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Original Article

METHOD DEVELOPMENT, VALIDATION AND STABILITY INDICATING STUDIES FOR DETERMINATION OF EBASTINE IN TABLET DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

RP- HPLC method is developed for estimation of Ebastinein the tablet dosage form. Employing a simple and stability-indicating HPLC method, using stress degradation studies, drug was well separated from the degradantswith good peak resolution. Mobile phasewas prepared with using ortho phosphoric acid and diethylaminebuffer, methanol and acetonitrile in the ratio of 10: 70: 20 v/v. The chromatographic separation was achieved by using Kromasil 100 C8, 250x4.6mm, 5 µLat a flow rate of 1.0 mL/min. the detection wavelength selected is 210 nm.The drug was subjected for degradation studies acid degradation, base degradation, peroxide degradation, photolytic degradation and thermal degradation. Ebastine was eluted at a retention time of 6 minutes. the developed method is used for assay of orodispersible tablets containing Ebastine. The percentage assay was found to be 99.82 %. Linearity of the drug for the developed method was found within a range of 80 µg/ml to 800 µg/ml. the method was precise with % RSD values below 2. The method is found accurate with % recoveries of 99.3 to 100.6 %. The method was validated as per ICH guidelines. Validation results confirm the applicability of the developed method for quality analysis and stability studies of the regular product on the manufacturing stream.

***Keywords**: Ebastine; degradation; RP-HPLC; method development; Validation;*

INTRODUCTION

Ebastine (EBA), chemically, 4– (4–benzhydryloxy–1–pipe ridyl) –1– (4–tert–butylphenyl) butan–1–one is a non sedating H1 antihistamine. Assay of Ebastine in bulk form is official in British Pharmacopoeia EBS is very soluble in methylene chloride and sparingly soluble in methanol. It is used in antihistaminic treatment. Ebastine, a piperidine derivative, is a long-acting, non-sedating, second-generation histamine receptor antagonist that binds preferentially to peripheral H1 receptors. It has antihistaminic, antiallergic activity and prevents histamine induced broncho-constriction. It does not have significant sedative or antimuscarinic actions. Ebastine is normally is available as orodispersible tablets dosage form with 20 mg. Figure 1 shows the chemical structure of Ebastine. The literature survey shows very few reports on analytical methods to analyse the Ebastine. Few methods were reported by HPLC in presence of its impuriti1es, LC/MS for metabolites, pharmacokinetic studywas available. But there is no single report on stability method development. The methods that are available suffer from few drawbacks regarding the retention time, linearity range etc1-6. The present method focus on establishing a method at a low retention time, a method which is applicable for extensive concentration range with a good reproducibility and accuracy.

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2.0 EXPERIMENTAL

2.1 Instruments:

The separation was achieved by using Kromasil 100 C8, 250x4.6mm, 5 µ columnat a flow rate of 1.0 mL/min..The Agilent-HPLC 1100 series containing quaternary pump, degasser, auto injector andUV detector the range 200-400 nm using empower 3 software. The Mettler Toledo analytical balances range from 1 mg 200 g used for the preparation of standard and samples.

2.2 Chemicals and Reagents:

The drug was procured from the MSN laboratories, Hyderabad, Telangana, India.AR grade Ortho phosphoric acid, diethylamine reagents purchased from the Merck, mumbai, india. The HPLC grade acetonitrile (ACN) and HPLC grade Methanol was procured from Merck chemicals. High purity water (HPLC grade) used for all the experiments.

2.3. Chromatographic conditions:

The Chromatographic separation was achieved by using the a buffer prepared by adding 11.9 g of Orthophosphoric acid in 50 mL volumetric flask and diluted with 25 mL of water and the volume was made up to the mark with water and mixed well, 25 mL of the above solution was added to mixture of 450 mL of water and 6 mL of diethyl amine and pH id adjusted to 6 using diethyl amine and volume was made up to 500 mL with water. The mobile phase consist of a mixture of the Buffer, Methanol and Acetonitrile in the ratio of 10: 70: 20 .the separation was achieved using Kromasil 100 C8, 250x4.6mm, 5 µ column. The detection wavelength is selected as 210 nm with 5µl as a injection volume.

2.4. Diluent

Mobile phase is used as Diluent.

2.5 Standard solution

Weigh accurately about 40 mg of Ebastine working standard and transfer into a 100 mL volumetric flask, add 70 mL of diluent sonicate it to dissolve and then make up to volume with same diluent.

2.6 Sample solution

Weight accurately not less than 20 tablets and note down the weight. Then calculate the average weight. Crush the tablets in to fine powder with mortar pestle then weigh accurately about 600 mg of powdered sample (equivalent to 40 mg of Ebastine) and transfer into a 100 mL volumetric flask, then add 70 mL of diluent, sonicate to 15 minutes with intermediate shaking, then make up to the volume with diluent and mix well. Centrifuge the above solution at 3500 rpm about 5 minutes (or) Filter through 0.45 µm PVDF or Nylon filter.

3.0 RESULTS AND DISCUSSION

3.1 Method development:

The main aim is to develop a simple stability indicating method for estimation of Ebastine with optimum resolution with the degradents by using the HPLC. The estimation of Ebastinein pharmaceutical dosage form by spectrophotometric methods is critical in terms of specificity due to presence of placebo, which contains different types of in active ingredients. Simple RP HPLC methods are preferable in quality control labsto get reproducibility and accurate results within short time. The initial method development started with selection buffer and pH. Based on the pka value and other physicochemical properties of Ebastine, decided to go with Orthophsphoric acid solution, further adjusted the pH 6.0 with diethylamine. To optimize the column two different manufactures with the same stationary phase used, in that Kromasol C8 column has given optimum resolution with symmetry peak shape. The sample is injected using the optimized chromatographic conditions and evaluated for the system suitability parameters. All the results (Table-2) were found satisfactory. Further, performed forced degradation analysis and verified interference placebo peaks, there was no interference was observed and impurities well resolved from each other.

3.3 Method validation

3.3.1 Specificity

Specificity was carried out by conducting different force degradation studies. Base degradation was performed with 1.0 N NaOH at 60°C for 2 hours. Acid degradation studies were performed with 1 NHCl at 60°C for 2hr. Other degradation studies were performed using dry heat at 50°C, humidity 90% RH, UV, Visible, peroxide at 60 oC for 2 hours and water degradation at 60°C for 5 hours. The interference of the placebo peaks and other degradation peaks were verified with help of peak purity. In all the conditions the peak purity was passed (Purity angle less than that of purity threshold). Specificity results were represented in table 4.

3.3.2 Linearity:

To demonstrate linearity of optimized method, prepared the standard solutions about concentration of 1000 µg mL-1. The stock solution was further diluted to a series of seven solutions from the range of 80 to 800 µg mL-1of Ebastine in the diluent. Further, a linearity graph containing peak response against the concentration was plotted. The Correlation coefficient was found more than 0.999 for the Ebastine.

3.3.3 Limit of Detection (LOD) and Limit of quantification (LOQ):

The LOD and LOQ values for APX and impurities were established by calibration curve method. LOD and LOQ were calculated by using the below following formula. (Table 3)

$$LOD = \frac{3.3 \times SD \text{ of y-intercept}}{\text{Slope of a calibration curve}}$$

$$LOQ = \frac{10 \times SD \text{ of y-intercept}}{\text{Slope of a calibration curve}}$$

3.3.4 Repeatability

The precision is decomposed into the repeatability of the system and the repeatability of the method and the intermediate precision. The repeatability of the system is demonstrated by injecting after equilibration of the chromatographic system, 6 replicates of a standard solution of Ebastine 0.4 mg/mL in the diluent. The repeatability of the assay method was demonstrated by analysing 6 replicate of samples prepared from Ebastine 20 mg, orodispersible tablets as per test method. The individual results are reported together with the mean value, the standard deviation, the relative standard deviation and the confidence limits. The intermediate precision has been demonstrated by analysing in triplicate the samples of Ebastine 10 mg and 20 mg, orodispersible tablets, as per test procedure on three different days with different analysts, different systems and different columns. The %RSD values for each individual impurity at 100% concentration level are found below 2.0% (Table-3 & Figure-2).

3.3.5 Accuracy

Accuracy was performed with freshly prepared samples at 20, 50, 80, 100 and 120 % levels of test concentration. The solutions were replicated with three preparations at each level. Results are tabulated in tables 3.From the 3 value groups, the mean % recovery rates, the mean value and the standard deviation for each concentration are reported.

3.3.6 Robustness

Robustness was performed by by altering the optimized chromatographic conditions. The robustness studies were performed by making deliberate changes in pH, composition of the mobile phase, variation in flow rate and variation in column oven temperature. The results were tabulated in tables 4-9

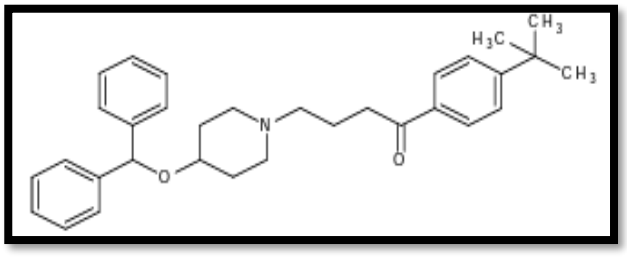

Figure 1. Chemical structure of Ebastine

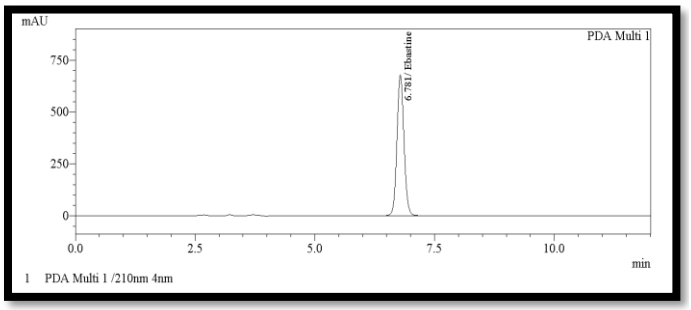

Figure 2.Chromatogram of test

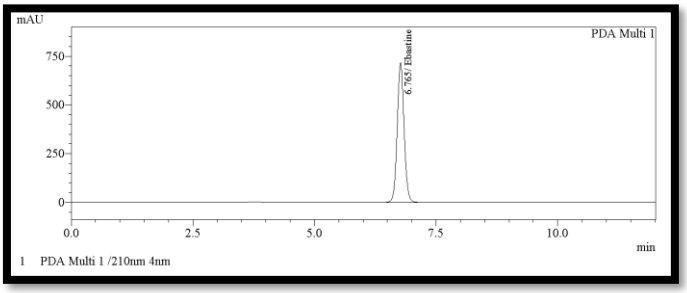

Figure 3. Chromatogram of test

Table 1. Results of Forced degradation conditions

Stress Condition	Drug Product		
	% Degradation	Purity Angle	Purity Threshold
1N HCl solution for 2 hours at 60°C (Acid)	12.86	0.257	0.260
1N NaOH solution for 2 hours at 60°C (Base)	0.00	0.263	0.265
1% Hydrogen Peroxide (H2O2) for 2 hours at 25°C (Peroxide)	8.39	0.248	0.260
Purified water for 5 hours at 60°C (Aqueous)	0.00	0.257	0.267
Sun-Light for about 1.2 Million.Lux.Hours	0.76	0.259	0.267
UV-Light for about 200 Watts/m2	4.65	0.258	0.265
Dry heat at 50°C for about 24 hours	0.62	0.257	0.265
Humidity at 25°C and 90% RH for about 7 days	0.00	0.266	0.270

Table 2. Results of linearity

Concentration in µg/ml		Response
Theoretical	Practical	
80.0	80.0157	1575972
200.0	200.0393	3899641
320.0	320.0628	6193009
400.0	400.0786	7654990
480.0	480.0943	9082162
600.0	600.1178	11499542
800.0	800.1571	15033216
Slope		18716.29721
Y-Intercept		146430.0122
Correlation Coefficient		0.999865

Table. 3: Results of System Precision for Assay

Injection N°	Response (mAu.) for Ebastine
01	7679950
02	7616988
03	7613860
04	7615636
05	7613802
06	7618211
Mean	7626408
Standard deviation	26287.206
Relative standard dev. (%)	0.3

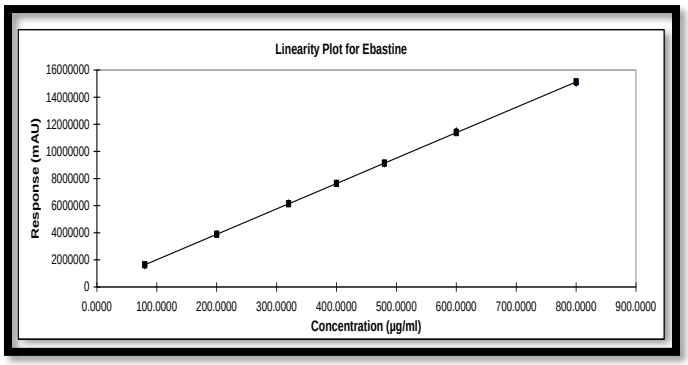

Figure. 2: Linearity graph of Ebastine

Table. 4: Results of Method Precision for Assay

Sample No	Obtaiined Quantity
01	20.10
02	20.33
03	20.35
04	20.08
05	20.07
06	20.31
Mean	20.21
Standard deviation	0.136
% Relative standard dev.	0.7
Confidence limits (%)	0.5
95% Confidence interval	20.10 – 20.32

Table. 5: Results of intermediate Precision for Assay

Series	Sample N°	Ebastine content (mg/Tablet)	Mean	Variance
1	1	20.10	20.26	0.0193
	2	20.33		
	3	20.35		
2	1	19.86	19.85	0.0017
	2	19.88		
	3	19.80		
3	1	20.18	20.12	0.0026
	2	20.08		
	3	20.11		
	Mean	20.08	Cochran test : Cexpt.= Varmax/∑var Cexpt.= 0.8178	
	Standard deviation	0.198		
	(%) Relative standard dev.	1.0		
	Confidence limits (%)	0.6		
	95% Confidence Interval	19.95 to 20.21		

Table 6: Robustness study showing variation in pH of mobile Phase

Series	Sample N°	Ebastine content (mg)		Percent recovery	Mean (%)	Variance
		Theoretical	Calculated			
20%	1	8.48	8.60	101.38	101.0	0.1157
	2	8.42	8.49	100.86		
	3	8.45	8.51	100.74		
50%	1	20.14	20.43	101.42	101.4	0.0052
	2	20.09	20.38	101.46		
	3	20.18	20.45	101.32		
80%	1	32.12	32.39	100.84	101.0	0.0387
	2	32.05	32.37	100.99		
	3	31.86	32.25	101.23		
100%	1	40.16	40.38	100.54	100.6	0.0040
	2	40.24	40.50	100.65		
	3	40.19	40.45	100.65		
120%	1	48.22	48.10	99.75	99.3	0.2122
	2	48.15	47.87	99.42		
	3	47.96	47.40	98.84		
150%	1	60.20	59.72	99.21	99.2	0.3484
	2	59.50	59.36	99.77		
	3	58.70	57.87	98.59		
200%	1	78.30	76.98	98.31	98.2	0.0186
	2	78.60	77.06	98.04		
	3	79.00	77.59	98.21		
Mean				100.1	Cochran test : Cexpt.= Varmax/∑var Cexpt.= 0.4690	
Std dev.				1.1725		
% RSD.				1.2		
Confidence limits (%)				0.6		
95% Confidence interval				99.5 to 100.7		

Table 7: Robustness study showing variation in pH, organic phase ratio of mobile Phase, flow rate and column oven temperature

pH Variation	**Average % Assay of Two Test preparations**	**Difference from Actual % Assay**
pH 5.9	99.6	0.1
pH 6.0	99.5	NA
pH 6.1	99.0	0.5
Organic Variation (Methanol)	Average % Assay of Two Test preparations	Difference from Actual % Assay
90%	100.0	0.7
100%	100.7	NA
110%	100.0	0.7
Organic Variation (Acetonitrile)	Average % Assay of Two Test preparations	Difference from Actual % Assay
90%	98.8	0.0
100%	98.8	NA
110%	97.4	1.4
Flow Rate	Average % Assay of Two Test preparations	Difference from Actual % Assay
0.9 ml/min	100.4	0.3
1.0 ml/min	100.7	NA
1.1 ml/min	99.9	0.8
Column Temperature	Average % Assay of Two Test preparations	Difference from Actual % Assay
20°C	99.6	1.1
25°C	100.7	NA
30°C	99.2	1.5

4.0 CONCLUSION

A robust stability indicating RP-HPLC method for Ebastineisdeveloped.Method validation was performed with specificity, precision, linearity, robustness, ruggedness, accuracy, limit of detection and limit of quantification. The specificity of the method is established by stress degradation studies. In the stressed conditions (acid, base, peroxide, aqueous, sunlight, humidity, UV light and dry heat) % degradation observed up to 12.86%. In all the conditions peak purity of ebastine was evaluated, and found that the ebastine peak was pure. This indicates that there is no interference and no co-elution of peaks due to impurities in quantifying the assay of ebastine in Ebastine 20 mg orodispersible tablets. The linearity of Ebastine has been demonstrated for concentration of 80 µg/ml to 800 µg/ml. the method is found precise demonstrating the % RSD values of 0.3 % for Repeatability, 0.7 % for method precision and 0.9 % for system precision. The method is found accurate with % recoveries of 99.3 to 100.6 %. The method is found robust after making the deliberate changes, it demonstrated that there is no change in the system suitability of the method. Thus it can be concluded that the method can be successfully employed in the routine assay of Ebastine from tablet dosage form.

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