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Research Article

Analytical Method Development and Validation for the Assay of Ebastine in Ebastine Mouth Dissolving tablets

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ABSTRACT

An accurate, simple, sensitive and selective reverse phase liquid chromatographic method has been developed for the determination of Ebastine in its pharmaceutical preparations. The proposed method depends on the reverse phase chromatography which was conducted using Octadecylsilane (C18) (250 × 4.6 mm id) column at 40º C temperature with UV-detection at 255 nm. A mobile phase containing a mixture of phosphoric acid buffer adjusted to pH 6.0 and Acetonitrile in the ratio of 400:600, has been used for the determination of Ebastine at a flow rate of 1.0 ml/min. It is also desirable to have less run time; Henceforth the analysis of assay sample will become fast and reliable. The method was validated to meet requirements of global regulatory filling.

Key words: Ebastine, reverse phase chromatographic technique, method validation.

INTRODUCTION

Ebastine is official in British pharmacopoeia. It is a second-generation H1 receptor antagonist that is indicated mainly for allergic rhinitis and chronic idiopathic utricaria. The antihistaminic action is mainly induced by the active metabolite, carebastine that is rapidly generated in the small intestine and in the liver. Ebastine is chemically 1-[4-(1,1-dimethyl ethyl) phenyl]-4-[4-(diphenyl methoxy)piperidin-1-yl]butan-1-one which has no effects on cardiovascular and psychomotor functions, which occurred during treatment with classical antihistamine agents such as chlorpheniramine and diphenhydramine Various methods like UV spectrophotometry[1,2] for estimation of Ebastine with other drugs , HPLC[3] , HPLC[4,5] for determination of Ebastine are reported in literature.

MATERIAL AND METHODS

Chemicals & Reagents: The standard and sample drug of Ebastine was obtained as a gift sample from Bal pharma Pvt Ltd, Bommasandra, Banglore. All other chemicals and the reagents used were of Merck grade.

Instrumentation: A High performance liquid chromatography (Make: Schimadzu, Model: LC2010 CHT) equipped with Auto Sampler and UV detector[6] with the software of LC Solutions. All weighing's were done on Analytical balance (Make: Shimadzu, Model: AY-220).

Chromatographic Conditions: The chromatographic conditions were optimized by using Octadecylsilane (C_{18}) column (250 x 4.6mm, particle size 5µ) with the mobile phase, buffer (Phosphoric acid buffer with pH adjusted to 6.0) : Acetonitrile in the ratio of 400:600. The flow rate of the mobile phase was maintained at 1.0 ml/min and the detection was carried out at 255 nm. All determinations were performed at constant column temperature (40^0C).

Buffer preparation: Accurately weigh and transfer 6.8 ml of phosphoric acid and 6 ml of diethylamine into a beaker containing 1000ml of water and mix. Adjust pH 6.0 with diethylamine or phosphoric acid

Preparation of Mobile Phase: Filtered and degassed mixture of buffer and Acetonitrile in the ratio of 400:600 v/v

Preparation of diluent: Mobile phase is used as diluent

Preparation of Standard solution: Accurately weigh and transfer about 40mg of Ebastine working standard into a 100ml of volumetric flask add about 35 ml of diluent, shake and sonicate for 5 minutes and make up to the mark with diluent.

Transfer 10.0 ml of above solution into a 100 ml volumetric flask and make up to the mark with diluent. Filter the solution through 0.45µm membrane filter.

Preparation of Sample solution: 20 tablets of Ebastine were weighed and powdered in glass mortar. Accurately

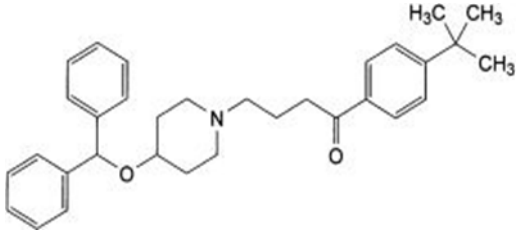

Fig.1. Chemical structure of Ebastine

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Table 1: System suitability data

Parameter	Ebastine
Tailing Factor	1.233
Theoretical Plates	11481
%RSD of Peak area	0.70

Table 2: Linearity data for Ebastine

Concentration	Peak Area
25.0%	331499
50.0%	697819
75.0%	1041675
100.0%	1374832
125.0%	1733042
150.0%	2105807
Slope	1406899
Intercept	0.01227
Correlation Coefficient	0.9999

Fig 2: Linearity plot for Ebastine

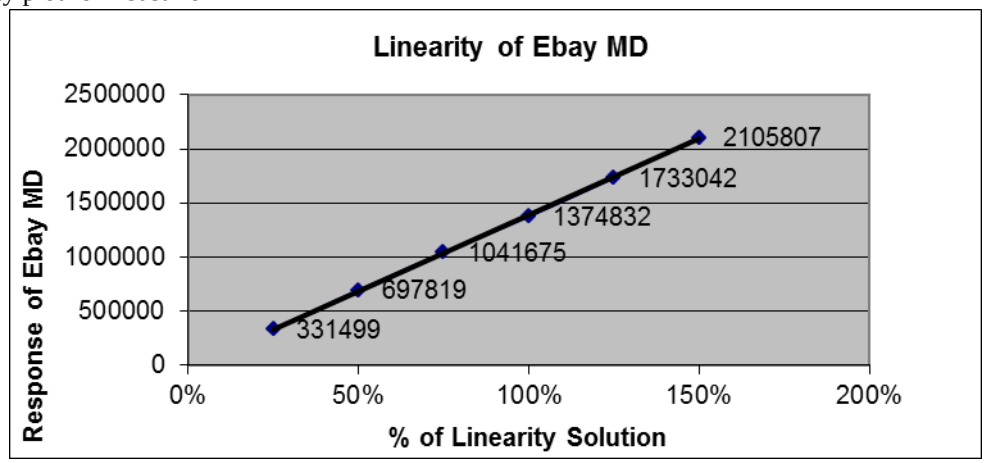

Table.3: Repeatability data

Injection No.	%Assay
1	102.0
2	102.0
3	101.9
4	101.8
5	101.9
6	102.2
Mean	102.2
SD	0.16
%RSD	0.16

weigh the powder blend sample equivalent to 40 mg of Ebastine into a 100 ml volumetric flask, add about 70 ml of diluent sonicate for 5 minutes with intermittent shaking and make up to the mark with diluent.

Transfer 10.0 ml of above solution into a 100 ml volumetric flask and make up to the mark with diluent. Filter the solution through 0.45µm membrane filter.

Preparation of Placebo Solution: Weigh and transfer about 440 mg of placebo powder into a 100 ml volumetric flask, add about 70 ml of diluent, shake and sonicate for 5 minutes. Make up to the mark with diluent. Transfer 10.0 ml of the resulting solution into a 100 ml volumetric flask and make up to the mark with diluent. Filter the solution through 0.45µm membrane filter.

Method validation[7]

1) System Suitability/System Precision: System Suitability was performed by injecting six replicate

The chromatograms for specificity were shown in Fig.3, 4, 5 and 6.

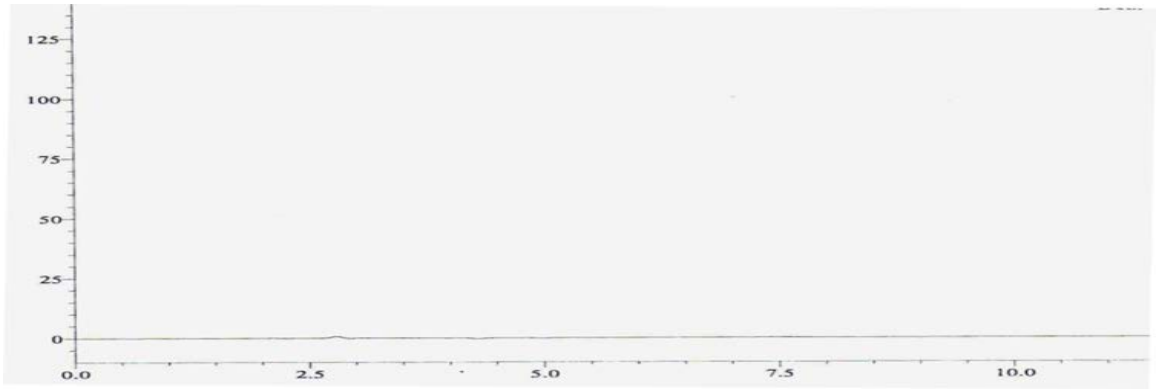

Fig.3.Blank chromatogram

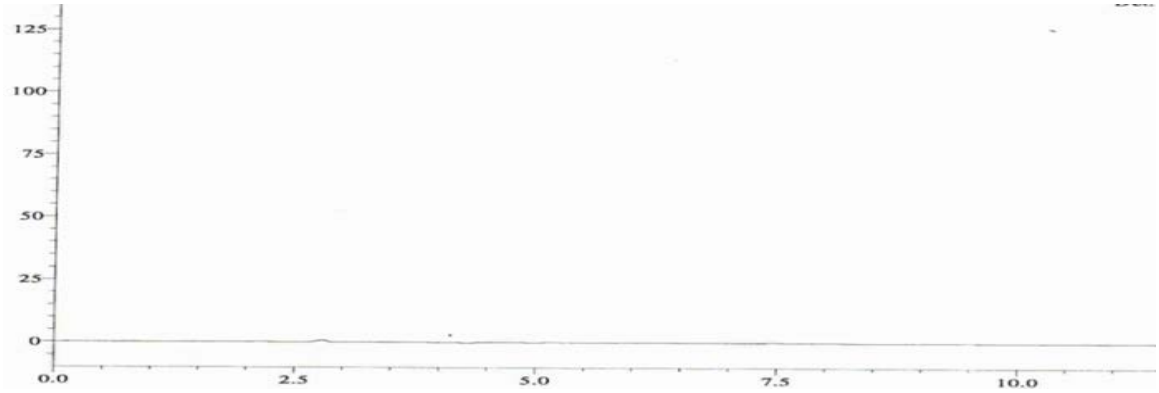

Fig.4.Placebo chromatogram

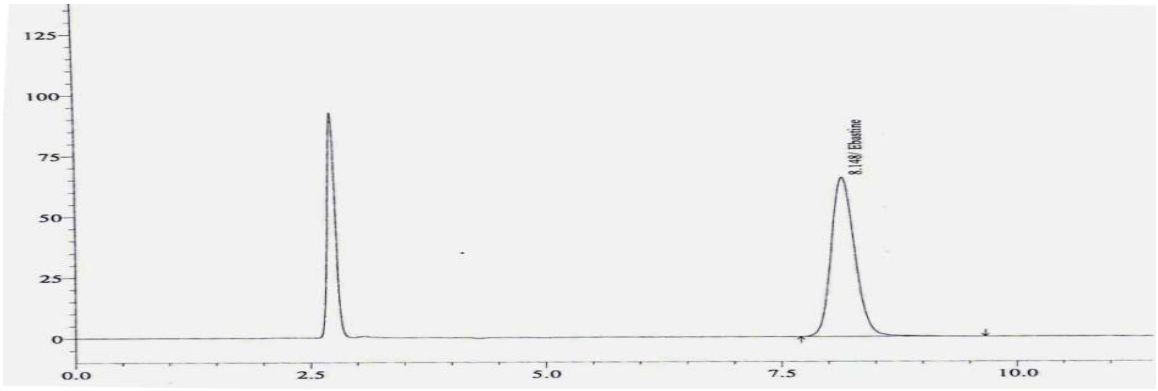

Fig.5.Standard chromatogram

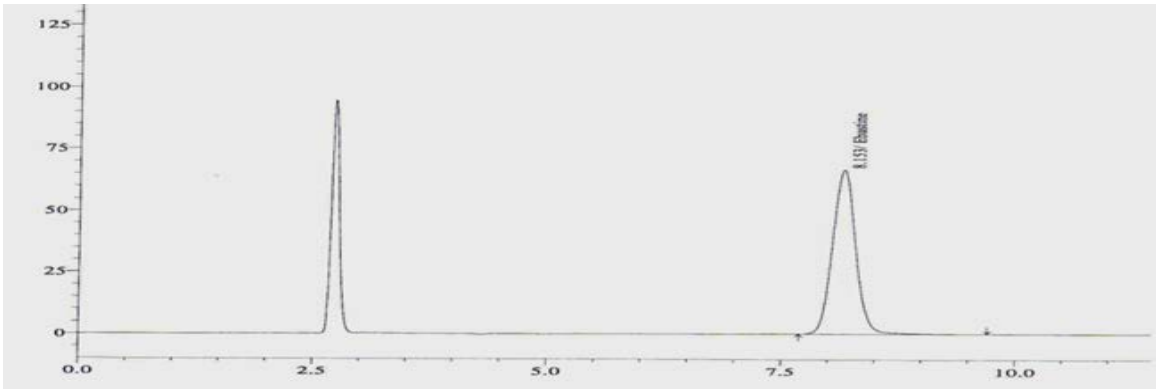

Fig.6.Sample chromatogram

Table.4: Accuracy data

Drug	%Level	Amount added (mg)	Amount found (mg)	Mean %Recovery	%RSD
Ebastine	25%	0.0253	0.0249	100.56%	0.70
	50%	0.0539	0.0533	101.4%	0.30
	100%	0.108	0.104	99.6%	0.75

Table.5. Robustness data

Parameter	RT of Ebastine	% Assay
Actual	8.216	101.5
High Flow Rate : 1.2 ml/min	7.286	101.4
Low Flow Rate : 0.8 ml/min	8.867	101.6
High Temperature : 45^0C	8.289	102.0
Low Temperature : 35^0C	8.183	101.7
High Buffer Composition : 6.20	8.195	101.7
Low Buffer Composition : 5.80	8.207	101.5

injections of standard solutions of Ebastine and expressed as %RSD of peak area.

2) Calibration Curve of Ebastine Standard solutions of various conc. of about 25-150% were injected into the chromatograph and calibration curve was constructed by plotting peak area against concentration in µg/ml.

3) Specificity: To demonstrate that diluents and placebo are not interfering with analytic peak. Solutions of blank, Ebastine standard, sample, and placebo are prepared individually and run chromatogram. The peak purity of analyte peak should be not less than 0.999.

4) Precision: [8] Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the standard injections.

5) Accuracy: [9] %Recovery studies were carried out at three different levels of 25%, 50% and 100% of standard solution in triplicate in each level.

6) Robustness: The robustness[8,10] of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate (±0.2ml/min), Buffer composition (±0.20%), column temperature (±5^0c) which may differ but the responses were still within the specified limits.

7) Solution stability: To eatablish the stability of analytical solutions by injecting the standard and sample solutions at periodic intervals upto 48 hours standard solutions and two test solutions will be prepared and injected and measure the %RSD.

RESULTS AND DISCUSSION

Optimization of the mobile phase was performed based on resolution, asymmetric factor & peak area obtained for Ebastine. The Mobile phase buffer (Phosphoric acid buffer with pH adjusted to 6.0): Acetonitrile in the ratio of 400:600 was found to be satisfactory & gave symmetric & well resolved peaks for Ebastine & system suitability results were summarized in Table.1.

The correlation coefficient was found to be 0.9999 & results were summarized in Table.2.

Precision was determined and the results are represented in the form of %RSD which is below 2 and shows that the proposed HPLC method was highly precise and results given in Table-3.

The percentage mean recovery was found as 101.52%. The results were summarized in Table.4.

As part of the robustness study deliberate changes in the flow rate, column temperature and buffer composition was made to the impact on the method. Retention time was significantly changed but within the acceptance limit and results given inTables.5.

Stability of analytical solutions by injecting the standard and sample solutions at periodic intervals up to 48 hours have been recorded and the %RSD is within the limit.

CONCLUSION

It can be concluded that the proposed RP-HPLC method is accurate, precise, sensitive, specific, robust, and reproducible for the analysis of Ebastine with less tailing and is also economical.

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