergic activity and also prevents histamine-induced bronchoconstriction. Ebastine and its active metabolite carebastine are selective histamine H_1 peripheral receptor antagonists. Thus it prevents the attachment of histamine on receptors and its activation (Activation of receptors of histamine on various tissues produce various allergic symptoms e.g. Runny nose)[1-3].

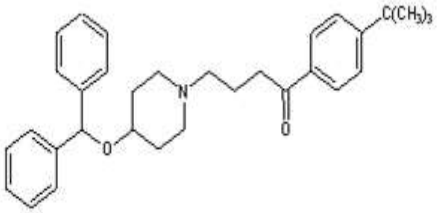

Figure 2: Chemical structure of Ebastine

Literature survey revealed that few analytical methods were reported so far for both drugs in combination or in alone likeHPTLC[4],RP-HPLC[5-7], LC-MS/MS method in biological fluids[8]and Spectrophotometric methods[9-10].The aim of the present study was to develop a simple, precise,

sensitive and selective stability indicating RP-HPLC method with PDA detection for the analysis of Phenylephrine and Ebastine in bulk and in combined tablet formulation.

MATERIALS AND METHOD

The Pharmaceutical grade pure samples of Phenylephrine and Ebastine were received as gift samples from Micro Labs Ltd., Bangalore. HPLC grade water, methanol and acetonitrile were purchased from E.Merck. Chem. ltd., Mumbai. All the chemicals used were of analytical reagent grade (Rankem). Fixed dose combination tablet formulation (Ebast-DC) containing 10 mg of Phenylephrine and 10 mg of Ebastine (Manufactured by Micro Labs Ltd., Bangalore)were procured from local market. Quantitative HPLC was performed on Waters Alliance e2695 and PDA Detector 2996series equipped with auto injector using empower software2.An UV-2400PC Series UV/Visible double beam spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements.

Chromatographic conditions

Parameter	Condition
Mobile phase composition	Phosphate buffer (adjusted to pH 5.0 with dilute OPA): acetonitrile: methanol in the ratio of 30:45:25 %v/v/v
Stationary phase	Kromasil C_{18}column (250 X 4.6mm, particle size 5µ)
UV detector wave length	211 nm
Run time	8 min
Flow rate	0.8 ml/min
Injection volume	10µl
Temperature	30^0C

Preparation of Phosphatebuffer

Accurately weighed quantity of 1.41gm of disodium hydrogen phosphate was transferred into a 1000ml volumetric flask. About 900ml of HPLC grade water was added and degassed by subjecting to sonication for 5min and final volume was made with water.Then pH of the solution was adjusted to 5.0 with dilute orthophosphoric acid solution. The buffer was filtered through 0.45 µ filter before use.

Preparation of Mobile phase

Phosphate buffer, acetonitrile and methanol were taken in the ratio 30:45:25%v/v/v and mixed and then degassed by subjecting to sonication for 10 min and resultant solution used as mobile phase after filtration using vacuum filtration assembly.

Preparation of diluent

Phosphate buffer and Methanol were taken in the ratio 30:70%v/v and used as diluent.

Preparation of standard solution

Accurately weighed and transferred each 50 mg of Phenylephrine and Ebastine working standards into a 50 ml clean and dry volumetric flask separately, 30 ml of diluents was added, sonicated to dissolve for 10 minutes and then made up to the final volume with diluent. From the above stock solution, 5ml each was pipette out in to a 50ml volumetric flask and then volume was made up to mark with diluent to obtain 100µg/ml solution of standard.

Sample solution preparation

20 tablets were accurately weighed and calculated the average weight of each tablet. The tablets were crushed to fine powder. Accurately weighed and transferred 531.2mg powder equivalent to 50mg of Phenylephrine and Ebastine into a 50ml volumetric flask, 30ml of diluent was added, sonicated for 10 min and volume was made up with diluent. Filtered through 0.45µ Millipore Nylon filter. From the filtered solution, 5ml was pipette out into a 50 ml volumetric flask and then volume was made up to mark with diluent to obtain each 100µg/ml solution of sample. Then Injected 10µl of filtered portion of the sample and standard preparation into the chromatograph. Recorded the responses for the major peaks. Calculated the content of Phenylephrine and Ebastine present in each tablet.

Method validation

Analytical validation parameters for this proposed method were determined according to ICH guidelines.

System suitability

System suitability was carried out by injecting 10 µl of the standard solutions five times into the chromatographic system. The system suitability parameters were then evaluated for tailing factor, retention time and theoretical plates of standard chromatograms. % RSD for peak area of five replicate injections of standard solutions (% RSD NMT 2) were within the limits. The results for system suitability studies are presented in table 1.

Specificity

The specificity of the method was performed by injecting blank, placebo, standard and sample preparations into the chromatograph. Chromatograms were recorded. Retention times obtained from standard and sample preparations were compared for identification of analytes.

Linearity

The linearity of an analytical method was determined on six concentration levels ranging from 25-150µg/ml for both Phenylephrine and Ebastine by diluting aliquots (0.25- 1.5 ml) of standard solution (100µg/ml) in to each 10 ml volumetric flasks separately made up to volume with diluent

and injected each concentration into the chromatographic system and the chromatograms were recorded. Calibration graphs were then plotted between concentration (µg/mL) and peak areas (mV) of Phenylephrine and Ebastine respectively. The linearity of the proposed method was then evaluated by linear regression analysis. The correlation coefficient, slope and intercept were calculated for both Phenylephrine and Ebastine as shown in Figure 4 and Figure 5.

Accuracy

The accuracy of the test method was demonstrated by % recovery across its range by making three different concentrations at 50%, 100% and 150 % levels using standard addition method, where sample preparations were spiked with known amount of standard preparations and then each concentration was injected triplicate into the chromatographic system.

PRECISION

System precision

System precision was established by six replicate injections of the standard solution into the chromatographic system. The corresponding peak areas were measured and % RSD was calculated.

Method precision

The method precision study was performed by injecting six sample preparations of marketed formulations into the chromatographic system. The corresponding peak areas were measured and % RSD was calculated.

Intermediate precision

A study was carried out by injecting six standard preparations on different days into the chromatographic system. The corresponding peak areas were measured and % RSD was calculated.

Robustness

Robustness of the method was determined by small deliberate changes in flow rate, mobile organic phase temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust.

Stability of the solution and Forced degradation studies

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results showed that for both the solutions, the retention time and peak area of Phenylephrine and Ebastine were remained almost similar (%RSD less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr., which was sufficient to complete

the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed. The results of the degradation studies are presented.

Acid degradation studies

To 1.0 ml of standard solution of Phenylephrine and Ebastine, 1.0 ml of 2N hydrochloric acid was added and refluxed for 30 mins at 60 °C and then neutralized with 1.0 ml of 2N NaOH solution.The resultant solution was suitably diluted with diluent to obtain 100µg/ml solution of Phenylephrine and Ebastine respectively. Then 10µl of the solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

Base degradation studies

To 1.0 ml of standard solution of Phenylephrine and Ebastine, 1.0 ml of 2N sodium hydroxide was added and refluxed for 30 mins at 60 °C and then neutralized with 1.0 ml of 2N HCL solution.The resultant solution was suitably diluted with diluent to obtain 100µg/ml solution of Phenylephrine and Ebastine respectively. Then 10µl of the solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

Peroxide degradation studies

To 1.0 ml of standard solution of Phenylephrine and Ebastine, 1.0 ml of 20 % hydrogen peroxide (H_2O_2) solution was added and the resultant solution was kept for 30 min at 60 °C. For HPLC study, the resultant solution was suitably diluted to obtain to obtain 100µg/ml solution of Phenylephrine and Ebastine respectively. Then 10µl of the solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation studies

1.0 ml of standard solution of Phenylephrine and Ebastine separately was placed in oven at 105 °C for 6 hr to study dry heat degradation. The resultant solution was suitably diluted to 100µg/ml solution of Phenylephrine and Ebastine respectively. Then 10µl of the solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the drug solution to UV light by keeping the beaker in UV chamber for 7 days or 200 Watt hours/m^2 in photo stability chamber. The resultant solution was suitably diluted to obtain 100µg/ml solution of Phenylephrine and Ebastine respectively. Then 10µl of the solutions were injected into the chromatographic system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION

From this study, it was found that a simple, precise, accurate, sensitive and efficient stability indicating RP-HPLC method has been developed and validated for the simultaneous estimation of Phenylephrine and Ebastine in bulk and pharmaceutical dosage form. Separation was done by using mobile phase composed of Phosphate buffer (adjusted to pH 5.0 with dilute OPA): acetonitrile: methanol in the ratio of 30:45:25 %v/v/v on Kromasil C_{18} column (250 X 4.6mm, 5µparticle size) at a flow rate 0.8 ml/min using PDA detection at 211 nm. The retention times were found to be2.295 min and 4.225 minfor Phenylephrine and Ebastine respectively. System suitability chromatogram as shown in figure 3 and results are shown in table 1.Linearity was evaluated in the concentration range of 25-150 µg/ml forboth Phenylephrine and Ebastine respectively. The calibration curves of Phenylephrine and Ebastine were described by the equation y = 22471.3x +528.39andy = 37234.7x +1187.7 with correlation coefficient of0.99996forPhenylephrine and 0.99987 for Ebastine as shown in figure 4 and figure 5 respectively. The standard and sample chromatograms in the specifity studies are shown in figure 6 and figure 7. The Limit of detection (LOD) and limit of quantification (LOQ) are shown in figure 8 and figure 9. The %RSD in precision, accuracy and robustness studies were found to be less than 2.0%, indicating that the proposed method was found to be precise, accurate and robust. Accuracy data as shown in table 2. The validation summary parameters and assay results obtained from the marketed formulation is shown in table 3 and table 4. The results of robustness studies are shown in table 5 and table 6. The stress testing chromatograms for both Phenylephrine and Ebastine are shown from figure 10 to figure 14 and results are shown in table 7 and table 8.

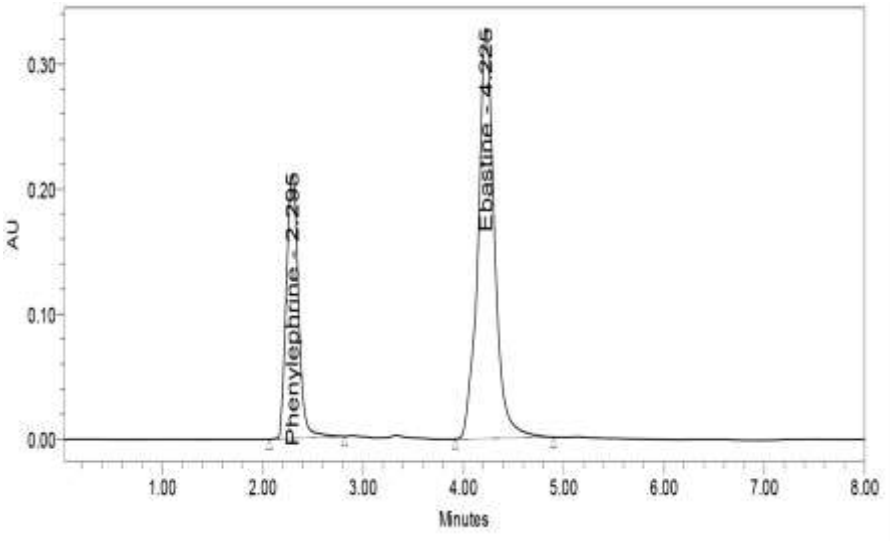

Figure 3: Typical chromatogram of system suitability solution

Table 1.System Suitability Results

S.No	System suitability parameters	Phenylephrine	Ebastine
1	Tailing factor(T_f)	1.11	1.06
2	Resolution (Rs)	6.22	
3	Retention time(Min)	2.295	4.225
4	Theoretical plates(N)	3790	3796

Table 2: Accuracy data

Sample	Level	Peak	Amount added	Amount recovered	Mean % Recovery
Phenylephrine	50%	1061021	5.00	4.98	99.72±0.42
	100%	2121682	10.00	9.99	99.97±0.56
	150%	3181562	15.00	15.02	100.16± 0.62
Ebastine	50%	1766159	5.00	4.99	99.96±0.22
	100%	3543883	10.00	10.00	100.02±0.43
	150%	5319364	15.00	15.02	100.19±0.71

***Mean of three determinations**

Linearity

The calibration curve was found to be linear over the concentration range of 25-150 µg/ml for both Phenylephrine and Ebastine. The correlation coefficient was found to be 0.999 for both Phenylephrine and Ebastine respectively.

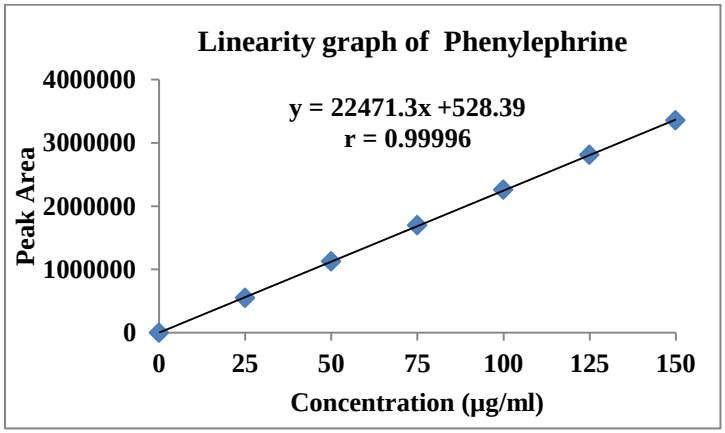

Figure 4: Linearity Graph of Phenylephrine(25-150 µg/ml)

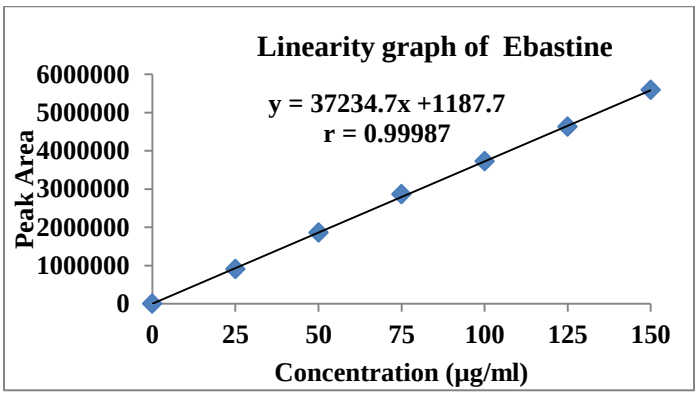

Figure 5: Linearity Graph of Ebastine (25-150 µg/ml)

Table 3: Validation Parameters of the proposed RP-HPLC Method

Parameter	Phenylephrine	Ebastine
Regression equation	y = 22471.3x +528.39	y = 37234.7x +1187.7
Correlation coefficient	0.99996	0.99987
LOD (µg/ml)	0.16	0.74
LOQ (µg/ml)	0.54	2.56
System precision (% RSD)	0.85	0.94
Method precision (% RSD)	0.37	0.53
Intermediate precision (% RSD)	0.76	0.64

Table 4: Results of Assay in Marketed Formulation

Brand	Drug	Standard peak area	Sample peak area	Labelled amount (mg/tab)	Amount found (mg/tab)	% Assay	%RSD*
Ebast-DC	Phenylephrine	2125122	2120410	10.0	9.96	99.64%	0.46
	Ebastine	3531214	3497920	10.0	9.98	99.88%	0.37

***Mean of two determinations**

Specificity studies

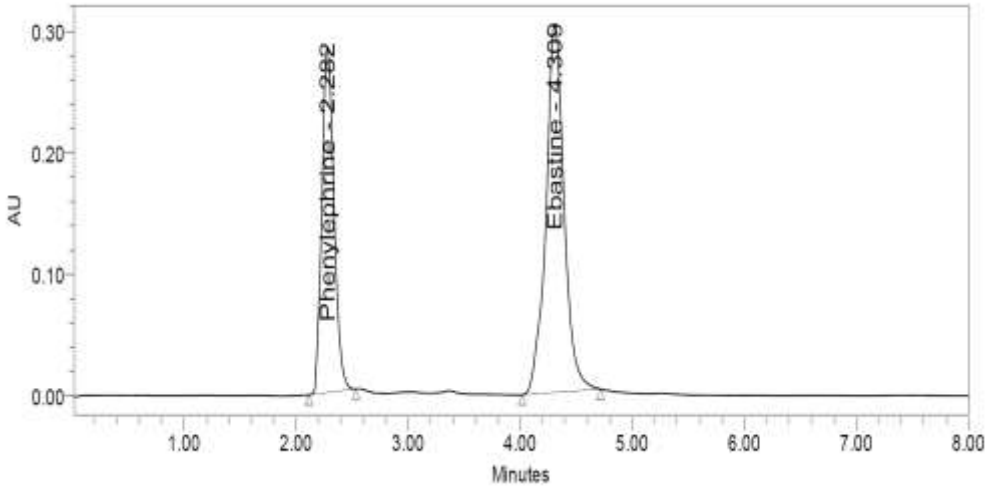

Figure 6: Typical chromatogram of standard

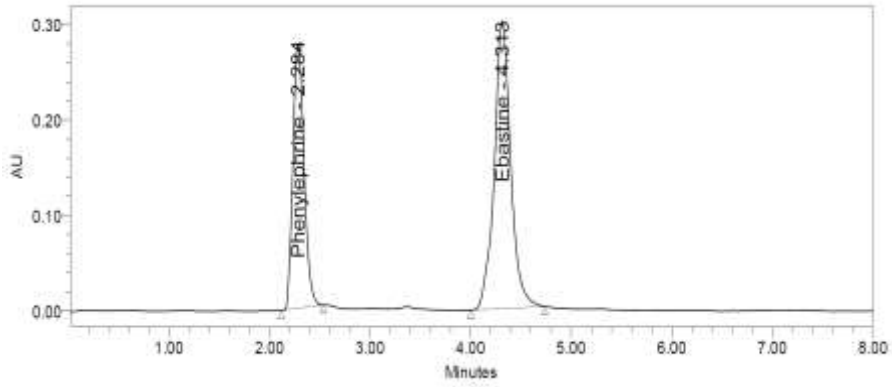

Figure 7: Typical chromatogram of sample

Limit of detection (LOD) and Limit of Quantification (LOQ)

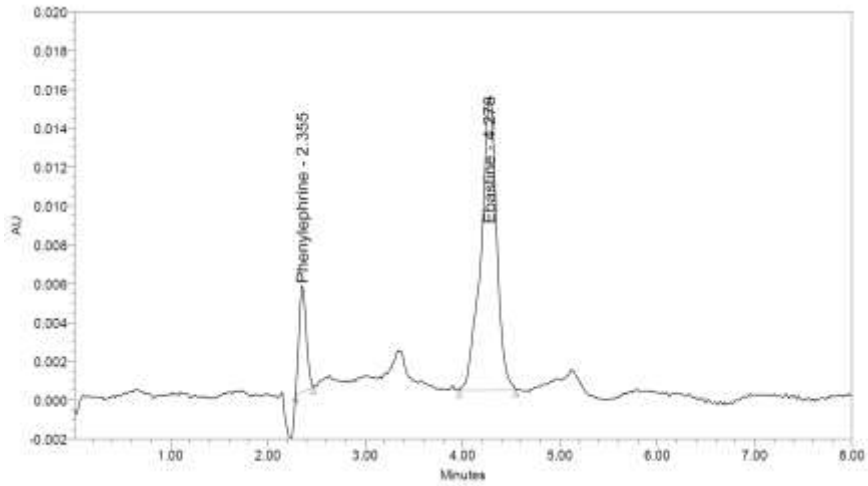

Figure 8: Typical chromatogram of Limit of detection (LOD) solution

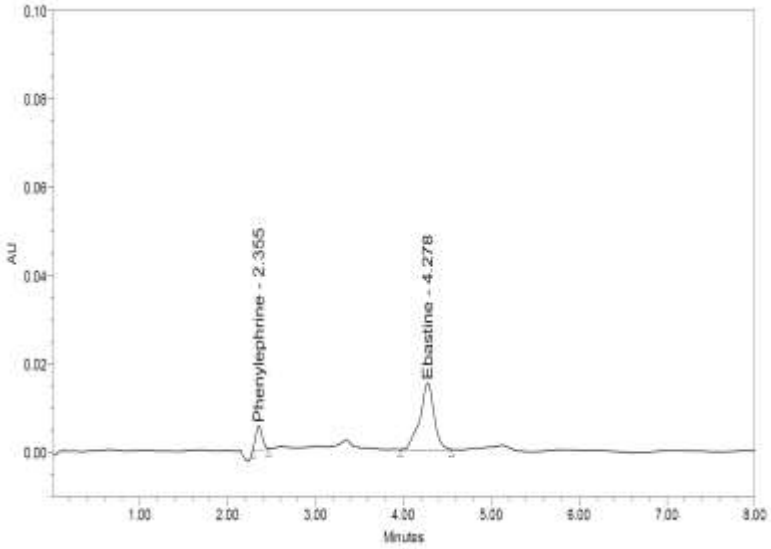

Figure 9:Typical chromatogram of Limit of Quantification (LOQ) solution

Robustness

The developed method is robust with deliberate changes in variation of mobile organic phase composition, flow rate and temperature for both Phenylephrine and Ebastine respectively.

Table 5: Results of robustness study of Phenylephrine

S. No.	Parameter	Change Level	Phenylephrine			
			Retention	Peak	USP	USP
1.	Flow rate (±0.1ml/min)	0.7	2.32	2140696	1.14	3849
		0.9	2.06	2131095	1.11	3979
2.	Mobile organic phase composition (±10%v/v/v)	Less	2.46	2138349	1.06	3906
		More	2.12	2139203	1.06	3721
3.	Temperature (±5°C)	25 ^{0}C	2.31	2138563	1.05	3858
		35 ^{0}C	2.17	2126401	1.06	3744

Table 6: Results of robustness study of Ebastine

S. No.	Parameter	Change Level	Ebastine			
			Retention time	Peak area	USP	USP Plate
1.	Flow rate (±0.1ml/min)	0.7	4.23	3557804	1.03	3668
		0.9	3.74	3559931	1.07	3530
2.	Mobile organic phase composition (±10%v/v/v)	Less	4.47	3541141	1.02	3811
		More	3.94	3553549	1.03	3748
3.	Temperature (±5°C)	25 ^{0}C	4.19	3553549	1.04	3718
		35 ^{0}C	4.06	3540432	1.06	3834

Forced degradation studies

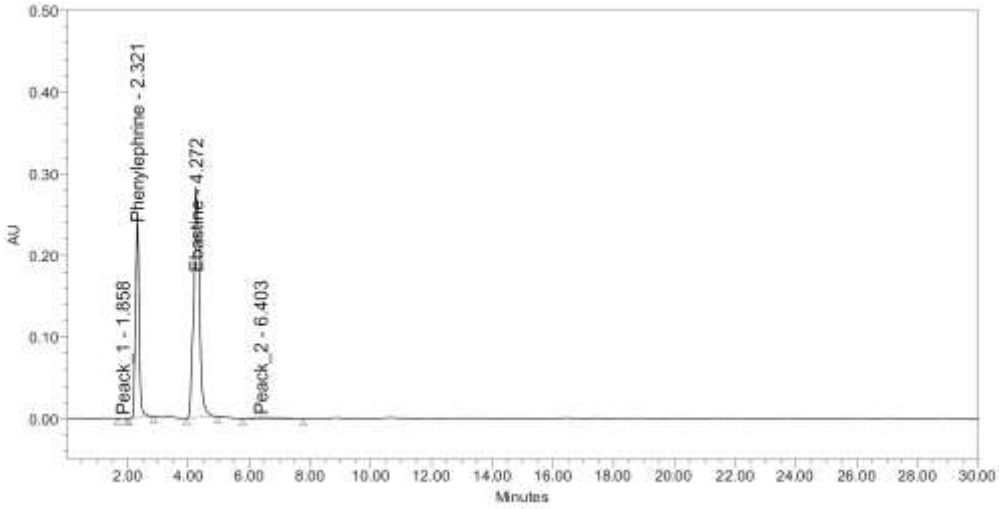

Figure 10: Chromatogram of Acid hydrolysis

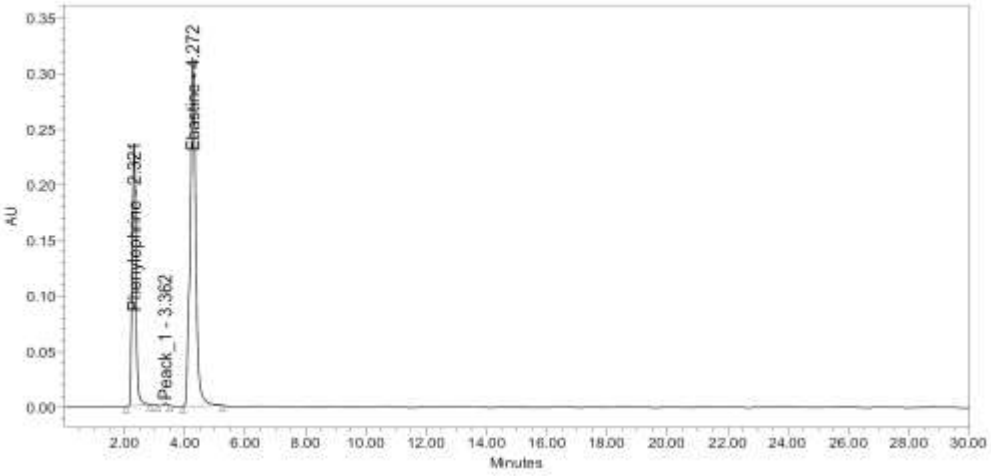

Figure 11: Chromatogram of Base hydrolysis

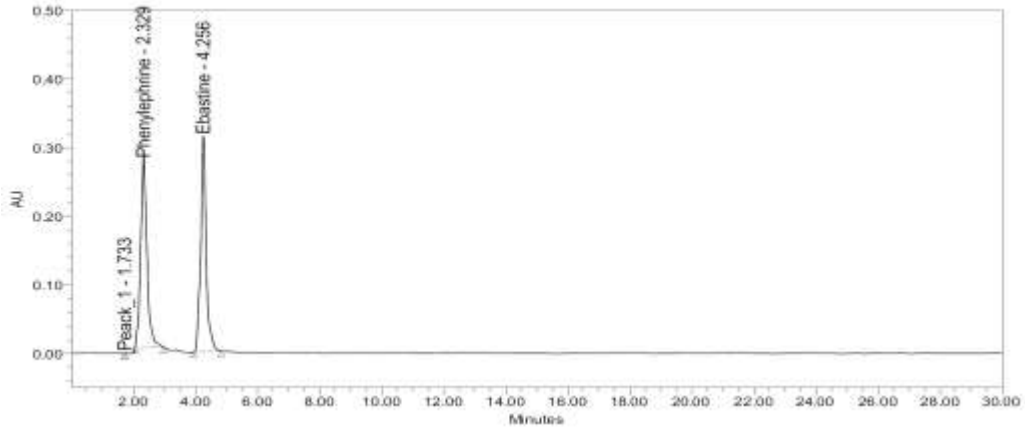

Figure 12: Chromatogram of Oxidation (peroxide)

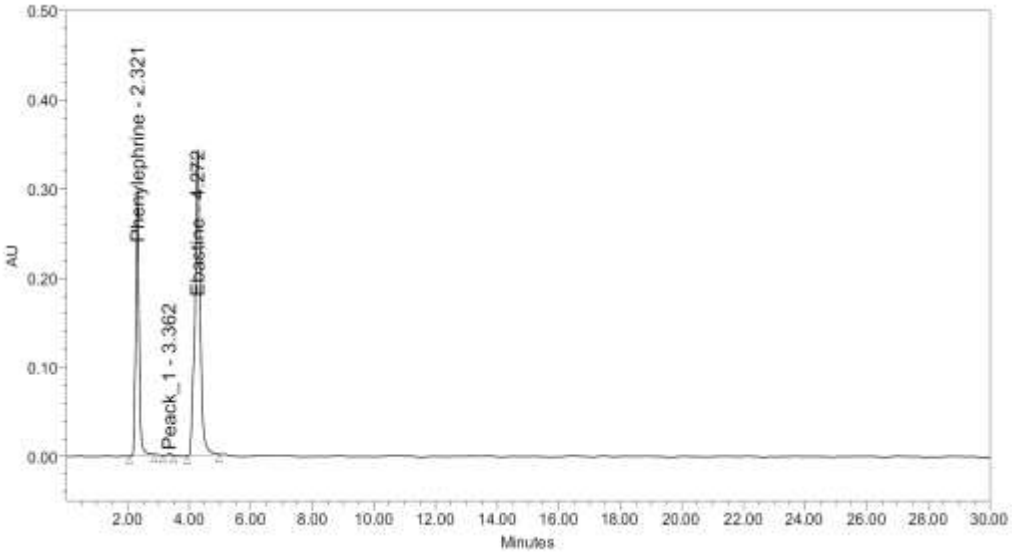

Figure 13: Chromatogram of Heat Exposure

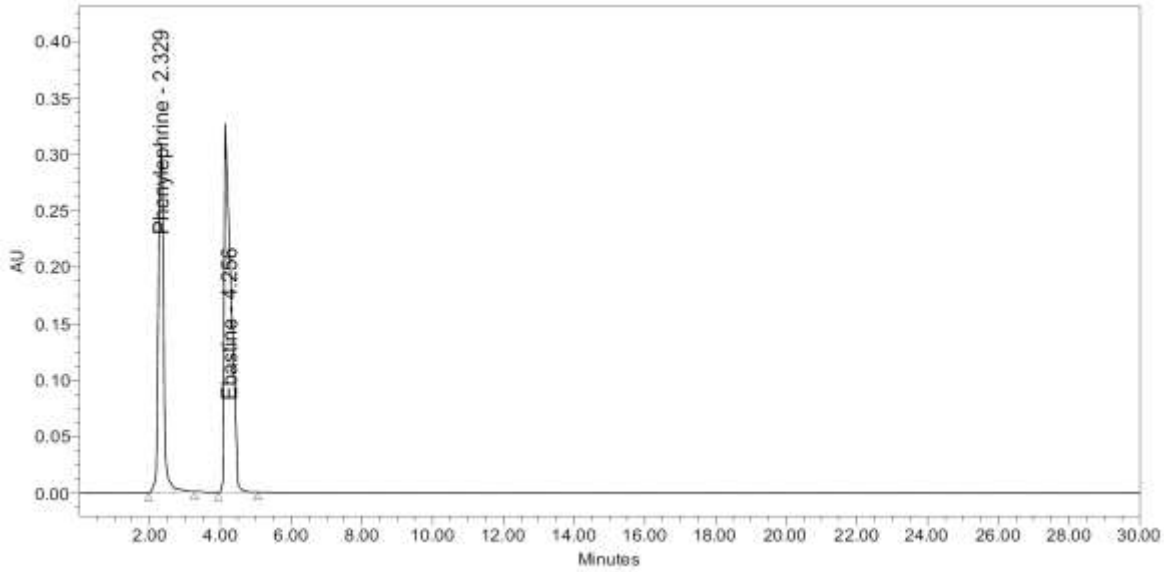

Figure 14: Chromatogram of UV Exposure

Table 7: Degradation Study of Phenylephrine

S.No.	Condition	Retention time	Peak	Purity	Purity
1	Acid degradation	2.321	1978354	0.208	0.475
2	Base Hydrolysis	2.321	1998452	0.308	0.475
3	Heat Exposure	2.321	2038354	0.208	0.475
4	Oxidation (peroxide)	2.329	2018268	0.132	0.369
5	UV Exposure	2.329	2038354	0.208	0.475

Table 8: Degradation Study of Ebastine

S. No.	Condition	Retention time(min)	Peak Area	Purity angle	Purity threshold
1	Acid degradation Hydrolysis	4.272	3182619	0.210	0.398
2	Base Hydrolysis	4.272	3207499	0.309	0.422
3	Heat Exposure	4.272	3242619	0.210	0.398
4	Oxidation (peroxide)	4.256	3222974	0.296	0.451
5	UV Exposure	4.2256	3242619	0.210	0.398

CONCLUSION

From this study, it is concluded that the proposed Stability Indicating RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of Phenylephrine andEbastine in bulk & Pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

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