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Short communication

A validated stability-indicating UPLC method for desloratadine and its impurities in pharmaceutical dosage forms

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ABSTRACT

A novel stability-indicating gradient reverse phase ultra-performance liquid chromatographic (RP-UPLC) method was developed for the determination of purity of desloratadine in presence of its impurities and forced degradation products. The method was developed using Waters Aquity BEH C18 column with mobile phase containing a gradient mixture of solvents A and B. The eluted compounds were monitored at 280 nm. The run time was 8 min within which desloratadine and its five impurities were well separated. Desloratadine was subjected to the stress conditions of oxidative, acid, base, hydrolytic, thermal and photolytic degradation. Desloratadine was found to degrade significantly in oxidative and thermal stress conditions and stable in acid, base, hydrolytic and photolytic degradation conditions. The degradation products were well resolved from main peak and its impurities, thus proved the stability-indicating power of the method. The developed method was validated as per ICH guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision and robustness. This method was also suitable for the assay determination of desloratadine in pharmaceutical dosage forms.

1. Introduction

Desloratadine is indicated for the relief of the nasal and non-nasal symptoms of seasonal allergic rhinitis in patients 2 years of age and older. Its chemical designation is 8-chloro-6,11-dihydro-11-(4-piperdinylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine (Fig. 1). It is available as 5 mg tablets and also available as syrup.

In the literature there were limited LC methods have been reported for determination of desloratadine in pharmaceutical preparations. The assay method [1] reported describes the separation of degradation impurities from deslaratadine formed through forced degradation studies, but it was out of scope because it did not separate and determine the impurities. A validated RP-HPLC method has been reported for quantification of desloratadine in pharmaceutical forms [2], a spectophotometric, spectrofluorometric and HPLC method has been reported for determination desloratadine in dosage forms and in human plasma [3] and a LC method has been reported for simultaneous determination of loratadine and desloratadine in pharmaceutical preparations using micro-emulsion as eluent [4], but forced degradation study and impurity details were not reported in these articles [2–4]. We have developed a stability-indicating RP-LC method that can separate and determine deslaratadine and its five impurities namely imp-A, imp-B, imp-C, imp-D and imp-E (Fig. 1).

Ultra-performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Literature indicates that UPLC system allows about ninefold decreases in analysis time as compared to the conventional HPLC system using 5 μm particle size analytical columns, and about threefold decrease in analysis time in comparison with 3 μm particle size analytical columns without compromise on overall separation [5–9].

Hence a rapid simple reproducible gradient stability-indicating RP-UPLC method was developed and validated for the quantitative determination of desloratadine and its five impurities in pharmaceutical dosage forms.

2. Experimental

2.1. Chemicals and reagents

The purity of all chemicals was above 98%. Tablets and standards of desloratadine (99.8%) and its five impurities namely imp-A (98.2%), imp-B (99.1%), imp-C (98.1%), imp-D (98.9%) and imp-E (99.5%) were supplied by Dr. Reddy's laboratories limited, Hyderabad, India. The HPLC grade acetonitrile, methanol, tertahydrofuran

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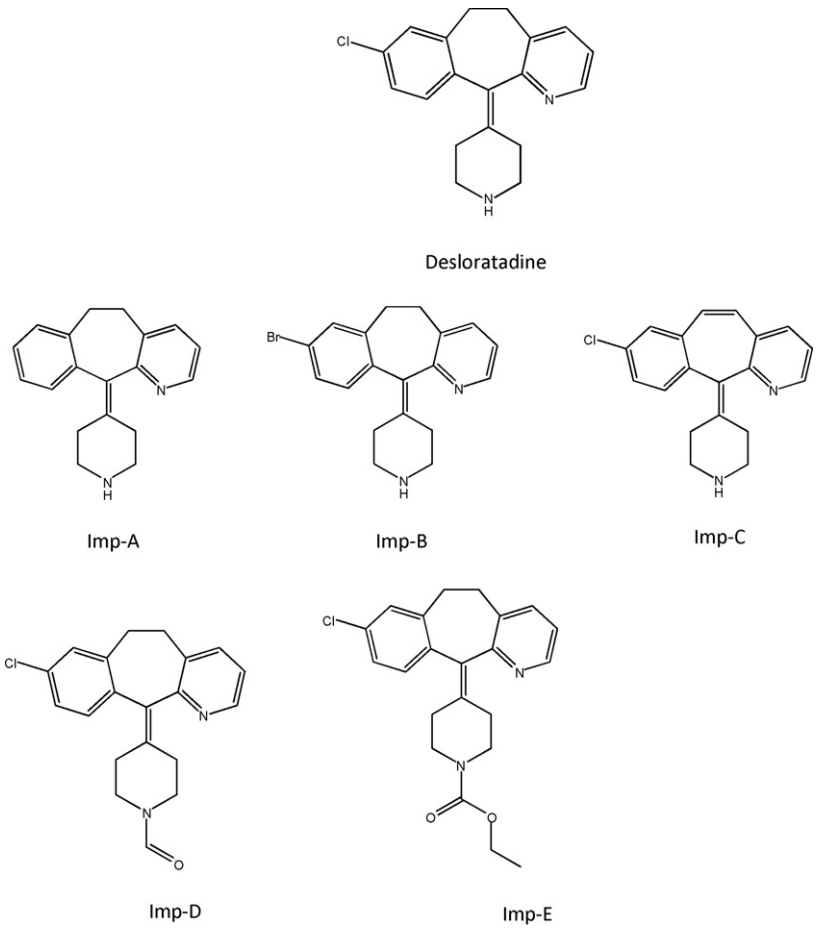

Fig. 1. Structures of desloratadine and its five impurities.

and analytical grade KH_2PO_4 and ortho phosphoric acid were purchased from Merck, Darmstadt, Germany. High purity water was prepared by using Milli Q Plus water purification system (Millipore, Milford, MA, USA).

2.2. Equipment

Acquity UPLC™ system (Waters, Milford, USA) we used consists of a binary solvent manager, a sample manager and a photodiode array (PDA) detector. The output signal was monitored and processed using empower2 software. Cintex digital water bath was used for hydrolysis studies. Photostability studies were carried out in a photostability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (Cintex, Mumbai, India). The pH of the solutions was measured by a pH meter (Thermo Orion Model 420 A, USA). All solutions were degassed by ultra sonication (Power sonic 420, Labtech, Korea) and filtered through a 0.45 μm Nylon 66 filter (PALL Life Sciences, USA). Intermediate precision was performed on different Acquity UPLC™ system (Waters, Milford, USA) consists of a binary solvent manager, a sample manager and a tunable ultraviolet (TUV) detector.

2.3. Chromatographic conditions

The method was developed using Waters Aquity BEH C18, 50 mm × 2.1 mm; 1.7 μm column (Waters, Milford, USA) with mobile phase containing a gradient mixture of solvents A and B. 0.01 M potassium dihydrogen orthophosphate buffer, pH adjusted to 2.5 with orthophosphoric acid was used as buffer. Buffer, methanol and acetonitrile in the ratio 80:15:5, v/v/v; was used as solvent A and buffer, tetrahydrofuran and acetonitrile in the ratio 30:5:70, v/v/v; was used as solvent B. The gradient program (T/%B) was set as 0/0, 1.5/0, 5.5/80, 6.5/80, 7.0/0 and 8.0/0. The mobile phase was filtered through a nylon 0.45 μm membrane filter. The flow rate of the mobile phase was 0.6 ml/min. The column temperature was maintained at 30 °C and the wavelength was monitored at 280 nm. The injection volume was 5 μl.

2.4. LC–MS/MS conditions

LC–MS/MS system (Waters 2695 Alliance liquid chromatograph coupled with quattromicro mass spectrometer with Mass Lynx software, Waters Corporation, Milford, USA) was used for the unknown compounds formed during forced degradation studies. Hypersil BDS C18, 250 mm × 4.6 mm, 5 μm column (Thermo Scientific, USA) was used as stationary phase. 0.01 M ammonium formate (Merck, Darmstadt, Germany) was used as buffer. Buffer, methanol and acetonitrile in the ratio 80:15:5, v/v/v; was used as solvent A and buffer and acetonitrile in the ratio 25:75, v/v/v; was used as solvent B. The gradient program (T/% solvent B) was set as 0/0, 10/0, 40/80, 50/80, 52/0 and 60/5. Solvent A was used as diluent. The flow rate was 1.0 ml/min. The analysis was performed in positive electro spray positive ionization mode. Capillary and cone voltages were 3.5 kV and 25 V, respectively. Source and dissolvation temperatures were 120 and 350 °C, respectively. Dissolvation gas flow was 650 l h^{-1}.

2.5. Preparation of stock solutions

A stock solution of desloratidine (1.0 mg/ml) was prepared by dissolving appropriate amount of drug in the solvent A. Working

solutions of 500 and 100 μg/ml were prepared from the above stock solution for the related substance determination and assay determination, respectively. A stock solution of impurity (mixture of imp-A, imp-B, imp-C, imp-D and imp-E) at 0.5 mg/ml was also prepared in the solvent A.

2.6. Preparation of sample solution

Tablet powder (5 mg tablets) equivalent to 100 mg drug was dissolved in solvent A with rotary shaking for 10 min and sonication for 10 min to give a solution containing 500 μg/ml. 5 ml of this solution was diluted to 25 ml with solvent A, to give a solution containing 100 μg/ml. These solutions were filtered through a 0.45 μm pore size Nylon 66 membrane filter.

2.7. Method validation

The proposed method was validated as per ICH guidelines [11].

2.7.1. Solution stability and mobile phase stability

The stability of desloratadine in solution was determined by leaving test solutions of the sample and reference standard in tightly capped volumetric flasks at room temperature for 48 h during which they were assayed at 12 h intervals. Stability of mobile phase was determined by analysis of freshly prepared sample solutions at 12 h intervals for 48 h and comparing the results with those obtained from freshly prepared reference standard solutions. The mobile phase was prepared at the beginning of the study period and not changed during the experiment. The % assay of the results was calculated for both the mobile phase and solution-stability experiments.

The stability of desloratadine and its impurities in solution for the related substance method was determined by leaving spiked sample solution in a tightly capped volumetric flask at room temperature for 48 h and measuring the amounts of the five impurities at every 12 h. The stability of mobile phase was also determined by analysis freshly prepared solution of desloratadine and its impurities at 12 h intervals for 48 h. The mobile phase was not changed during the study period.

2.7.2. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [10]. The specificity of the developed LC method for desloratadine was carried out in the presence of its five impurities. Stress studies were performed at an initial concentration 500 μg/ml of desloratadine on tablets to provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted to stress condition of UV light (254 nm), heat (60 °C), acid (0.5N HCl at 50 °C), base (0.5N NaOH at 50 °C), hydrolytic (60 °C) and oxidation (3.0% H_2O_2 at 40 °C) to evaluate the ability of the proposed method to separate desloratadine from its degradation products. For heat and light studies, study period was 10 days whereas for hydrolytic, acid, base and oxidation, it was 24 h. Peak purity test was carried out for the desloratadine peak by using PDA detector in stress samples.

Assay of stressed samples was performed (at 100 μg/ml) by comparison with qualified reference standard and the mass balance (% assay + % impurities + % degradation products) was calculated. Assay was also calculated for desloratadine sample by spiking all five impurities at the specification level (i.e. 0.20%).

2.7.3. Linearity

Linearity test solutions for the assay method were prepared from desloratadine stock solutions at five concentration levels from 50 to 150% of assay analyte concentration (50, 75, 100, 125 and 150 μg/ml). The peak area versus concentration data was treated by least-squares linear regression analysis. Linearity test solutions for the related substance method were prepared by diluting stock solutions to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 150% of the specification level (LOQ, 0.075, 0.15, 0.20, 0.25 and 0.30%).

2.7.4. Limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ for desloratadine and its impurities were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations [11]. Precision study was also carried out at the LOQ level by injecting six individual preparations and calculating the %RSD of the area.

2.7.5. Accuracy

The accuracy of the assay method was evaluated in triplicate using three concentration levels 50, 100 and 150 μg/ml on real sample (5 mg tablets). Standard addition and recovery experiments were conducted on real sample to determine accuracy of the related substance method. The study was carried out in triplicate using four concentration levels LOQ, 0.5, 1.0 and 1.5 μg/ml. The percentages of recoveries for desloratadine and its impurities were calculated.

2.7.6. Precision

The precision of the method verified by repeatability and by intermediate precision. Repeatability was checked by (Waters Acquity UPLC™ system with PDA detector, Milford, USA) injecting six individual preparations of desloratadine real sample (5 mg tablets) spiked with 0.20% of its five impurities (0.20% of impurities with respect to 0.5 mg/ml desloratadine). %RSD of area for each impurity was calculated. The intermediate precision of the method was also evaluated using different analyst and different instrument (Waters Acquity UPLC™ system with TUV detector, Milford, USA), and performing the analysis on different days.

Assay method precision was evaluated by carrying out six independent assays of real sample of desloratadine at 0.1 mg/ml level against qualified reference standard. The intermediate precision of the assay method was evaluated by different analysts.

Table 1
Chromatographic performance data.

Compound	RT (min)	RRT[a] ($n = 3$)[c]	Resolution[b] ($n = 3$)[c]	Tailing factor ($n = 3$)[c]
Imp-A	0.89	0.34 ± 0.01	–	1.2 ± 0.06
Desloratadine	2.63	1.00 ± 0.00	27.1 ± 1.51	1.2 ± 0.00
Imp-B	2.84	1.08 ± 0.01	4.1 ± 0.26	1.2 ± 0.04
Imp-C	3.24	1.23 ± 0.02	11.1 ± 0.31	1.2 ± 0.08
Imp-D	3.40	1.29 ± 0.02	4.3 ± 0.31	1.1 ± 0.05
Imp-E	4.53	1.72 ± 0.03	28.2 ± 1.76	1.0 ± 0.00

[a] Relative retention times (RRT) were calculated against the retention time (RT) of desloratadine.
[b] Resolutions were calculated between two adjacent peaks.
[c] Mean ± SD.

2.7.7. Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between desloratadine and its impurities and tailing factor for desloratadine and its impurities were recorded. The flow rate of the mobile phase was 0.6 ml/min. To study the effect of flow rate on the resolution, flow was changed by 0.1 units from 0.5 to 0.7 ml/min. The effect of the column temperature on resolution was studied at 25 and 35 °C instead of 30 °C. The effect of the percent organic strength on resolution was studied by varying acetonitrile by −3 to +3% while other mobile phase components were held constant as stated in Section 2.3.

3. Results and discussion

3.1. Method development and optimization

The main objective of the chromatographic method was to separate critical closely eluting pair of compounds desloratadine and imp-B; and imp-C and imp-D and to elute desloratadine as a symmetrical peak. The blend containing 500 μg/ml of desloratadine and 1 μg/ml of each five impurities was prepared in the solvent A. All the impurities of desloratadine were subjected to separation by reversed-phase LC on a Waters Aquity BEH C18, 50 mm × 2.1 mm, 1.7 μm column with pH 2.5, 0.01 M potassium dihydrogen orthophosphate buffer–methanol in 80:20 ratio as a solvent A and pH 2.5, 0.01 M potassium dihydrogen orthophosphate buffer–acetonitrile in 30:70 ratio as solvent B. Two compounds viz., imp-C and imp-D were merged together, impurity B was co-eluted with desloratadine and also imp-E was strongly retained. With addition of 5% acetonitrile to solvent A, three compounds viz., imp-B, imp-C and imp-D were well separated but still imp-E was strongly retained. When 5% of tetra hydro furan was added to solvent B, imp-E was eluted with desired separation and retention time.

Attempts were made with gradient elution with solvents A and B (Section 2.3) using different C18 UPLC columns (Inertsil ODS-3, 50 mm × 2.1 mm, 2 μm particles and Zorbax XDB C-18, 50 mm × 4.6 mm, containing 1.8 μm particles, 0.6 ml min^{-1}), using different buffer pH (4.5 and 7.0) conditions and using isocratic elution with solvent A as a mobile phase (0.6 ml min^{-1}). But at all above conditions, separation of impurities was not satisfactory (Fig. 2).

It was found that use of buffer prepared by adjusting the pH of 0.01 M potassium dihydrogen phosphate to 2.5 with orthophosphoric acid (solvent A: buffer, methanol and acetonitrile in the ratio 80:15:5, v/v/v and solvent B: buffer, acetonitrile and tetrahydrofuran in the ratio 30:70:5, v/v/v) with gradient elution (time (min)/% solvent B: 0/0, 1.5/0, 5.5/80, 6.5/80, 7.0/0 and 8/0) gives enabled separation for all pair compounds and eluted desloratadine as a symmetrical peak (Fig. 3 and Table 1). Interference from the excipients was also checked, no interference was observed.

3.2. Validation of the method

3.2.1. Solution stability and mobile phase stability

Assay (%) of desloratadine during solution stability and mobile phase stability experiments was within ±1%. The variability in the estimation of desloratadine impurities was within ±10% during solution stability and mobile phase experiments when performed using the related substances method. The results from solution stability and mobile phase stability experiments confirmed that standard solutions and solutions in the mobile phase were stable up to 48 for assay and related substances analysis.

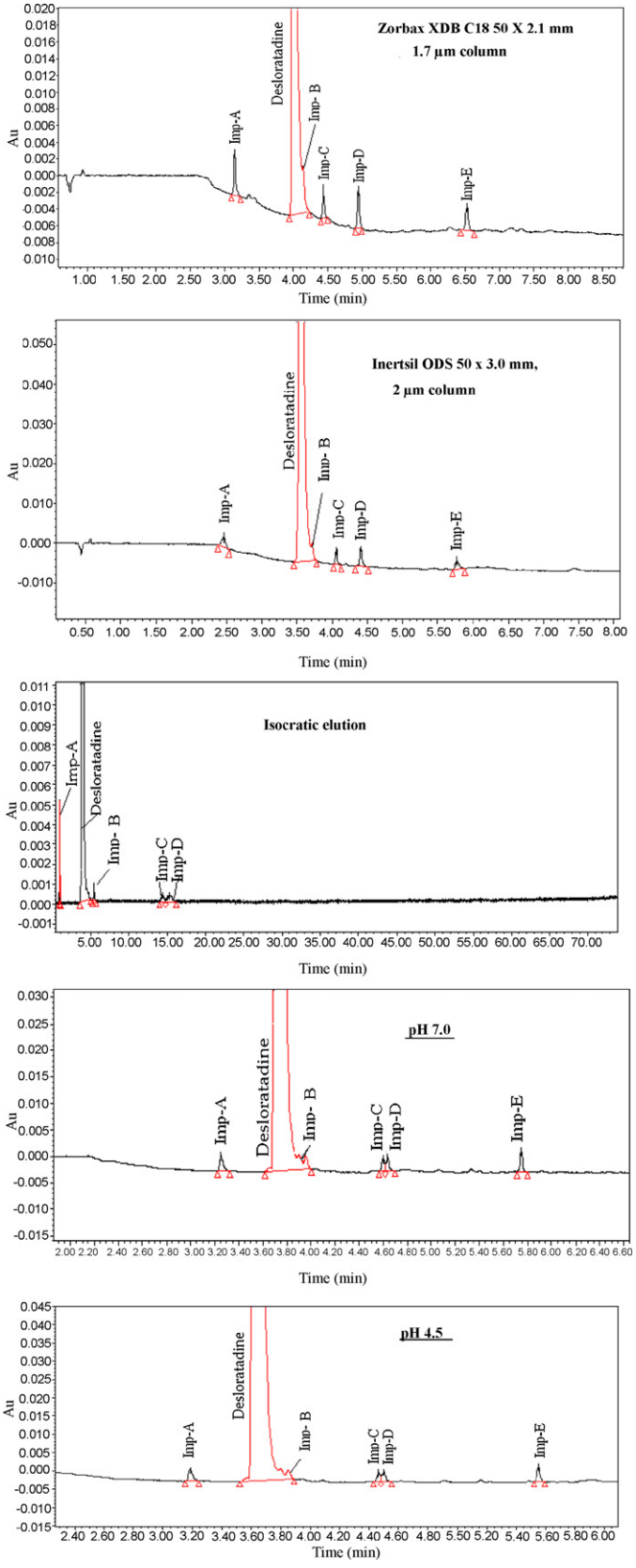

Fig. 2. Typical chromatograms of desloratadine spiked with its five impurities from method development trial.

3.2.2. Results of forced degradation studies

All forced degradation samples were analyzed at an initial concentration 500 μg/ml of desloratadine with UPLC conditions mentioned in Section 2.3 using PDA detector to ensure the homogeneity and purity of desloratadine peak. Significant degradation of desloratadine was observed in oxidative (3.0% H_2O_2 at 40 °C for 24 h) and thermal (60 °C for 10 days) conditions leading to the formation of imp-D (Fig. 3). This was confirmed by co-injecting imp-D standard to these degraded samples and by LC–MS/MS analysis. LC–MS/MS analysis was performed as per Section 2.4 and mass of the impurity was 311.4 which was corresponding to the mass of imp-D. Desloratadine was found to be stable under hydrolytic

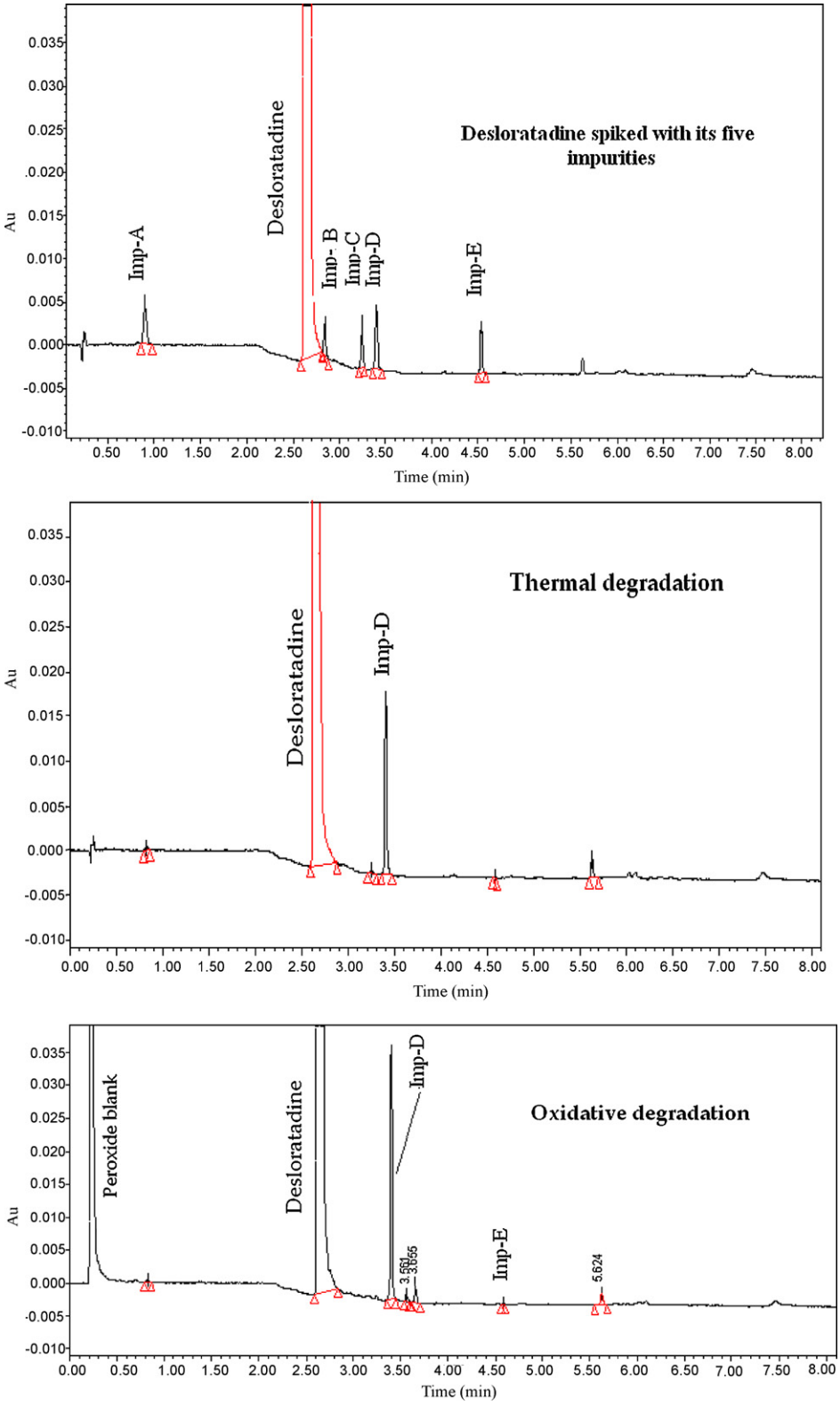

Fig. 3. Typical chromatograms of desloratadine spiked with its five impurities and its forced degradation samples.

Table 2
Regression and precision data.

Parameter	Desloratadine	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E
LOD (μg/ml)	0.055	0.075	0.070	0.055	0.050	0.065
LOQ (μg/ml)	0.160	0.205	0.210	0.175	0.165	0.180
Regression equation (y)						
Slope (b)	8571.8	68,935.0	52,477.0	53,683.6	59,725.0	46,943.2
Intercept (a)	−6185.4	38.9	148.8	331.0	425.5	184.8
Correlation coefficient	0.9999	0.9992	0.9994	0.9995	0.9994	0.9988
Precision (%RSD)	0.85	1.06	1.32	0.52	0.39	0.44
Intermediate precision (%RSD)[a]	0.71	0.79	1.81	0.89	0.55	1.05

Linearity range is LOQ-150% with respect to 0.5 mg/ml of desloratadine for impurities; linearity range is 50–150% with respect to 0.1 mg/ml of desloratadine for assay.
[a] Six determinations using LOQ solution for impurities and 0.1 mg/ml for assay of desloratadine.

Table 3
Evaluation of accuracy.

Amount spiked[a]	% Recovery[b]					
	Desloratadine	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E
LOQ	98.9 ± 0.89	98.1 ± 0.32	98.5 ± 0.22	98.9 ± 0.33	99.1 ± 0.35	99.3 ± 0.18
50%	100.5 ± 0.25	99.1 ± 0.28	98.1 ± 0.23	98.5 ± 0.11	100.5 ± 0.15	99.9 ± 0.37
100%	99.3 ± 0.31	97.5 ± 0.42	99.9 ± 0.33	100.1 ± 0.17	99.5 ± 0.23	98.6 ± 0.39
150%	100.1 ± 0.15	99.1 ± 0.28	102.1 ± 0.36	98.7 ± 0.45	98.9 ± 0.29	100.5 ± 0.19

[a] Amount of five impurities spiked with respect to 0.20% specification level individually to 0.5 mg/ml of desloratadine.
[b] Mean ± %RSD for three determinations.

(60 °C for 24 h), acid (0.5N HCl at 50 °C for 24 h), base (0.5N NaOH at 50 °C for 24 h) and photolytic (10 days) degradation conditions.

Assay studies were carried out for stress samples (at 100 μg/ml) against desloratadine qualified reference standard. The mass balance (% assay + % sum of all compounds + % sum of all degradants) results were calculated for all stressed samples and found to be more than 99%. The purity and assay of desloratadine was unaffected by the presence of its impurities and degradation products and thus confirms the stability-indicating power of the developed method.

3.2.3. Linearity

The linearity calibration plot for the assay method was obtained over the calibration ranges tested, i.e. 50–150 μg/ml and correlation coefficient obtained was greater than 0.999. The result shows that an excellent correlation existed between the peak area and concentration of the analyte.

Linear calibration plot for the related substance method was obtained over the calibration ranges tested, i.e. LOQ to 0.30% for impurities. The correlation coefficient obtained was greater than 0.998 (Table 2). The above result shows that an excellent correlation existed between the peak area and the concentration of imp-A, imp-B, imp-C, imp-D and imp-E.

3.2.4. Limits of detection and quantification

The determined limit of detection, limit of quantification and precision at LOQ values for desloratadine and its five impurities are reported in Table 2.

3.2.5. Accuracy

The percentage recovery of desloratadine from tablets was ranged from 98.9 to 100.5%. The percentage recovery of impurities in desloratadine samples varied from 97.5 to 102.1%. The LC chromatogram of spiked sample at 0.20% level of all five impurities in desloratadine tablets sample is shown in Fig. 3. The % recovery values for desloratadine and impurities are presented in Table 3.

3.2.6. Precision

The %RSD of assay of desloratadine during the assay method repeatability study was 0.85% and the %RSD for the area of imp-A, imp-B, imp-C, imp-D and imp-E in related substance method repeatability study was within 1.06%. The %RSD of the assay results obtained in the intermediate precision study was within 0.71% and the %RSD for the area of imp-A, imp-B, imp-C, imp-D and imp-E were well within 1.81%, conforming good precision of the method. The %RSD values are presented in Table 2.

3.2.7. Robustness

In all the deliberate varied chromatographic conditions (flow rate, column temperature and composition of organic solvent), all analytes were adequately resolved and elution orders remained unchanged. The resolution between critical pairs, i.e. for desloratadine and imp-B; and for imp-C and imp-D was greater than 3.0 and tailing factor for desloratadine and its impurities was less than 1.2. The assay variability of desloratadine was within ±1%. The variability in the estimation of desloratadine impurities was within ±10%.

4. Conclusions

The rapid gradient RP-UPLC method developed for quantitative analysis of desloratadine and related substances in pharmaceutical dosage forms is precise, accurate, linear, robust and specific. Satisfactory results were obtained from validation of the method. The method is stability indicating and can be used for routine analysis of production samples and to check the stability of samples of desloratadine.

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