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Stability-indicating RP-HPLC Method Applied to the Quantification of anti-histaminic drug Ebastine in Its Oral Suspension Dosage Form

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For the quantification of ebastine in pharmaceutical suspension, a simple, quick, accurate, and exact stability-indicating HPLC approach was developed and validated. The drug was determined using a phase reverse system and the separation was performed in an analytical C18 column (250 mm x 4.6 mm, 5 µm). The mobile phase consists of 0.1% orthophosphoric acid and methanol in a 25:75 v/v ratio. Using a concentration range of 10–90 µg mL^{-1}, the technique demonstrated a strong linear response (r=0.999). Effluents were measured at 262 nm while the flow rate was kept at 1.0 mL min^{-1}. There was a retention time of 3.506 min. The method was statistically validated to determine its accuracy, precision, linearity, ruggedness, robustness, solution stability, selectivity, and forced degradation assessments. The stresses that were used were acid, alkali hydrolysis, water stress, oxidation, photolysis, and heat. Since the degradation products did not affect the capacity to identify ebastine, this technique may be taken as a stability indication. This methodology may be utilized for the analysis of Ebastine in pharmaceutical suspension, since the findings obtained were within the limits set by ICH standards.

Keywords: ebastine; RP-HPLC; stability-indicating; pharmaceutical suspension

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Introduction

Ebastine (Figure 1) has the chemical formula 4-(4-benzhydryloxy-1-piperidyl)-1-(4-tert-butylphenyl)butan-1-one and $C_{32}H_{39}NO_2$ is the molecular formula. It is a white powder with a molecular mass of 469.66 g/mol and is soluble in dichloromethane, partially soluble in methanol and insoluble in water (1). It has a melting point of 86 °C (1). The logP of the ebastine is 6.8 while the most basic pKa is 8.19 (1). Ebastine is an inverse histamine agonist rather than an antagonist of H1-receptors (2). Its antagonistic effect often prevents histamine activation, particularly in cases of acute hypersensitivity. It impacts on the bronchi, capillaries, and other smooth muscles to prevent or treat motion sickness, seasonal rhinitis, and allergic dermatitis. The ebastine is recognized by a number of pharmacopeias, including the BP (3), EP (4)and JP XVI (5).

Solely the Japanese Pharmacopeia has a monograph for ebastine drug ingredient, tablets, and orally disintegrating tablets; the first two compendia only cover raw materials and impurities. Tablets of 10 mg and 20 mg, fast-dissolving tablets, and a syrup in a 1 mg mL^{-1} dosage are all available forms of ebastine. It is also available in other active ingredient combinations, including "Co-Aleva" (Ebastine and Betamethasone), "Ebast-DC" (Ebastine and Phenylephrine), "Ebastel D" (Ebastine and Pseudoephedrine), and "Ebast-M" (Ebastine and Montelukast). Ebastine is also available in suspension form as Ebast suspension, which contains 5 mg/60 mL of ebastine.

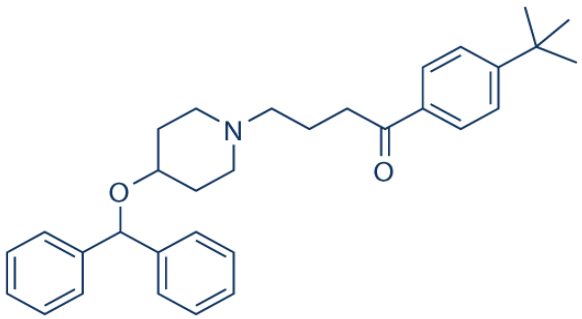

Figure 1. Chemical structure of ebastine

A thorough study revealed that numerous analytical procedures for estimating ebastine in tablet and syrup dosage forms, either alone or in combination with other

medications, have been described, including spectrophotometry(6-9), HPTLC (10), HPLC (11-16), and LC-MS/MS(17-19). There have been very few reports of RP-HPLC techniques(20-22) for estimating ebastine in tablet and syrup dose forms that are stable. However, no RP-HPLC techniques for estimating ebastine in suspension dose form have been published to date. All medications must be evaluated using a stability-indicating assay technique before release, according to Current Good Manufacturing Practices. The determination of ebastine in the active pharmaceutical ingredient and in the pharmaceutical dosage forms, as well as the separation of the drug from the degradation products, under the conditions advised by the International Conference on Harmonization (ICH), are necessary. Therefore, the development of a stability-indicating liquid chromatography (LC) technology (23) is recommended. The main goal of this research was to develop an innovative, inexpensive, accurate, repeatable, and stability-indicating RP-HPLC approach for assessing ebastine in pharmaceutical dosage forms (oral suspensions) and bulk medication.

Experimental section

Chemicals

A free sample of the reference standard ebastine (purity >99.8%) was acquired from RA Chem Pharma Ltd. (Hyderabad,India). Acetonitrile and water of HPLC grade were supplied by MERCK India Ltd. Methanol of an HPLC-grade quality was purchased from standard reagent Pvt. Ltd. Hyderabad. HCl, NaOH, and H_2O_2 were purchased from SD Fine chemicals in Mumbai, India, and were of analytical quality. The 0.2 µm and 0.45 µm nylon membrane filters came from PALL Life Sciences in Mumbai, India.

Equipment

The equipment utilized in the research included an electronic balance from Apex in India, a sonicator from LAB India Ltd. in Mumbai, a hot air oven from Accumax in India, and a digita pH meter (Elico LI 120). Using LC solutions software, HPLC (Shimadzu) was integrated and monitored. In addition, a Hamilton (Rheodyne-20 µL) syringe and a "Himedia Syringe-driven filter" (0.22 µm) syringe were employed.

Chromatographic conditions

Shimadzu HPLC with an SPD-20A detector with programmable variable wavelength, a SCL 20A system controller, and an LC-20AD binary gradient pump and Rheodyne injector 7725i fitted with a 20 µL loop comprised the chromatographic system utilized for the development and validation of the technique. Data were acquired and analysed using LC solutions software version 5.0. At ambient temperature, separation was carried out using an Enable C18G (250 x 4.6 mm i.d.,5 µm). The best mobile phase for the ideal chromatographic separation of Ebastine was discovered to be a mixture of 0.1 % Orthophosphoric acid and methanol in a ratio of 25: 75 v/v. Before use, the solvent mixture was sonicated and processed through a membrane filter with a pore size of 0.45 µm filtered . It was pushed through the column at a rate of 1.0 mL min^{-1}. The column was kept at room temperature, and 20 µL was injected. After pumping the mobile phase through the column for at least 30 minutes, the column was equilibrated for the injection of the drug solution. At 262 nm, the drug's detection was seen. The 10 minute runtime was chosen. Table 1 displays the ideal chromatographic conditions.

Preparation of mobile phase

The mobile phase was created by combining 900 mL of methanol of HPLC quality with 100 mL of 0.1 % ortho-phosphoric acid. The ortho-phosphoric acid was made by combining 0.1 mL of ortho-phosphoric acid with 100 mL of water of HPLC quality. After sonicating the mobile phase for 10 min, a 0.45 µm membrane filter was used to filter the solution.

Preparation of standard stock solutions

By combining 50 mg of the pure drug with 50 mL of mobile phase in a volumetric flask, the standard stock solution of the drug at 1000 µg mL^{-1} was produced. The 100 µg mL^{-1} concentration was achieved by diluting the stock solution with mobile phase. The above prepared standard solution aliquots were added to a volumetric flask of capacity 10 mL and mark with mobile phase until the desired concentration was reached. The drug's final concentrations were in the range of 10-90 µg mL^{-1} as a result.

Preparation of sample solution

The whole of one container was removed and carefully mixed in order to ascertain the amount of ebastine in oral suspension dose form (5 mg/60 mL according to the label). It was measured out and mingled in methanol (50 mL) in a quantity equal to 5 mg of ebastine. Following this, the resulting solution, which was 5 mL, was poured into a volumetric flask measuring 10 mL, and mobile phase was used to dilute it to the appropriate level. A 0.45 µm membrane filter was used as an injection filter to filter the final solution. The filtrate was added in a volume of 20 µL to the chromatographic device. Utilizing an equation for linear regression discovered from the calibration curve, the ebastine concentration was measured. The placebo formulation contains: citric acid, sorbic acid, deionized water, cellulose

microcrystalline, hydroxyethylcellulose, propylparaben, methylparaben, propylene glycol, saccharin sodium.

Method validation

By assessing "linearity, accuracy, precision, robustness, ruggedness, detection limit, quantification limit, and stability", accordign to oficial giuidelines (24), the proposed technique was shown to be valid.

System Suitability Test

System suitability test was conducted by examining the peak areas' relative standard deviation (RSD), capacity factor, tailing factor and theoretical plate number.

Stability

After being left at room temperature for 48 hours, QC standard solutions were examined in order to determine stability. Results were examined as recovery values and compared with newly made solutions.

Linearity

Mobile phase was used to create a 1000 µg mL^{-1} stock solution of ebastine. From it, different working standard solutions ranging from 10 to 120 µg mL^{-1} were prepared after which it was feed into HPLC. It was shown that the chosen medication showed linearity between 10-90 µg mL^{-1}. Replicate study (n=9) at all concentration levels produced the calibration plot (peak area of ebastine vs ebastine concentration), and the linear connection was assessed using the least squares technique inside the Microsoft Excel® application. The linearity results were analyzed by the testone-way ANOVA with post Tukey test and significance level of α=0.05 (95% of confidence interval). The analysis of variance (ANOVA) allowed evaluating the linearity of the method and the validity of the linear regression.

Accuracy

Standard addition procedures were used at 80 %, 100 %, and 120 % concentrations, and the approach's accuracy was evaluated by calculating the deviation between the theoretical value (spiking value) and the observed value.

Precision

As an evaluation of the method's precision, the standard deviation was calculated by measuring the peak area of six replicates of a fixed concentration of the medication (50 µg mL^{-1}) prepared from ebastine suspension dosage form. The assay's precision was evaluated by comparing the intra- and inter-day fluctuations in peak areas of a series of drug solutions (50 µg mL^{-1}) prepared from the suspension dosage form over the course of three separate days. Using relative standard deviation(RSD), we calculated the daily and hourly variations in the medication solution's peak area. Reproducibility was studied by inter-laboratory variations.

Robustness

Using modest variations in flow rate, pH, and mobile phase ratio, the suggested technique for ebastine was tested for robustness. Ebastine's percentage of recovery and RSD were reported.

Ruggedness

The test solutions (50 µg mL^{-1}) was prepared from the suspension dosage form and injected under varying conditions. Different analysts investigated the robustness of the approach.

Detection limit and quantification limit

Limits of detection (LOD) and quantitation (LOQ) were calculated using the following formulae, which were based on the parameters of the calibration curve:

The limits of detection (LOD) are 3.3σ/s and the limits of quantification (LOQ) are 10σ/s, where 's' is the slope of the calibration curve and 'σ' is the standard deviation of the y-intercept of the regression line.

Forced degradation studies

Forced degradation experiments on the sample employing oxidative, thermal, photolytic, acid, alkaline, and UV degradations was used to show the method's specificity. demonstrating that the technique effectively isolated degradation products from pure active ingredient, the sample was subjected to these conditions. The drug was subjected to hydrolytic degradation(neutral, acidic, and basic pH) , oxidative degrdation , photolytic degradation , UV degrdation and thermal degradation.

Degradation in neutral condition

Using pure water, ebastine was submitted to neutral degradation. Accurately weighing ten micrograms of ebastine mass, it was then put into a tidy volumetric flask of capacity 10 ml. The volumetric flask's contents were mixed with 5 mL of distilled water. The volumetric flask was then heated to 80 °C in a water bath. For various time periods, including 0 min, 30 min, 1 h, 2 h, and 4 h, samples were produced. Several sample solutions were collected at various periods, and then 5 mL of HPLC-grade methanol was added to each. After sonicating the sample solution for 5 minutes, it was diluted to a concentration of 50 µg mL^{-1}. After filtering it via a 0.22 µm filter, 20 µL was added for analysis in the HPLC. The acquired chromatogram was checked for any degradation that could have happened over time.

Degradation in acidic condition

Using 0.1 M HCl, ebastine was acidically degraded. Accurately weighing ten micrograms of ebastine mass, it was then put into a clean volumetric flask of capacity 10 mL. The volumetric flask's contents were dissolved in 5 mL of 0.1 M HCl and heated to 80 °C on a water bath. For various time periods, including 0 min, 30 min, 1 h, 2 h, and 4 h, samples were produced. At different periods, various sample solutions were withdrawn, and 5 mL of HPLC-grade methanol was subsequently added. The sample solution was then subjected to a 5-minute sonication , and diluted to a concentration of 50 µg mL^{-1}. After that, it was put into the HPLC after being filtered with a 0.22 µm filter. The resultant chromatogram was evaluated for any degradation happening throughout the period.

Degradation in basic condition

With 0.1 M NaOH, ebastine was subjected to an alkaline degradation process. Accurately weighing ten micrograms of ebastine mass, it was then put into a tidy volumetric flask of capacity 10 ml. The volumetric flask's contents were dissolved in 5 mL of 0.1 M NaOH and heated to 80 °C on water bath. For various time periods, including 0 min, 30 min, 1 h, 2 h, and 4 h, samples were produced. At different periods, various sample solutions were withdrawn, and 5 mL of HPLC-grade methanol was subsequently added. The sample solution was then subjected to a 5-minute sonication , and diluted to a concentration of 50 µg mL^{-1}. After that, it was put into the HPLC after being filtered with a 0.22 µm filter. The generated chromatogram was examined for any deterioration that could have occurred over time.

Oxidative degradation

Hydrogen peroxide (3,0 % concentration) was the agent used for oxidation. Accurately weighing ten milligrams of ebastine mass, it was then put into a tidy volumetric flask of capacity 10 mL. The contents of the volumetric flask added to 5 ml of H_2O_2. It was then allowed to degrade at room temperature. For various time periods, including 0/30/60/120/240 min samples were produced. At different periods, various sample solutions were withdrawn, and 5 mL of HPLC-grade methanol was subsequently added. The sample solution was then subjected to a 5-minute sonication , and diluted to a concentration of 50 µg mL^{-1}. After that, it was put into the HPLC after being filtered with a 0.22 µm filter. The acquired chromatogram was examined for any deterioration that could have occurred throughout the allotted period.

Photolytic degradation

Carefully weighing 100 mg of ebastine mass and placing it in a clean petri dish for photolysis. The closed petri dish was then exposed to direct sunshine to degrade. At various time periods, 10 mg of material was extracted. It was used to make a 1000 µg mL^{-1} stock solution. It was then subjected to a 5 min sonication and diluted to create a workable solution of 50 µg mL^{-1} in the mobile phase. Following that, it was filtered via a 0.22 µm filter and put into the HPLC. The resulting chromatogram was analyzed for any degradation that occurred throughout the course of the experiment.

UV-degradation

Ebastine, weighed to the 100 mg , was placed in a clean petri dish and exposed to ultraviolet light. After that, the petri dish was placed in a UV chamber 30 cm away from the UV light. The petridish's lid was removed to allow for deterioration. The UV light was turned off after 3 hours, and 10 mg of material was extracted. A stock solution of 1000 µg mL^{-1} was created using the mobile phase, from which a working solution of 50 µg mL^{-1} was obtained. It was sonicated , filtered through a 0.22 µm filter and feed into HPLC(20 µL).

Thermal degradation

The ebastine mass was thermally degraded by putting it in a hot air oven set to 40 °C. At periodically, samples were obtained. The weighted sample was sonicated for 5 minutes after being combined with 5 mL of HPLC grade methanol. The standard stock solution (at 1000 µg mL^{-1}) was made in methanol. The stock was diluted with mobile phase to provide a working standard solution at a concentration of 50 µg mL^{-1}. It was put through a 0.22 µm filter after being sonicated and fed into the HPLC in 20 µL batches. The chromatogram obtained was evaluated for any deterioration that occurred over time.

Results and Discussion

Optimization of chromatographic conditions

To develop the HPLC technique for the ebastine within the presence of the degradation products, optimization studies were conducted. In this case, a number of parameters were investigated, including the mobile phase composition, the stationary phase, the wavelength, the flow rate, and the volume of injection. Based on peak shapes and retention duration values, the ideal parameters were identified. Selection of wavelength was based on the UV spectrum of ebastine, which showed maximum absorbance at 262 nm. Various mobile phases were evaluated during the method's development phase. According to the literature, ACN and methanol were the most often used organic solvents for the measurement of ebastine. Good system suitability parameters were reported with methanol, hence methanol was selected as the organic phase. It was found that for ebastine(pka=8.19) to be in ion-suppressed form and be absorbed by the column, the mobile phase needed to be acidic. Therefore, ebastine exhibited enhanced retention

at low pH values. According to the literature, the aqueous mobile phases used with methanol combinations are water, water (pH 3.5), phosphate buffers, orthophosphoric acid, and ammonium acetate. To achieve an acidic pH, we experimented with 0.1% ortho phosphoric acid. Therefore, experiments were conducted with different ratios of methanol: 0.1% ortho phosphoric acid. As the amount of methanol increased, the retention period reduced, but the number of theoretical plates fell as well. This composition is optimized because the mobile phase containing methanol and 0.1% ortho phosphoric acid 75:25, v/v) produced acceptable peak form and retention. We evaluated injection volumes of 5, 10, 15, and 20 µL. Peak area values rose as injection volume increased, and improved peak forms were achieved. Consequently, 20 µL was determined to be the optimal injection volume. Flow rates of 0.8, 1.0, and 1.2 mL min^{-1} were compared to identify the best flow rate. There was no significant difference in the peak area values that were obtained. With a flow rate of 0.8 mL min^{-1}, the peak area values were the greatest; however, peak tailing was slightly greater than with other flow rates. Because an increase in flow rate led to a rise in column pressure, the lowest feasible flow rate was chosen. This is the rationale for the choice of 1 mL min^{-1} as the procedure's flow rate. Figure 2 illustrates a typical chromatogram generated under optimal conditions. Table 1 displays the optimum chromatographic conditions.

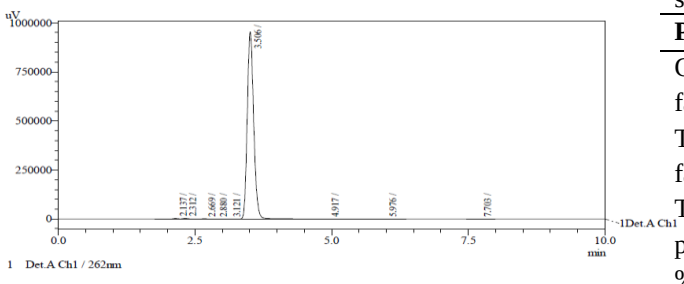

Figure 2. Representative chromatogram of standard solution of ebastine using optimized HPLC conditions.

Method validation

System Suitability Test

The system suitability parameters for the developed approach were calculated once the ideal circumstances had been established, and they were compared to suggested limits. Six injections were used in the investigation using a standard solution at a concentration of 50 µg/ml to ascertain the parameters. Table 2 shows the method's system suitability parameters. The approach was determined to be appropriate for the analysis based on the results which showed that all of the metrics for the system's suitability were well within the recommended limits.

Table 1. Optimized Chromatographic conditions for HPLC method in the determination of ebastine oral suspension.

Parameters	**Conditions**
Stationary Phase(Column)	C_{18} (250 × 4.6 mm i.d.,5µ)
Mobile Phase	Methanol: 0.1% Orthophosphoric acid (75:25,v/v)
Flow rate(ml/min)	1.0 mL min^{-1}
Run time(min)	10 min
Column temperature (°C)	ambient
Volume of injection loop(µL)	20
Detection wavelength(nm))	262 nm
Retention time(min)	3.506

Table 2. Results of system suitability test (n = 6) for HPLC method in the determination of ebastine oral suspension.

Parameter	**Criteria**	**Result**
Capacity factor(k')	$k' > 2$	3.824
Tailing factor (T)	T < 2	1.488
Theoretical plates (N)	N> 2000	4320
% RSD (peak area)	% RSD ≤ 1	0.74

Stability

The stability of the sample solution was evaluated by repeatedly injecting it at 0 hours, 12 hours, 24 hours, and 48 hours.The developed approach did not show the same change. Additionally, the findings (RSD <2) were judged to be within acceptable bounds. Data presented on Table 3.

Table 3. Analytical stability data of Ebastine (standard solutions) during HPLC method development for quantitation.

Time (hr)	Assay(%)	% Difference
Initial	100.08	----
After 12 hr	100.02	0.05
After 24 hr	99.87	0.21
After 36 hr	99.16	0.92
After 48 hr	98.32	1.76

Linearity and sensitivity

Using calibration standards with concentrations of 10, 20, 30, 40, 50, 60 , 70 , 80 and 90 µg mL^{-1}, a linearity study was carried out. Three separate injections of the standards were carried out. The peak areas were plotted against the preset concentrations, which resulted in the creation of calibration curves. The determination coefficient was used to assess the calibration curve. The calibration curves' determination coefficient (R^2) was 0.9997. As a result, it was discovered that the calibration curve for ebastine was linear within the concentration range of 10-90 µg mL^{-1} (Figure 3). The calibration graphs were used to compute the regression equations. The analysis of variance (ANOVA) allowed evaluating the linearity of the method and the validity of the regression. The peak area was divided by the corresponding concentration, and then the values obtained were analyzed by the test one-way ANOVA with post test Tukey using a significant level of α=0.05 (95% of confidence interval). The value obtained for the $F_{(calculated)}$ is lower than the $F_{(tabulated)}$, indicating no significant difference between the values obtained (peak area/corresponding concentration) from the calibration curves. Limits of detection (LOD) and quantitation were used to assess the analytical method's sensitivity (LOQ) were found to be 0.09 and 0.21 µg mL^{-1} .

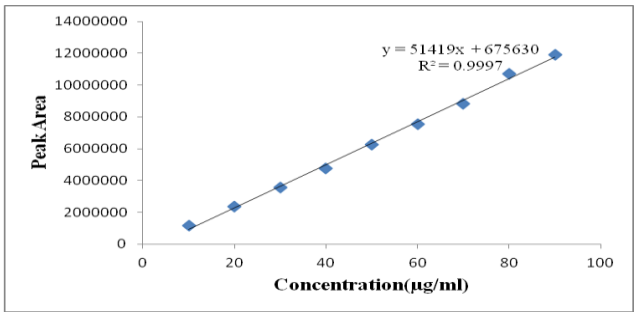

Figure 3. Linearity curve obtained from validation study applyed to HPLC method for ebastine in suspension formulation.

Accuracy

Experiments on recovery were conducted to investigate the reliability, appropriateness, and precision of the approach. Using the suggested approach, samples containing 80, 100, and 120 % of the drug were created by mixing known quantities of pure medication to placebo The results of calculating accuracy as the percentage of recovery are shown in Table 4.

Precision

Repetition, intermediate precision, and reproducibility (between laboratories' precision) were the three levels at which the precision was shown. Three sequential replicate injections at three different concentrations—40, 50, and 60 µg mL^{-1}—were used to test each level of precision. Using the relative standard deviation (RSD) or coefficient of variation, the precision was described. Table 5 displays the outcomes of three precision levels.

Robustness and ruggedness

By modifying analytical parameters such as flow rate (0.9 to 1.1 mL min^{-1}), mobile phase composition and detection wavelength, the robustness was evaluated. The results are shown in Tables 6. Several analysts evaluated the robustness of the procedure. The results are shown in Table 7.

Mobile phase stability

The mobile phase was kept at 4 to 8 °C for a week in order to test its stability. A freshly prepared mobile phase and an older one were compared. At 4–8 °C, the mobile phase remained stable for up to a week.

Forced degradation studies

The HPLC findings demonstrated that when stress conditions were applied to ebastine, there was no interaction between the tested drug and the degradation products, demonstrating the specificity and stability indicating of the procedure. The drug degraded extensively under oxidative conditions, alkali hydrolysis, heat conditions and UV. Literature survey reveals ebastine degraded more in oxidative and UV conditions. There is one reports on characterization of degradation products from ebastine by UV degradation (25). From our assumption the extensive degradation under oxidative conditions seen might be due to oxidation of piperidine ring. The chromatograms obtained following deterioration under various stress settings are shown in Figure 4. The percntage of degradation of ebastine in various conditions was presented in Figure 5.

Table 4. Accuracy data obtained during HPLC method development for quantitative determination of ebastine in oral suspension.

% Level	Concentration(µg mL^{-1})		Recovery(%)	Statistical Results		
	Formulation	SQR		Mean	SD	%RSD
80	50	40	98.4			
80	50	40	98.1	99.1	1.47	1.48
80	50	40	100.8			
100	50	50	100.5			
100	50	50	101.9	101.3	0.72	0.71
100	50	50	98.8			
120	50	60	99.4			
120	50	60	99.7	99.8	0.51	0.52
120	50	60	100.4			

Table 5. Precision data obtained from HPLC method development for quantitative determination of ebastine in oral suspension.

Precision parameters	Results		
	Concentration(µg mL^{-1})	RSD of Peak area	RSD of retention Time
Repeatability	40	0.89	0.021
	50	1.21	0.088
	60	1.11	0.123
Intermediate precision	40	1.42	0.087
	50	0.75	0.066
	60	0.67	0.062
Reproducibility	40	1.64	0.111
	50	0.78	0.17
	60	0.85	0.094

Table 6. Robustness data obtained from HPLC method development for quantitative determination of ebastine in oral suspension.

Parameter	Variation	Observed value			
		RSD % of area	RSD% of retention time	Tailing fator (T)	Theoretical plates(N)
Flow rate (mL min^{-1})	0.9	0.47	0.097	1.52	4112
	1.1	0.65	0.076	1.53	4225
Mobile phase composition	80% methanol	0.79	0.044	1.54	4187
	70 % methanol	0.81	0.132	1.55	4302
Wavelength of detection	264 nm	0.66	0.076	1.52	4221
	260 nm	0.92	0.026	1.51	4107

Table 7. Ruggedness data obtained from HPLC method development for quantitative determination of ebastine in oral suspension.

Analyst	Observed value			
	RSD % of area	RSD% of R.T	Tailing factor(T)	Theroteical plates(N)
Analyst I	0.45	0.078	1.492	4286
Analyst II	0.52	0.066	1.488	4311

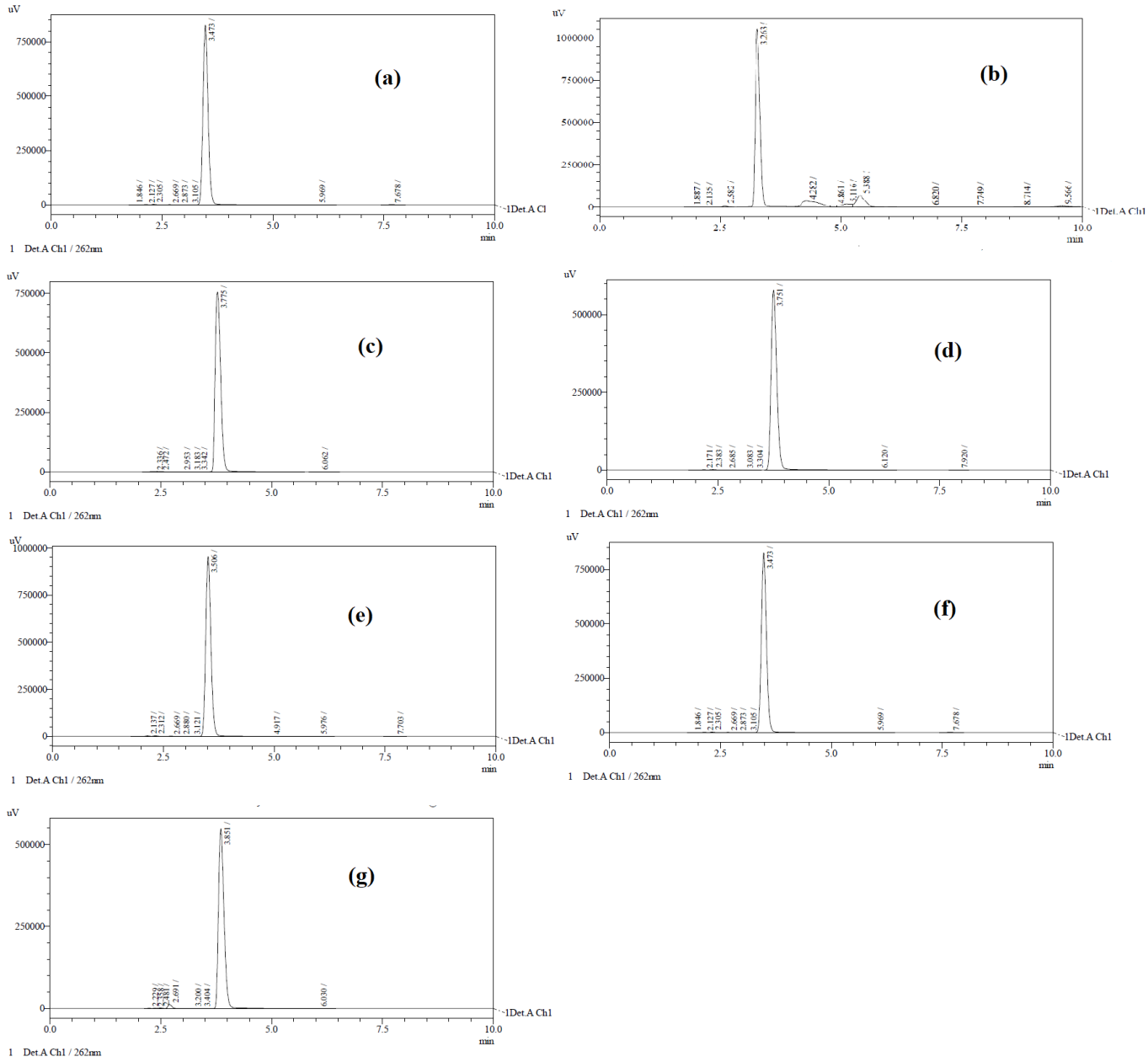

Figure 4. Representative chromatograms from forced degradation studies developed from validation of HPLC method applied to ebastine in suspension formulation. (a) neutral conditon; (b) acidic condition; (c) basic condition; (d) oxidative condition; (e) photolytic condition; (f) UV degradtion; (g) thermal condition.

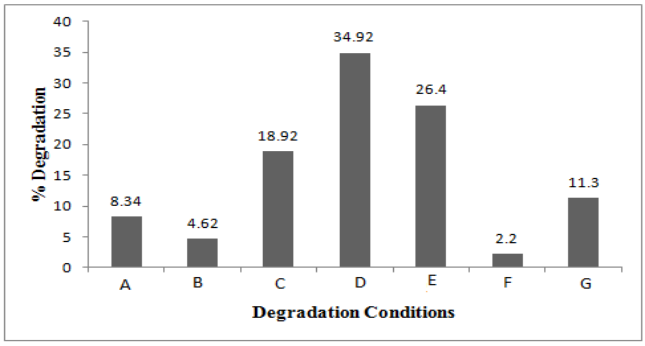

Figure 5. Degradation % of ebastine in suspension formulation, through qunatitation by HPLC. A-neutral condition, B- acidic condition , C- basic condition, D-oxidative , E- Thermal , F- Photolytic (sunlight) , G- UV.

Sample Analysis

The developed and validated method was used to the analysis of ebastine-containing oral suspensions. The material was examined three times. Using a calibration curve, the analysis outcomes were reviewed. Estimates of ebastine concentrations in the samples were calculated using the calibration curve equation, and values for recovery and relative standard deviation were also calculated. The recoveries of ebastine from suspension dosage form was found to be 98.4 % ± 0.92. The recoveries were consistent with the claims made on the label. The resultant chromatogram was shown in Figure 6. It was determined that the approach could be effectively used to the analysis of ebastine in suspension form.

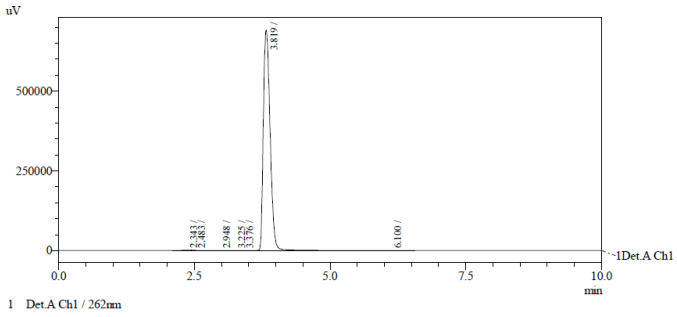

Figure 6. Representative chromatogram from ebastine analysis from suspension dosage form, applying the HPLC method developed.

Conclusion

For the estimation of ebastine in suspension dosage forms in the presence of degradation products, a simple, selective, repeatable, economical, stability indicating RP-HPLC technique has been designed and validated in accordance with ICH requirements. The percentage recovery was observed within the acceptance criteria, so it indicated method was accurate. Ebastine was subjected to a variety of stresses in order to undertake forced degradation tests to ascertain the stability-indicating nature of the analytic approach. Stability demonstrating forced degradation established studies revealed findings that there is no influence from any degraded products and it did not interact with ingredients in the formulation. The detection of ebastine and the conducted procedure are therefore specific stability-indicating. The technique is excellent for routine quantification of ebastine with high precision and accuracy because to its broad linearity range, sensitivity, accuracy, short retention period, and easy mobile phase.

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Conflict of interest

The authors declare no conflicts of interest.

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