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RP- HPLC Method Development and Validation for In vitro-dissolution of Ebastine in tablet dosage form

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ABSTRACT
RP- HPLC method is developed for estimation of Ebastine in the tablet dosage form. Employing a simple and stability-indicating HPLC method, using stress degradation studies, drug was well separated from the degradants with good peak resolution. Mobile phase was prepared with using ortho phosphoric acid and diethylamine buffer, 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 µL at a flow rate of 1.0 mL/min. the detection wavelength selected is 210 nm. The drug was subjected for dissolution according to European Pharmacopoeia. 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;

I. INTRODUCTION

Ebastine (EBA), chemically, 4−(4−benzhydryloxy−1−piperidyl)−1−(4−tert−butylphenyl) butan−1−one is a nonsedating 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 impuriti[1]es, LC/MS for metabolites, pharmacokinetic study was 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 etc[1-6]. The present method focus on establishing a method for dissolution of Ebastine in ODT tablet.at a low retention time, a method which is applicable for extensive concentration range with a good reproducibility and accuracy.

II. MATERIALS AND METHODS

2.1 Instruments:

The separation was achieved by using Kromasil 100 C8, 250x4.6mm, 5 µ column at a flow rate of 1.0 mL/min.. The Agilent-HPLC 1100 series containing quaternary pump, degasser, auto injector and UV 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, mubai, 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:

To achieve optimal separation of the compounds, a specific mobile phase and chromatographic setup were meticulously employed. The mobile phase itself involved a

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carefully crafted buffer solution: 11.9 g of Orthophosphoric acid dissolved in water, diluted further, and then mixed with diethylamine to reach a specific pH. This buffer was then combined with specific ratios of methanol and acetonitrile for the final mobile phase. The separation itself utilized a Kromasil 100 C8 column with precise dimensions and particle size, ensuring optimal interaction between the mobile phase and the compounds. Finally, the detection wavelength and injection volume were carefully chosen to maximize the sensitivity and accuracy of the analysis. This meticulous approach resulted in successful separation of the compounds, laying the groundwork for further investigation

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 Preparation of Dissolution sample

The European Pharmacopoeia (Ph. Eur.) describes two methods for the dissolution testing of Ebastine ODT tablets. Method I (basket-rod apparatus) uses 900 mL of a phosphate buffer solution (pH 6.8) as Dissolution medium. one tablet is placed in the basket and the apparatus is operated at 37 ± 1°C at a stirring rate of 100 rpm. Samples were Withdrawn at specified time points (e.g., 5, 10, 15, 20, 30 minutes) using a suitable calibrated sampling device without filtration.

The withdrawn volume is replaced with dissolution medium maintained at 37°C. The samples were Analyzed appropriately, the amount of dissolved Ebastine is determined using HPLC

2.7. Method development:

The main aim is to develop a simple method for dissolution of Ebastine with optimal resolution from its degradation products using HPLC. This method was preferred in quality control labs due to its repeatability and ability to deliver accurate results quickly.

The initial development started with the selection of buffer and pH. Based on Ebastine's pKa and physicochemical properties, an orthophosphoric acid solution was chosen and adjusted to pH 6.0 with diethylamine. To optimize the column, two different manufacturers with the same stationary phase were used, and the Kromasil C8 column provided the best resolution and peak symmetry.

The sample was injected using the optimized chromatographic conditions and evaluated for system suitability parameters. Drug release of Ebastine was carried out using an isocratic HPLC procedure (Ph. Eur.2.2.29.) coupled to ultraviolet detection at 255 nm. The validation of the HPLC method during the dissolution test was included and followed the ICH guidelines.

2.8. Method validation

2.8.1 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 N HCl at 60°C for 2hr. Other degradation studies were performed using dry heat at 50°C, humidity 90% RH, UV, Visible, peroxide at 60 ^{o}C 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.

2.8.2 Linearity: The linearity study is carried out, on the drug substance Ebastine working standard. Linearity of the response is checked from a 6-points calibration curve with a range from 0.110 µg/mL to 3.300µg/mL of Ebastine working standard (5% to 150% of target concentration)

2.8.3 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)

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

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

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2.8.4 Precision

Repeatability:

The precision is decomposed into the repeatability of the system, 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 at 0.0011 mg/ml and 0.0022 mg/ml in the diluent. The individual values, the mean response, the standard deviation and the relative standard deviation are presented in the table below. The relative standard deviation is less than the acceptance criterion of 2.0%. The homogeneity of the variances has been verified by a Cochran test using the following equation:

$$C\ expt. = \frac{Var_{max}}{\sum var}$$

Where:

Var = square of the standard deviation for each of the three means.

Varmax = square of the greatest standard deviation

Cochran values of Ebastine have been calculated for Ebastine 10 mg and 20 mg, orodispersible tablets. These values are less than the critical value for C (0.05, 3, 2) of 0.8709. Therefore the variances are of the same population and can be pooled to calculate the overall intermediate precision.

Thus the grand mean, standard deviation, relative standard deviation and the confidence limits have been calculated. The confidence limits are not greater than ± 0.5% and include the theoretical value of 100%. These values meet the acceptance criteria of ± 5.0% and bracketing the 100% theoretical content. (Table-3 & Figure-2).

Intermediate precision

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 individual values are reported in the table given below together with grand mean, standard deviation, relative standard deviation and the confidence limits.

2.8.5 Accuracy

The Accuracy is demonstrated by adding the Ebastine drug substance and Placebo at 5%, 50%, 80%, 100%, 120% and 150% of the Ebastine 20 mg, orodispersible tablets initial test concentration and analyzed in triplicate. The individual values, the percent recovery at each concentration, the mean value, the standard deviation, % relative standard deviation and confidence limits are reported. The homogeneity of the variances has been verified by a Cochran test using the following equation:

$$C\ expt. = \frac{Var_{max}}{\sum var}$$

Where:

Var = square of the standard deviation for each of the three means.

Varmax = square of the greatest standard deviation.

Calculate the grand mean, standard deviation, coefficient of variation and the confidence limits. The confidence limits should not be more ± 5.0% and should cover the theoretical recovery of 100%.

The mean recovery should not be more than ± 5% of the amount added.

2.8.6 Robustness

A. Validation on effect of variation of buffer pH in mobile phase:

The effect of variation in buffer pH in mobile phase was conducted by using two mobile phases, one containing pH 5.9 buffer and other containing pH 6.1 buffer. Standard solution of Ebastine 20 mg, orodispersible tablet was prepared as per the test method and injected into HPLC system.

B. Validation on effect of variation in mobile phase composition (Acetonitrile):

The effect of variation in mobile phase composition was conducted by using two mobile phases, one containing 90% of the method organic phase (Acetonitrile) composition and other containing 110% of the method organic phase (Acetonitrile) composition. Standard solution of Ebastine 20 mg, orodispersible tablets was prepared as per the test method and injected into HPLC system.

C. Validation on effect of variation in flow rate:

The effect of variation in flow rate was conducted. Standard solution of Ebastine 20 mg, orodispersible tablets was prepared as per the test method and injected into HPLC system with flow rate 1.4 ml/min and 1.6 ml/min

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D. Validation on effect of variation in column oven temperature:

The effect of variation in column oven temperature was conducted. Standard solution of Ebastine 20 mg, orodispersible tablets was prepared as per the test method and injected into HPLC system at 35°C and at 45°C.

III. RESULTS AND DISCUSSION

3.1. Method validation

3.1.1. Specificity There is no interference between the drug substance and the other components of the formulation. The absence of interference of the excipients is checked from a placebo. No peak from the chromatogram of placebo solution is observed except blank peaks. The HPLC method related to drug release of Ebastine during the dissolution test is therefore specific. Specificity results were represented in table 4.

3.12 Linearity: The correlation coefficient is 0.999985. The value of 1.0857 for the Student's t, for the comparison of the y-intercept to zero is less than the critical value of 2.5706, thus demonstrating that the y-intercept is not significantly different from zero. The residuals are randomly distributed. The linearity of Ebastine has been demonstrated for concentration range of 0.110 µg/ml to 3.294 µg/ml of Ebastine in Ebastine 20 mg, orodispersible tablets. The results for linearity is shown in Table-1 and Figure-2

3.1.3 Precision (Repeatability): The repeatability of the dissolution method was demonstrated by analysing 6 replicate of samples of Ebastine 10 mg and 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. These values meet the acceptance criteria of ± 5.0% and bracketing the 100% theoretical content. The results for repeatability was shown in Table-2.

3.1.4. Intermediate precision: Considering the acceptable standard deviation, relative standard deviation and confidence interval values of repeatability and of intermediate precision, the precision of the HPLC procedure, during the dissolution test, is proved. The results for intermediate precision was shown in Table-3.

3.1.5 Accuracy: The mean % recovery rate for Ebastine is close to 100.0%.

Hence, the procedure used during the dissolution test is considered as being accurate. The results for Accuracy was shown in Table-4.

3.1.6 Robustness: A. Validation on effect of variation of buffer pH in mobile phase:

The effect of variation in buffer pH in mobile phase was conducted by using two mobile phases, one containing pH 5.9 buffer and other containing pH 6.1 buffer. From the above data the allowable variation of buffer pH in mobile phase is from pH –5.9 to pH – 6.1. The results for robustness was shown in Table-5.

B. Validation on effect of variation in mobile phase composition (Acetonitrile):

The effect of variation in mobile phase composition was conducted by using two mobile phases, one containing 90% of the method organic phase (Acetonitrile) composition and other containing 110% of the method organic phase (Acetonitrile) composition. From the results obtained, the allowable variation in method organic phase composition (Acdetonitrile) in mobile phase is from 90% to 110%.

C. Validation on effect of variation in flow rate:

The effect of variation in flow rate was conducted. Standard solution of Ebastine 20 mg, orodispersible tablets was prepared as per the test method and injected into HPLC system. From the results obtained the allowable variation in flow rate is from 1.4 ml/min to 1.6 ml/min.

D. Validation on effect of variation in column oven temperature:

The effect of variation in column oven temperature was conducted. Standard solution of Ebastine 20 mg, orodispersible tablets was prepared as per the test method and injected into HPLC system. From the results obtained, the allowable variation in column oven temperature is from 35°C to 45°C.

IV. CONCLUSIONS

A robust stability indicating RP-HPLC method for Ebastine is developed. 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

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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.

REFERENCES

[1]. Sastre J. Ebastine in allergic rhinitis and chronic idiopathic urticaria. J Allergy 2008;63 (89):1-20.

[2]. Prabu SL, Kumar CD, Shirwaikar A, Shirwaikar A. Determination of ebastine in pharmaceutical formulations by HPLC. Indian J Pharm Sci. 2008;70(3):406-407. doi:10.4103/0250-474X.43022

[3]. N. S. Rana ,K. S. Rajesh, nikita N. Patel, P. R. Patel, U. Limbachiya and T. Y. Pasha. Development and validation of rp-hplc method for the simultaneous estimation of montelukast sodium and ebastine in tablet dosage form. Indian J Pharm Sci 2013;75(5):599-602

[4]. Lakshmana Prabu, Sakthivel & Kumar Chellappan, Dinesh & Shirwaikar, Annie. (2008). Determination of Ebastine in Pharmaceutical Formulations by HPLC. Indian journal of pharmaceutical sciences. 70. 406-7. 10.4103/0250-474X.43022.

[5]. Baokar Shrikrishna, Ranpise Nisharani. Analytical Method Development and Validation for Simultaneous Estimation of Montelukast and Ebastine by HPLC. Research J. Pharm. and Tech. 8(1): Jan. 2015; Page 01-05. doi: 10.5958/0974-360X.2015.00001.3

[6]. Om M. Yadav, Hemant Kumar Jain. RP-HPLC Method development and validation for simultaneous estimation of Phenylephrine Hydrochloride and Ebastine in tablet dosage form. Int J Pharm Pharm Sci, Vol 6, Issue 8, 466-470

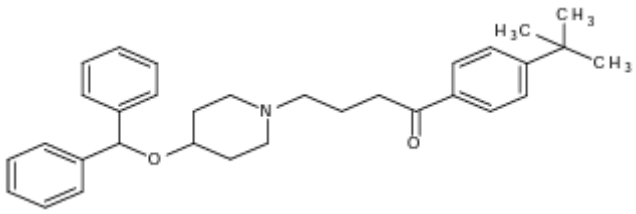

Figure 1. Chemical structure of Ebasttine

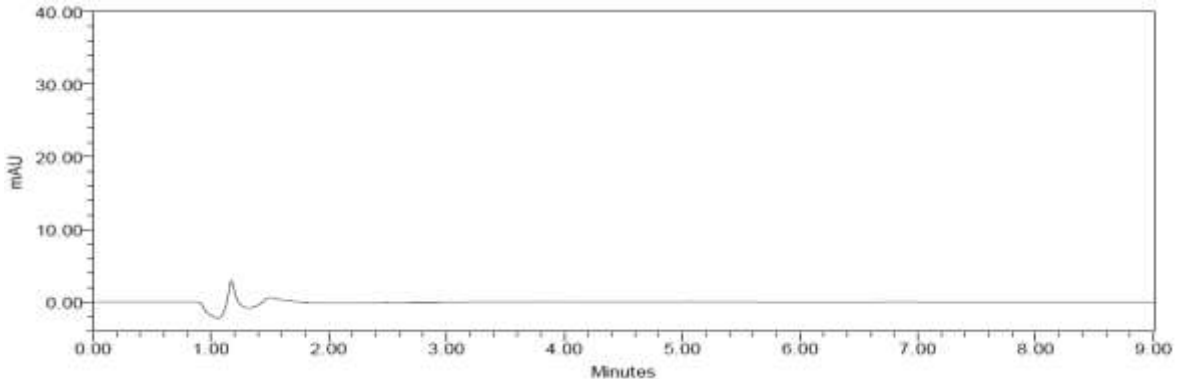

Figure 2. Chromatogram from placebo solution

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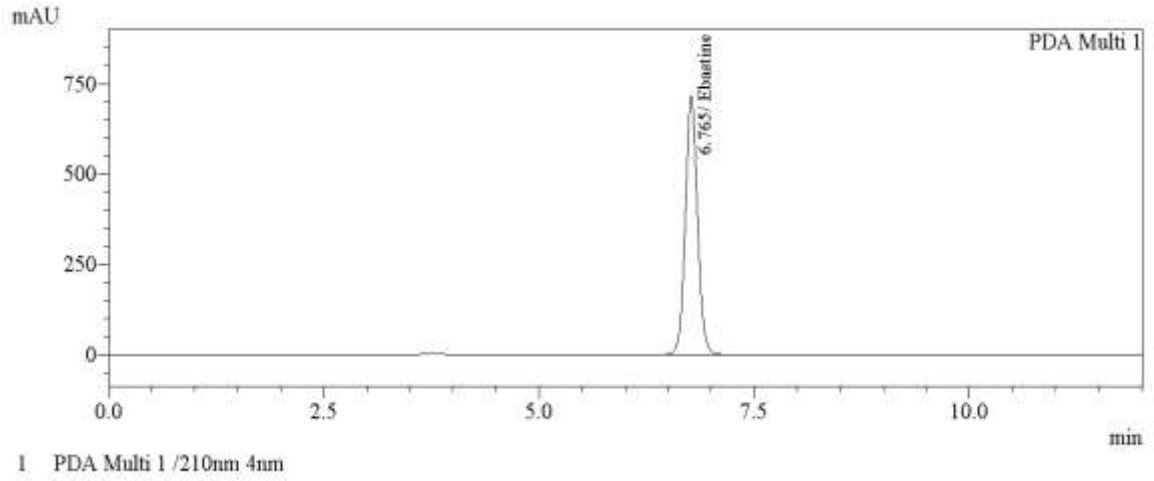

Figure 3. Chromatogram of standard

Table 1. Results showing Linearity

Level	Concentration in µg/ml	Stock solution (ml)
20%	80.0	1575972
50%	200.0	3899641
80%	320.0	6193009
100%	400.0	7654990
120%	480.0	9082162
150%	600.0	11499542
200%	800.0	15033216
Slope		18716.29721
Y-Intercept		146430.0122
Correlation Coefficient		0.999865

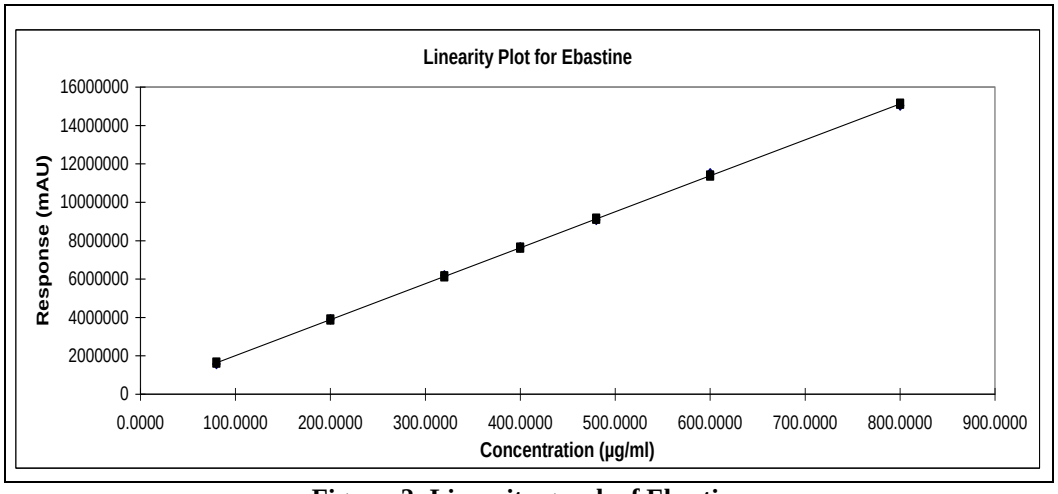

Figure. 2: Linearity graph of Ebastine

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Table. 2: Results of Repeatability

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. 3: Results of intermediate Precision

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		9.95	**Cochran test :** Cexpt.= Varmax /∑var Cexpt.= 0.8178	
Standard deviation		0.087		
(%) Relative standard dev.		0.9		
Confidence limits (%)		0.6		
95% Confidence Interval		9.89 to 10.01		

Table 4: 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		

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Table 5: Robustness study showing variation in pH, organic phase ratio of mobile Phase and flow rate

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	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	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