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Research

RP-HPLC Method Development and Validation of Ebastine in Bulk and Injectable Dosage Form

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Abstract:
The current study presents the development and validation of a novel reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of Ebastine in injectable formulations. A comprehensive literature review revealed the absence of reported RP-HPLC methods for the determination of Ebastine in this dosage form. The method was developed using an InertSustain AQ-C18 column (150 × 4.6 mm, 5 µm) and a mobile phase comprising a pH 5.5 buffer and acetonitrile in a 30:70 (% v/v) ratio. The mobile phase was pumped at a flow rate of 2.0 mL/min, and detection was carried out at 255 nm using a PDA detector. Ebastine exhibited a distinct retention time at 4.072 minutes. The method was validated in accordance with ICH guidelines for parameters including system suitability, specificity, linearity, accuracy, precision, robustness, and filter validation. Results demonstrated excellent linearity ($R^2 = 0.9998$), high recovery (100.4%), and satisfactory system suitability with %RSD of 0.041, a symmetry factor of 0.94, and 6029 theoretical plates. Specificity studies confirmed no interference from diluent, placebo, or degradation products. The method was robust and filter validation indicated minimal assay variability among different filter types. The developed method is accurate, precise, rapid, and cost-effective, making it suitable for routine quality control analysis of Ebastine in injectable dosage forms.

Keywords: RP-HPLC, Ebastine, Method development, Method validation, Bulk drug, Injectable dosage form, Pharmaceutical analysis, Linearity, Precision, Accuracy, Specificity, ICH guidelines

1. INTRODUCTION

Ebastine is a potent, second-generation, non-sedating H1-antihistamine used extensively in the management of allergic disorders such as rhinitis, urticaria, and bronchial asthma (Canonica et al., 2000; Tripathi, 2019). Due to its high selectivity for peripheral H1-receptors and negligible penetration across the blood-brain barrier, Ebastine exhibits minimal sedative effects, thereby offering significant therapeutic advantages over first-generation antihistamines (Bachert et al., 1996). While its use in oral dosage forms is well established, the development of injectable formulations of Ebastine has garnered attention for its rapid onset of action and improved bioavailability in acute allergic conditions.

Analytical method development and validation are critical components in the pharmaceutical quality assurance process. Among the various analytical techniques, reverse-phase high-performance liquid chromatography (RP-HPLC) is widely employed due to its accuracy, reproducibility, and suitability for routine analysis of pharmaceutical compounds (Sharma et al., 2010). Despite the clinical and commercial significance of Ebastine, a thorough literature review reveals the absence of specific, validated RP-HPLC methods for the quantification of Ebastine in injectable dosage forms (Kumar et al., 2013). Existing methods are primarily focused on oral formulations or biological matrices, underscoring the necessity for a dedicated method tailored to the physicochemical characteristics and formulation matrix of injectables.

In light of this gap, the present study was undertaken to develop and validate a novel RP-HPLC method for the estimation of Ebastine in both bulk drug and injectable dosage form. The method was optimized to be simple, rapid, precise, accurate, and cost-effective, making it suitable for application in quality control laboratories. Validation of the developed method was carried out in accordance with the International Council for Harmonisation (ICH, 2005), evaluating parameters such as system suitability, specificity, linearity, accuracy, precision, robustness, and filter compatibility.

This work aims to contribute a reliable analytical approach for routine quality assessment and regulatory compliance in the manufacture of Ebastine injectable products (Indian Pharmacopoeia Commission, 2018).

2. Materials and Methods

2.1. Chemicals and Reagents

Ebastine reference standard (purity ≥99.8%) was obtained from a certified pharmaceutical supplier. Acetonitrile (HPLC grade) and all other reagents were of analytical grade and procured from Merck (India). Milli-Q water was used throughout the study. The buffer solution (pH 5.5) was prepared using potassium dihydrogen phosphate and adjusted with orthophosphoric acid in accordance with standard chromatographic practices (Indian Pharmacopoeia Commission, 2018).

2.2. Instrumentation and Chromatographic Conditions

A high-performance liquid chromatography system equipped with a photodiode array (PDA) detector was employed for analysis. Chromatographic separation was achieved using an Inertsil Sustain AQ-C18 column (150 mm × 4.6 mm, 5 µm). The mobile phase consisted of a pH 5.5 phosphate buffer and acetonitrile in a 30:70 (v/v) ratio. The flow rate was set at 2.0 mL/min with a fixed injection volume of 10 µL. Detection was carried out at a wavelength of 255 nm, and the column temperature was maintained at ambient conditions, following optimization based on prior HPLC literature for similar compounds (Sharma et al., 2010; Kumar et al., 2013).

2.3. Preparation of Standard and Sample Solutions

A standard stock solution of Ebastine was prepared by dissolving 10 mg of the reference substance in methanol and diluting to 10 mL to achieve a concentration of 1000 µg/mL. Working standard solutions were prepared via serial dilution using the mobile phase.

Injectable formulations were analyzed by transferring a volume equivalent to 10 mg of Ebastine into a 10 mL volumetric flask. The solution was diluted with methanol and sonicated for 10 minutes to ensure complete dissolution, then filtered through a 0.45 µm membrane filter. This preparation approach is consistent with validated procedures for assay sample preparation in pharmaceutical quality control (Canonica et al., 2000).

2.4. Method Validation

The method was validated in accordance with International Council for Harmonisation (ICH) guidelines Q2(R1) (ICH, 2005), covering the following parameters:

- **System Suitability**: Evaluated by injecting six replicates of the standard solution. Key parameters assessed included percent relative standard deviation (%RSD) of peak area, peak symmetry, and the number of theoretical plates.
- **Specificity**: Determined by injecting diluent, placebo, and forced degradation samples to verify the absence of interfering peaks at the retention time of Ebastine.
- **Linearity**: Established by preparing and analyzing solutions across a concentration range of 50–150 µg/mL. A calibration curve was plotted and the correlation coefficient (R^2) was calculated.
- **Accuracy**: Performed via recovery studies at three concentration levels: 50%, 100%, and 150% of the target level, in triplicate. Mean recovery percentages were calculated.
- **Precision**: Assessed through repeatability testing using six independently prepared samples at a fixed concentration. Results were expressed as %RSD.

- **Robustness**: Studied by intentionally varying chromatographic parameters such as flow rate (±0.2 mL/min), mobile phase composition, and detection wavelength, to ensure consistency under minor deviations.
- **Filter Validation**: Conducted by comparing assay results of samples filtered through different membrane types (PVDF, Nylon, GF/C) with a centrifuged sample. Variability was evaluated in accordance with acceptable pharmaceutical validation standards (Sharma et al., 2010).

A straightforward, rapid, precise, and accurate HPLC technique was devised and validated for the quantification of Ebastine in injectable dosage form. Solubility experiments of Ebastine were conducted in various polar and non-polar solvents to assess the drug's dissolution. Based on the solubility investigations, a diluent comprising a 35:65 (v/v) ratio of pH 5.5 OPA buffer and acetonitrile was selected. Individual samples and standard solutions of 100 µg/mL Ebastine in OPA buffer at pH 5.5 were generated and subsequently scanned in the UV area within the wavelength range of 200 to 400 nm using the same OPA buffer at pH 5.5. From the spectra, 255 nm was chosen for the quantification of Ebastine in OPA buffer at pH 5.5, free from any interference. The optimal concentration of Ebastine was determined to be 100 µg/mL in OPA buffer at pH 5.5: ACN. The linearity was established by producing concentrations ranging from 25 to 150 µg/mL in OPA buffer at pH 5.5 and acetonitrile in a 30:70 v/v ratio, with peak regions presented in Table No. 14. The calibration curve for Ebastine is illustrated in Figure 21. The correlation coefficient was determined to be 0.999. Consequently, the concentrations were determined to be linear. The precision was assessed by analyzing Ebastine solutions at the working concentration level six times. The % RSD value for method precision was determined to be 1.5 in OPA buffer and ACN. The results are shown in Table No. 16. The quantity in the injectable formulation was consistent with the label stated. The results validated the precision of the procedures.The method's accuracy was evaluated by recovery studies. A specified number of Ebastine raw material solutions was included at varying concentrations (50%, 100%, and 150%). The solution area was quantified, and the recovery % was computed. The recovery percentage was determined to be between 100.29% and 100.4%. The low percentage relative standard deviation of medication solutions signifies that these procedures were highly accurate. The recovery data is presented in Table No. 18. The robustness was evaluated using working standards and sample solutions of Ebastine under varying flow rates and pH conditions. The low % RSD readings signify that the established procedures exhibited greater robustness. The results are shown in Table No. 19. The method's specificity is determined by utilizing a known concentration of Ebastine in various solvents and assessed according to the analytical procedure. The low % RSD values signify that the developed approach exhibited more specificity.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined based on linearity and precision. The limit of detection (LOD) for Ebastine was determined to be 0.0004, while the limit of quantification (LOQ) was established at 0.0013.

3. RESULT:

METHOD DEVELOPMENT:

Trail – I:

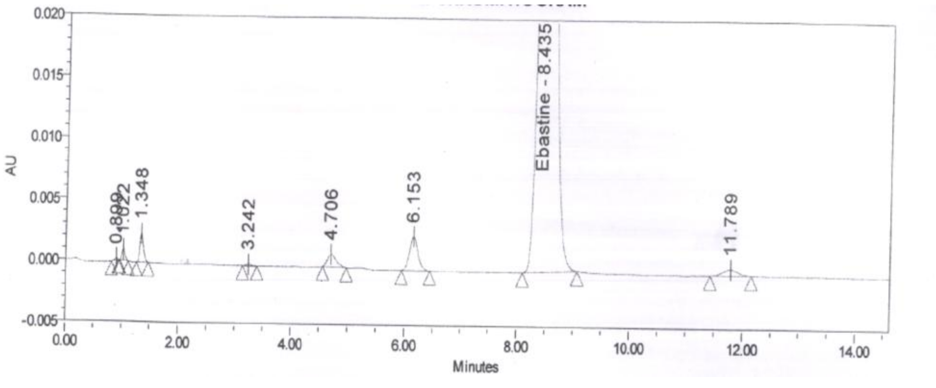

Fig No: 6 - Chromatogram of Trail-I

Table No: 8 Trail-I results:

Peak name	**R_t**	**Area**	**EP Plate count**	**Symmetry factor**
Impurity-C	0.899	496	1590	0.74
Impurity-A	1.022	3663	1492	1.20
Impurity-D	1.348	10679	2076	1.25
Impurity-G	3.242	921	6561	1.21
Impurity-F	4.706	11359	4308	1.32
Impurity-B	6.153	28968	8292	1.10
Ebastine	**8.435**	**1339771**	**6096**	**1.43**
Impurity-E	11.789	10092	7453	0.99

Observation:

The resolution between first two peaks was poor, still more trials was required for better resolution.

Trail-II:

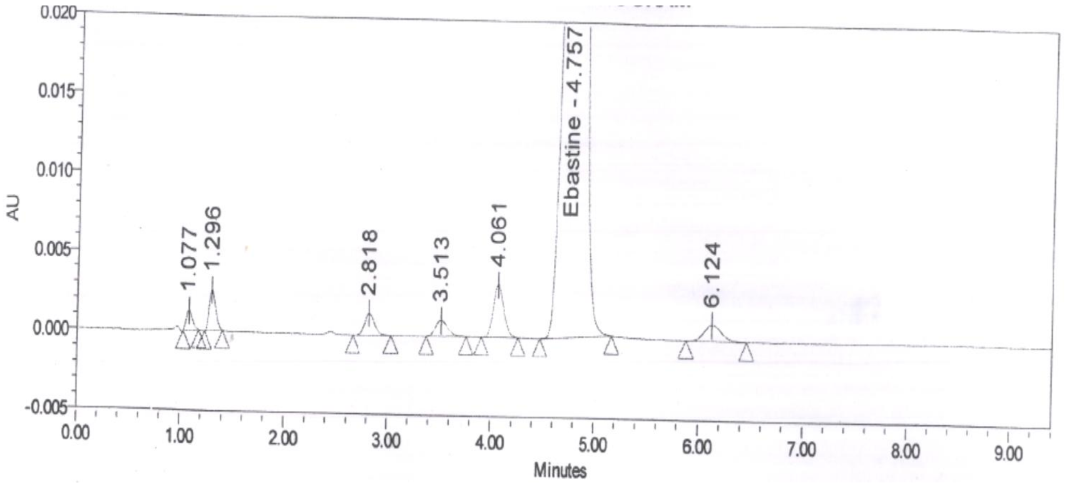

Fig No: 7 - Chromatogram for Trail-II

Table No: 9 Trail-II results:

Peak name	**Retention time**	**Area**	**EP plate count**	**Symmetry factor**
Impurity-D	1.307	6130	1505	1.42
Impurity-A	1.621	14330	2263	1.26
Impurity-G	3.275	11388	5159	1.19
Impurity-F	4.006	10444	5136	1.28
Impurity-B	4.481	27632	8895	1.17
Ebastine	**6.641**	**1716822**	**7689**	**0.95**
Impurity-E	8.476	12470	8938	1.07

Observation:

The separation was better, peak shape was good, still more trials was required for better separation.

Trail-III

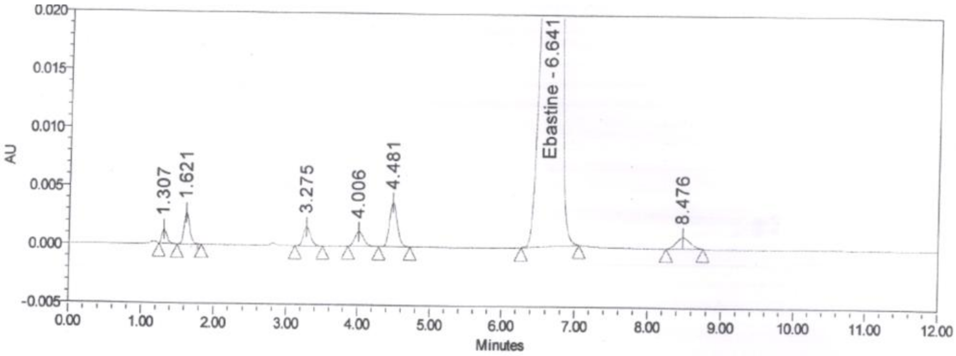

Fig No: 8-Chromatogram for Trail-III

Table No: 10 Trail-III

Peak name	Retention time	Area	EP Plate count	Symmetry factor
Impurity-D	1.077	5303	1441	1.30
Impurity-A	1.296	10966	2072	1.24
Impurity-G	2.818	9461	4109	1.19
Impurity-F	3.513	8364	4379	1.24
Impurity-B	4.061	23032	7878	1.15
Ebastine	**4.757**	**1472616**	**5810**	**1.10**
Impurity-E	6.124	12037	6468	1.19

Observation:

The peak shape and separation was good. But retention time was high, still more trials was required for better resolution with less retention time.

Trail-IV:

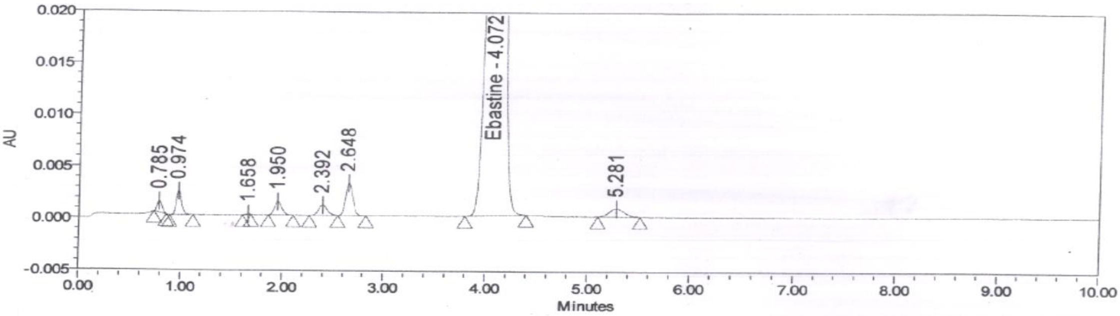

Fig No: 9 Chromatogram of Trail-IV

Table No: 11 Trail-IV results

Peak name	Retention time	Area	EP plate count	Symmetry
Impurity-C	0.785	3576	1245	1.30
Impurity-A	0.974	8367	1796	1.20
Impurity-D	1.658	279	7098	0.79
Impurity-G	1.950	6550	3629	1.11
Impurity-F	2.392	5983	3892	1.17
Impurity-B	2.648	15817	6556	1.17
Ebastine	**4.072**	**995002**	**5648**	**0.98**
Impurity-E	5.281	8264	6092	1.13

Observation:

The retention time, separation of all peaks was good, tailing factor was less than 2, and theoretical plates were more than 2000. Hence this trial was taken as **optimized method**.

METHOD VALIDATION:

SYSTEM SUITABILITY:

System suitability test was carried out to verify that the analytical system is working properly and can give accurate and precise results. System suitability results were tabulated in table no12:

System suitability results of Ebastine:

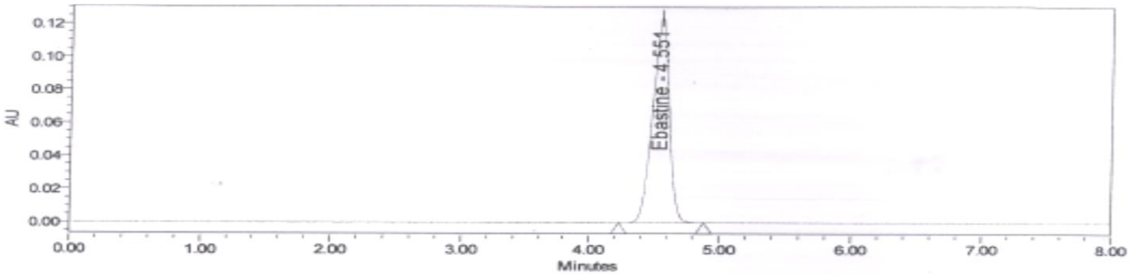

Fig No: 10 System suitability chromatogram

Table No: 12 System suitability results

Name of the peak	Ebastine
Retention time	4.551
Area	1108167
Symmetry factor	0.94
EP plate count	6029

The System suitability parameters are Retention time, theoretical plates, peak area and % RSD of number of injections are within the limits. So, the system is suitable for all sample sequence and conditions outlined in the method.

The finalized system suitability parameters are:

- RSD for the peak areas of Ebastine in five replicate injections of Ebastine standard solution is not more than 2.0%
- EP plate count for the peak due to Ebastine in standard solution is not less than 2000.
- Symmetry factor for the peak due to Ebastine in standard solution is not more than 2.0.

SPECIFICITY:

Blank Interference:

Blank was prepared and injected. It was observed that no blank peaks were eluting at the retention time of Ebastine.

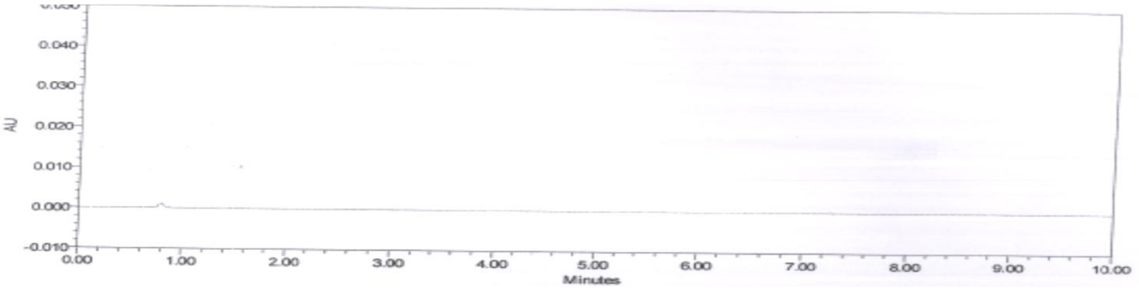

Fig No: 11 Chromatogram for specificity of Blank

Placebo Interferences:

Placebo is spiked at their specification level with known concentration of standard and sample solution and unspiked sample solution and standard solution was analysed. It was observed that no placebo peaks were eluting at the retention time of Ebastine in the spiked standard and spiked sample solution and was found to be within the acceptable limits.

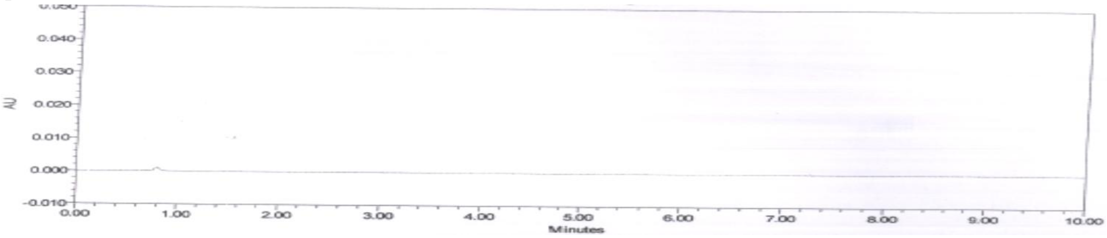

Fig No: 12 Chromatogram for specificity of Placebo

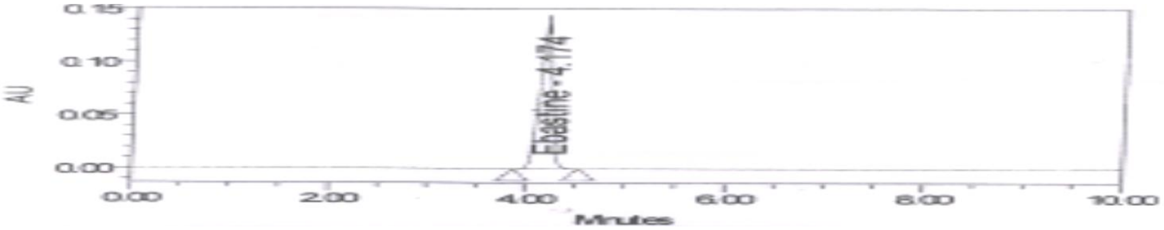

Fig No: 13 Chromatogram for specificity of Standard:

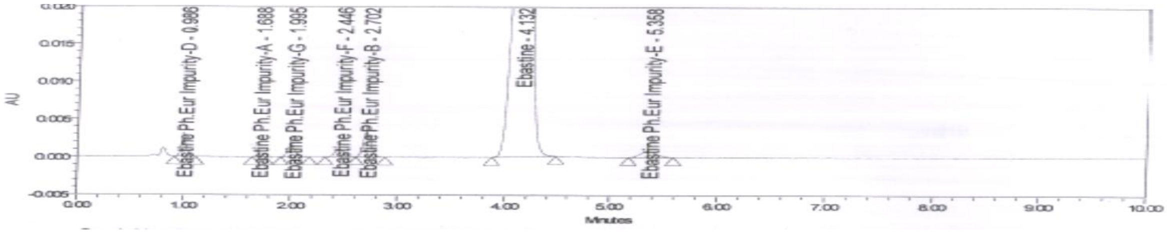

Fig No: 14 Chromatogram for specificity of sample

Table No: 13 Specificity data for Ebastine

Peak name	Retention time	Area	Resolution
Ebastine impurity-C	0.986	0.79	---
Ebastine impurity-A	1.688	471	7.39
Ebastine impurity-G	1.996	6799	2.66
Ebastine impurity-F	2.446	6296	3.19
Ebastine impurity-B	2.702	16176	1.82
Ebastine	**4.132**	**1020552**	**8.21**
Ebastine impurity-E	5.368	8423	4.97

Acceptance criteria:

No interference should be observed at the retention time of main peak due to diluents, placebo and individual impurities.

From the blank and placebo chromatograms, it was concluded that no peak was observed at the retention time of Ebastine peak. Hence the method is specific.

LINEARITY:

A series of Ebastine solution were prepared in the range of about 25 to 150% and injected to HPLC system. Linearity was established by plotting graph of concentration versus response of Ebastine. Linearity results were tabulated in Table No 14.

And chromatograms for linearity are shown in below figure: Calibration curve are shown in

Table No:14 Linearity data for Ebastine

Sample name	RT	Area	EP plate Count	Symmetry Factor
Standard linearity level-1 25%	4.115	295217	5679.23	1.06

Standard linearity level-2 50%	4.131	581856	5703.83	1.03
Standard linearity level-3 75%	4.133	866602	5633.38	0.99
Standard linearity level-4 100%	4.145	1188752	5665.27	0.95
Standard linearity level-5 125%	4.162	1398007	5646.91	0.93
Standard linearity level-6 150%	4.168	1740699	5549.24	0.90
Mean		1011856		
%RSD		52.87		

Table N0:15 Calibration curve data for Ebastine

Level	**Concentration (µg/ml)**	**Area**
25%	25.09	295217.000
50%	50.18	581856.00
75%	75.27	866602.00
100%	100.36	1188752.000
120%	120.43	1398007.000
150%	150.54	1740699.000
Correlation coefficient		0.9996

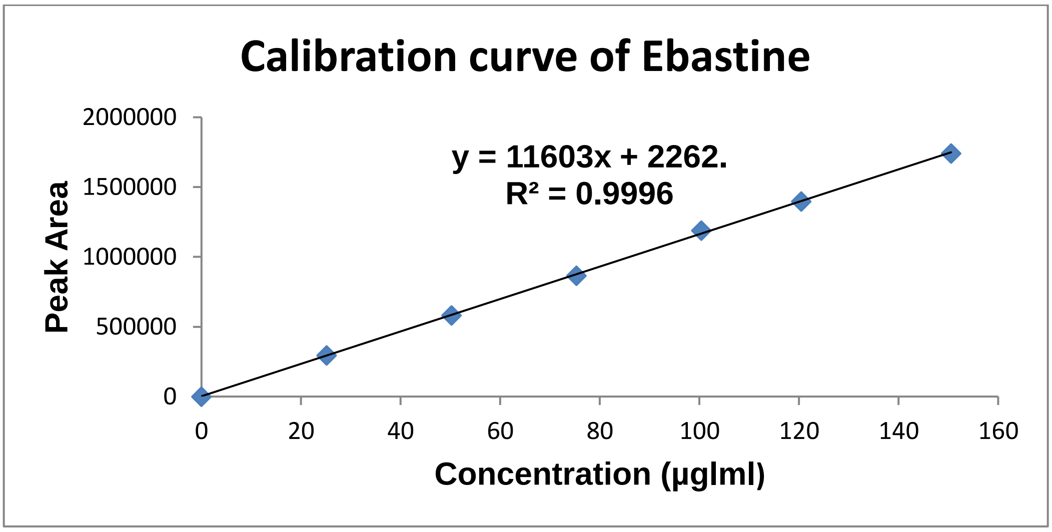

Fig No: 21 Calibration curve of Ebastine

The peak response of Ebastine is linear over the concentration range from 25% to 150% of the test concentration.

PRECISION:

Method precision

To evaluate the method precision for Ebastine method six samples solutions were prepared as per test procedure and analysed. % recovery and % RSD of six samples were calculated and found to be within the acceptable limits. Method precision results were tabulated in Table No: 16, Chromatogram is shown in Fig No:22.

Procedure:

Six test sample solutions were prepared individually as per method injected the solutions into HPLC as per methodology.

Acceptance criteria:

Assay values should be in the range with RSD NMT 2.0%

Table No: 16 Method Precision results for Ebastine

Peak name	Injection	Retention time
Method precision sample-1	1	4.161
Method precision sample-1	2	4.158
Method precision sample-2	1	4.139
Method precision sample-2	2	4.132
Method precision sample-3	1	4.143
Method precision sample-3	2	4.137
Method precision sample-4	1	4.139
Method precision sample-4	2	4.138
Method precision sample-5	1	4.135
Method precision sample-5	2	4.132
Method precision sample-6	1	4.131
Method precision sample-6	2	4.134

Table No: 17 Ebastine Assay results (Method Precision results)

S. No	No .of Vials	%Assay	Statistical analysis	
1	10	100.8	Mean	98.3
2	10	99.8		
3	10	99.9	SD	1.49
4	10	98.1		
5	10	100.0	%RSD	1.5
6	10	101.0		

Acceptance criteria:

98% to 102% recover y. Test results are showing that the method is precise.

ACCURACY:

A series of solutions were prepared in duplicate by spiking the placebo on sample at 50%, 100%, 150% level of test concentration and injected into the HPLC system and analysed. Individual % recovery, mean % recovery, % RSD was calculated at each level and found to be within the acceptable limits. Accuracy results were tabulated in Table No: 18, Chromatograms for accuracy are shown in Fig. No 33

Table No: 18 Accuracy results for Ebastine

Concentration	Amount added	Amount recovered	% Recovery	**Mean** 100.04
50% level	50.03	50.08	100.29	SD 0.09
100% level	100.53	100.78	100.45	% RSD
150% level	150.28	150.62	100.43	0.08

Acceptance criteria:

Recovery should be in the range of 98.0% to 102.0% of the added amount. The recovery results indicating that the method has an acceptable level of accuracy for the assay of Ebastine from 50% to 150% of sample concentration.

ROBUSTNESS:

Robustness indicates reliability of the procedure during the normal usage. Robustness chromatograms are shown in Fig No: 39. Results are tabulated in Table No: 19.

Change in flow rate:-

When robustness was carried out by changing the flow rate, (Flow plus) the retention time for Ebastine was shifted to Retention time from 4.551 to 3.767 and on decreasing the flow rate(flow minus) Retention time shifted to 4.649.

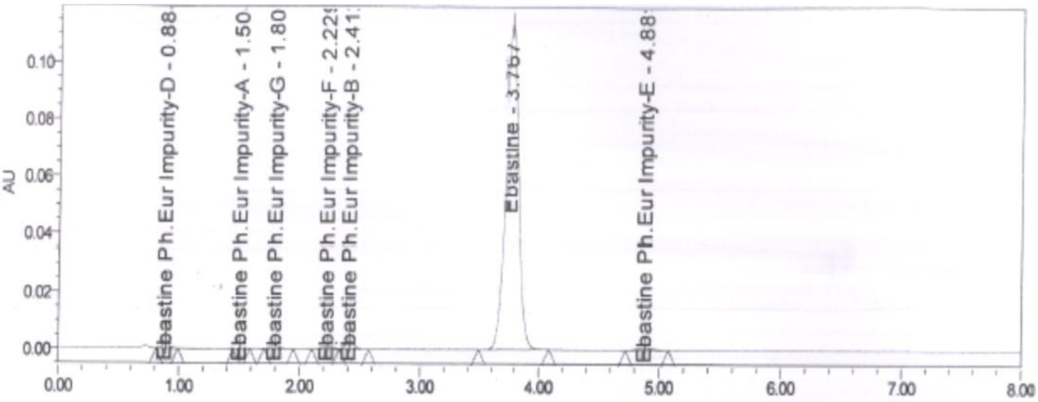

Fig No: 40 Chromatogram of Robustness Flow plus-(2.2mL/min)

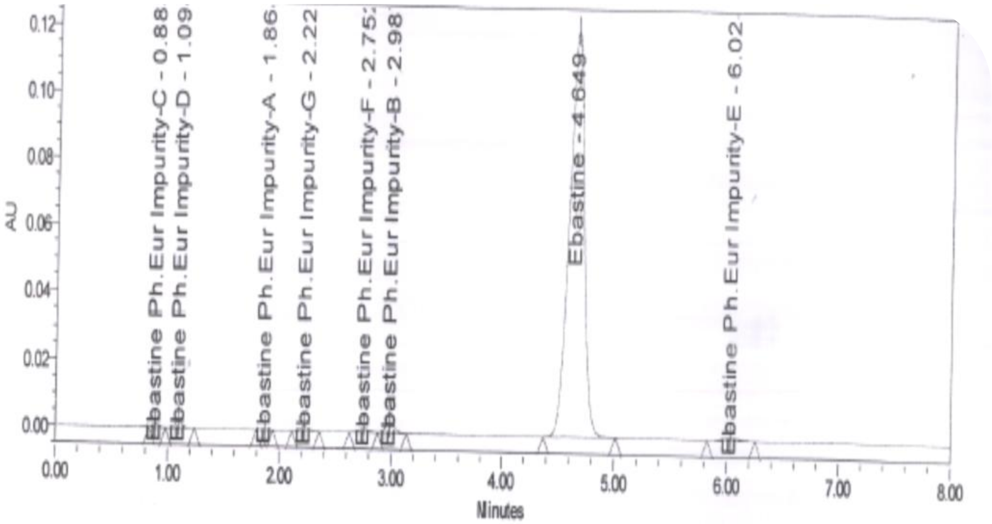

Fig No: 41 Chromatogram of Robustness Flow minus-(1.8mL/min)

Change in Oven temperature:-

When robustness was carried out by changing the oven temperature, (temperature plus) the retention time for Ebastine was shifted to from 4.551 to 4.553 and on decreasing the oven temperature (temperature minus) Retention time shifted to 3.745.

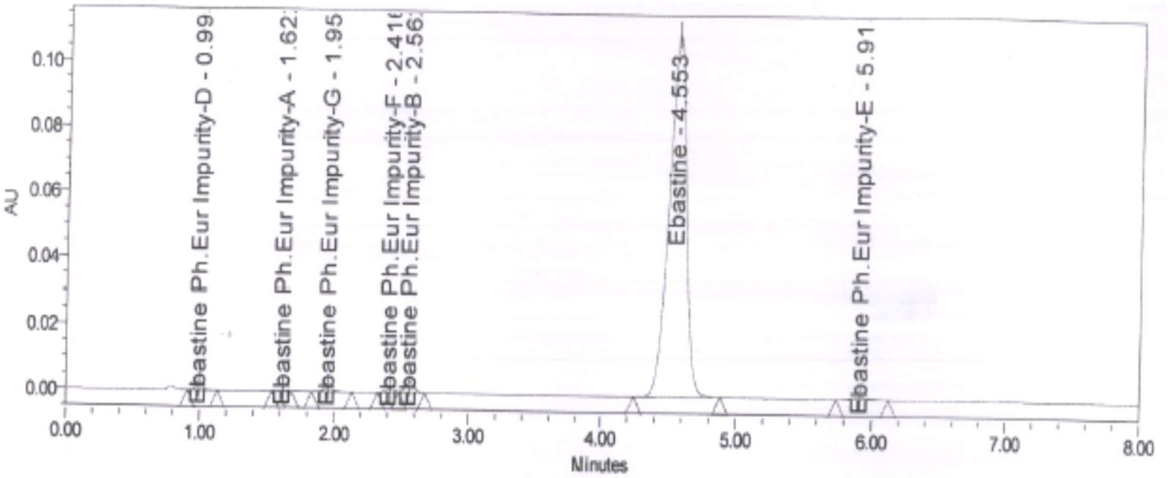

Fig No: 42 Chromatogram of Robustness temperature (temperature plus).

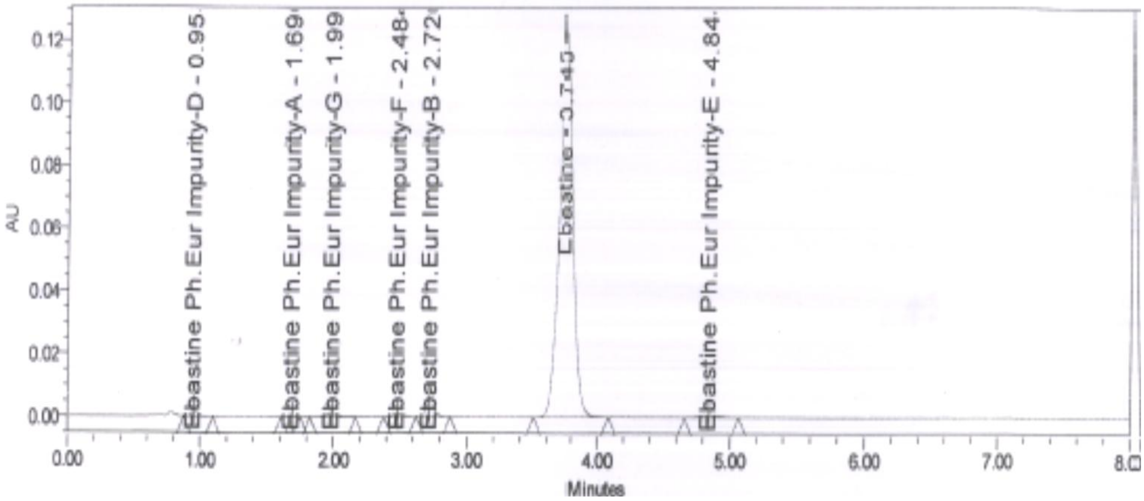

Fig No: 43 Chromatogram of Robustness temperature (Temperature minus)

Change in Oven temperature:-

When robustness was carried out by changing the pH of mobile phase, the retention time for Ebastine was shifted to from 4.551 to 5.325 and on decreasing the oven temperature Retention time shifted to 3.667.

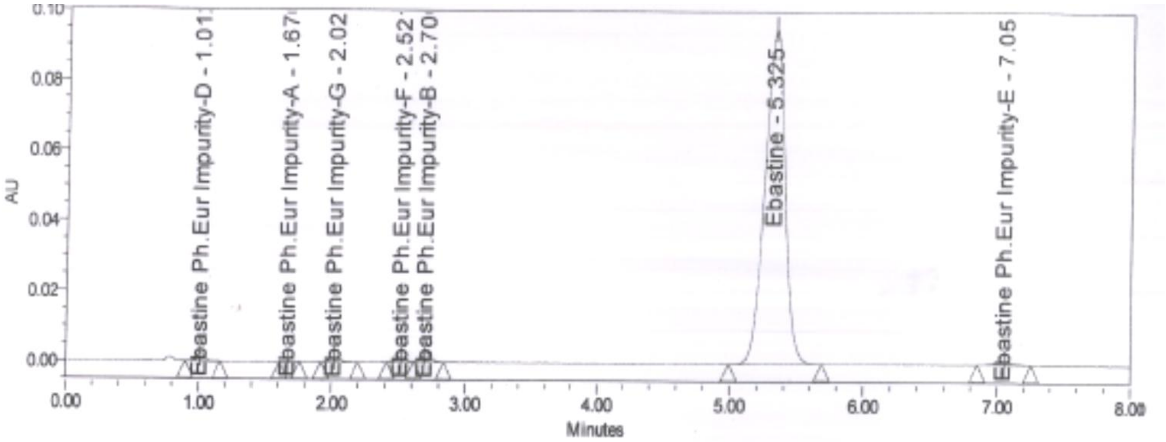

Fig No: 44 Chromatogram of Robustness pH plus (pH 5.7)

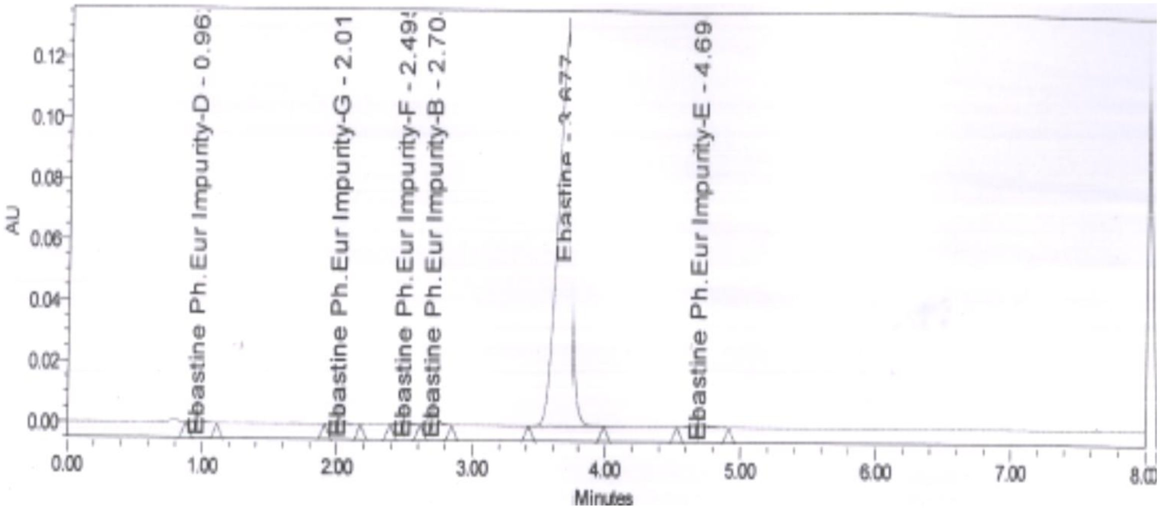

Fig No: 45 Chromatogram of robustness pH minus (pH 5.3)

Change in Organic concentration of mobile phase:-

When robustness was carried out by changing the organic concentration of mobile phase, the retention time for Ebastine was shifted to from 4.551 to 3.971 and on decreasing the oven temperature Retention time shifted to 4.868.

Table No: 19 Robustness results for Ebastine

Peak name	**Retention Time**	**Area**	**EP plate Count**	**Symmetry factor**
Ebastine –Flow plus	3.767	897258	5309	0.99
Ebastine-Flow minus	4.649	1114971	5959	0.98
Ebastine-Temperature plus	4.553	984704	6089	0.95
Ebastine-Temperature minus	3.745	983546	5189	1.03
Ebastine-pH plus	5.325	988183	6104	0.99
Ebastine-pH minus	3.677	997700	5299	0.92
Ebastine-Organic plus	3.917	979758	5344	0.90
Ebastine-Organic minus	4.868	982017	5794	0.98

The results are indicating that the method has an acceptable level of Robustness.

FORCED DEGRADATION STUDIES:

Table No: 20 Forced degradation results of Ebastine

Peak Name - Degradation sample	**Retention Time**	**Area**	**EP plate Count**	**Symmetry factor**
Ebastine-Acid	4.4	1132023	5557	0.90
Ebastine-Alkali	4.422	1154749	5533	0.90
Ebastine-peroxide	4.439	966508	5676	0.92
Ebastine-thermal	4.421	1127852	5691	0.90

FILTER PAPER VARIABILITY:

Procedure:

Standard solutions of Ebastine and sample solutions of Ebastine were prepared. These solutions were filtered by Millipore PVDF filter, Nylon filters and Whatmann GF/C Filter. Samples are analyzed as per methodology.

Acceptance criteria:

% difference between the absorbance obtained by Unfiltered by standard or centrifuged sample solution and filtered standard or sample solution should not be more than 2.0.

Results:

Table No: 21 Filter paper variability for Ebastine standard solution:

Sample	Area	% Difference
Unfiltered	1160323.000	-
Millipore PVDF filtered	1169679.000	0.81
Nylon filtered	1175891.000	1.34
GF/C Filter	1167420.000	0.61

Table No: 22 Filter paper variability for Ebastine sample solution:

Sample	Area	% Difference
Unfiltered	1121658.00	-
Millipore PVDF filtered	1100804.000	1.86
Nylon filtered	1117937.000	0.33
GF/C Filter	1115687.000	0.53

From the above results, it can be concluded that Millipore PVDF filter, Nylon and Whatmann GF/C filters are suitable for sample and standard preparation.

4. CONCLUSION

An uncomplicated, accurate, and exact HPLC technique for quantifying Ebastine in injectable dose form was established using a pH 5.5 ortho phosphoric acid buffer and acetonitrile in a 30:70% v/v ratio. The current analytical method was validated according to ICH Q2 (R1) principles and satisfies particular acceptance requirements. The optimal concentration of Ebastine was determined to be 100 µg/mL. The retention time of Ebastine was determined to be 4.1399 at 255 nm. The analysis of validation factors, including system appropriateness, accuracy, precision (SD and %RSD), specificity, robustness, and linearity and range, determined that the approach is quick, specific, accurate, exact, and reproducible. The developed procedures have undergone statistical evaluation, yielding results that are accurate, exact, and devoid of influence from other excipients in the formulation.

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