Original Article

RP-HPLC Method Development And Validation For The Determination Of Inherent Impurities In Mizolastine Bulk And Dosage Forms

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Abstract

Objective: The purpose of the present study is to develop a simple, novel, accurate and robust reverse phase High Performance Liquid Chromatography (RP-HPLC) method for estimation of five inherent impurities in Mizolastine bulk and dosage forms.
Method: Chromatographic approach was optimised and achieved separation on Inertsil 250 x 4.6 mm, 5µm particle size Column by using 0.1% Phosphoric acid buffer as mobile phase-A and acetonitrile as Mobile phase B with gradient elution at flow rate of 1.0ml/min by using Ultraviolet (UV) detector at 220nm.Specificity, linearity, recovery, accuracy, robustness and ruggedness was determined as per Validation and the results were found to be within acceptable limits.
Results: The developed method was validated as per International Conference on Harmonization (ICH) guidelines. Individual impurities peaks were resolved clearly and Calibration curves plotted were linear with regression coefficient values as 0.99 for all the Mizolastine and its inherent impurities.
Conclusion: The validated method is suitable for quantification of five inherent impurities in Mizolastine bulk and dosage forms, Since there is no HPLC method reported in the literature fore estimation of Mizolastine and its five inherent impurities, the developed method can be used for routine analysis in pharma industries and there is a need to develop quantitative methods for determining more inherent impurities in Mizolastine bulk and dosage forms.

Keywords: Mizolastine, Impurity D, Inherent, Method development, Impurity A

Introduction

Antihistamines [1] are the medicines used to relieve symptoms of allergies, such as hay fever, hives, conjunctivitis and reactions to insect bites or stings. H1 antagonists called as H, blockers are class of medications that block the action of histamine at the H1 Receptor, helping to relieve allergic reactions. H1 antihistamine discovered was Iperoxan by Ernest Fourneau and Daniel Bovet (1933) efforts to develop a guinea pig animal model for anaphylaxis at the Pasteur institute. Second generation H1 antihistamines are novel drugs that are more selective for peripheral H1 receptors as opposed to the central nervous system. Mizolastine (Fig. 1) is a second-generation piperidine H1-antihistamine. H1-antihistamines interfere with the agonist action of histamine at the H1 receptor and are administered to reduce inflammatory process in order to treat conditions such as allergic rhinitis and allergic conjunctivitis.

IUPAC name of Mizolastine is 2-[[1-[1-[(4-fluorophenyl) methyl] benzimidazol-2-yl] piperidin-4-yl]-methyl amino]-1H-pyrimidin-6-one with molecular formula of $C_{24}H_{25}FN_6O$ and molecular weight of 432.49 gm/mol.

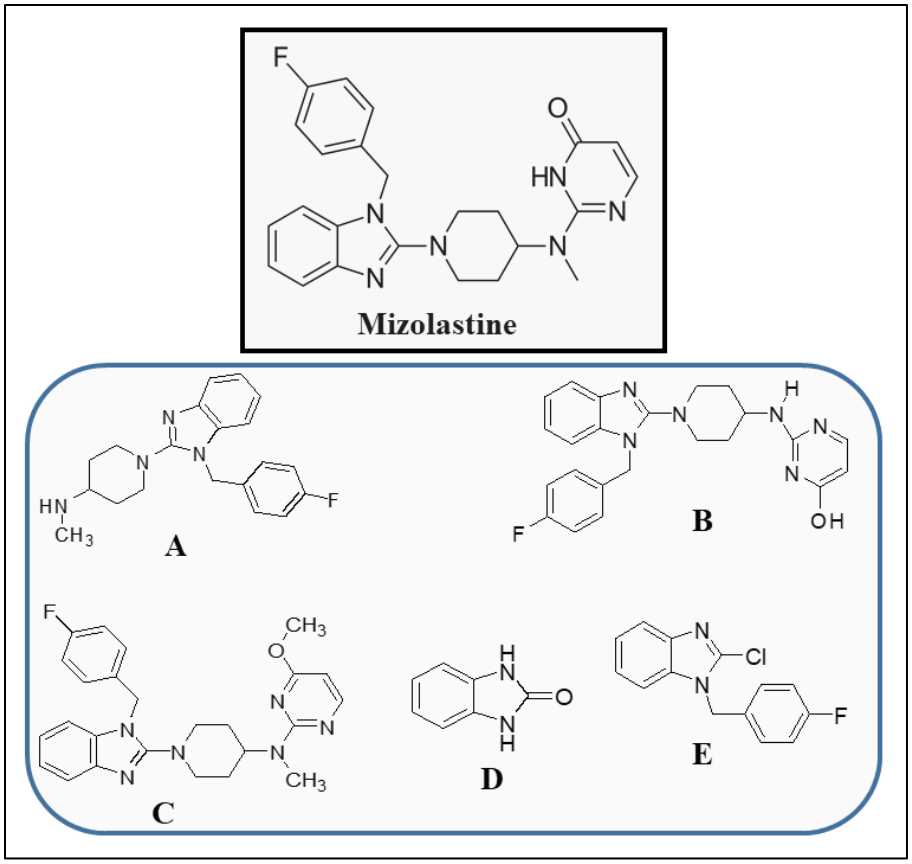

Fig. 1. Structure of Mizolastine and Impurities A, B, C, D, and E

A detailed literature survey shows that various analytical methods were developed for determination of Mizolastine and its impurities by UV-Visible, High performance liquid chromatography (HPLC) [2,3,6, 7, 8,9,15,16,17], Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) Method, Electrophoresis method[5], Liquid Chromatography with Electrospray Ionization Mass Spectrometry LC-ESI-MS Method [4] , Mizolastine determination in human plasma[11,12] and pharmacokinetics studies [10,13,14,18]were verified but there is no validated method available for determining five inherent impurities (Fig. 1) in Mizolastine bulk and dosage forms.

The present study describes a novel method of determination of five inherent impurities in Mizolastine which is accurate, simple, reproducible and cost saving method which can be adopted for routine analysis at quality control labs in pharma industries, which is in line with ICH-Q2 Guidelines.

Material and Methods

Chemicals and Reagents

Mizolastine Standards and Impurities were gifted by M/s Shodhana labs, Hyderabad and Mizolastine tablets (Elina tablets of Dr. Reddys Labs) were purchased from local market. Acetonitrile, Methanol(HPLC Grade) were purchased from M/s Merck Chemicals division and that of Potassium dihydrogen Ortho phosphate, Triethyl Amine, Ortho-Phosphoric acid and Sodium dihydrogen Ortho phosphate was procured from M/s Rankem avantor.

Instruments used

Electronics balance of Denver, Ultrasonicator of Labman, and Vacuum pump of Crompton and High performance liquid chromatography 2695 system with PDA detector with Empower 2 software was used for Method validation.

Preparation of Mobile Phase

Preparation of 0.1% Ortho Phosphoric acid buffer:

Transfered 1ml of Ortho Phosphoric acid solution to a 1000ml volumetric flask and then added 500ml of water, sonicated for mixing and finally made up the volume to 1000ml with Water and filtered through 0.45 µm membrane filter and degassed.

Mobile phase A

Used 0.1% Ortho Phosphoric acid buffer as mobile phase solution A.

Mobile phase B

Used Acetonitrile as Mobile phase B

Diluent

Water: Acetonitrile (50:50 %v/v)

Standard Preparation

Weighted [23]10mg of Mizolastine Standard to a 50ml volumetric flask, dissolved with dilute and made up to the volume with diluent. Further transferred 2.5ml of above stock to a 50ml volumetric flask and diluted to volume with diluent and further diluted above solution 5.0ml to a 50ml volumetric flask, diluted to volume with diluent.

Sample preparation

Crushed not less than 10 tablets into fine powder, weighed equivalent to 25mg of Mizolastine and transferred to a 50ml volumetric flask and then added about 35 mL of diluent, sonicated for 20 minutes with intermittent shaking and after attaining to room temperature diluted up to the volume with diluent and mixed well.

Placebo solution

Weighted and transferred placebo powder equivalent to 25 mg of Mizolastine to a 50 mL of volumetric flask and added about 35ml of diluent, sonicated for 20minutes with intermittent shanking for attaining to room temperature and then further diluted up to the volume with diluent and mix well.

Chromatographic conditions

Mobile phase **:** Gradient mobile phase
Flow rate **:** 1 ml/min
Column **:** Inertsil 250 mm x 4.6mm, 5µ.
Detector wave length **:** 220 nm
Column temperature **:** 30°C
Injection volume **:** 10µL
Run time **:** 25min
Diluent **:** Water: Acetonitrile 50:50
Gradient programme **:** Table 1

Table 1. Gradient programmed

Sr. No.	Time (mins)	Flow (mL)	%A	%B
1	---	1.0	70.0	30.0
2	6.0	1.0	70.0	30.0
3	10.0	1.0	50.0	50.0
4	14.0	1.0	20.0	80.0

5	16.5	1.0	50.0	50.0
6	18.0	1.0	70.0	30.0
7	25.0	1.0	70.0	30.0

Method development and optimization

In method development the solubility of the active pharmaceutical ingredient was checked in different solvents like water, methanol and acetonitrile. Finally the standard and samples are diluted by Acetonitrile and water in the ratio 50:50. During development different mobile phases like phosphate buffer, Orth phosphoric acid and Acetonitrile were used in various compositions with isocratic pump mode at a flow rate of 1.0 ml/min but impurities peaks were not separated.

The chromatographic analysis was tested by using different columns like Intersil 250 X 4.6mm, 5µm particle size columns maintained at different temperatures 25°C, 30°C and 35°C were used, but the impurities separation was not achieved. The actual chromatography analysis achieved on Inertsil 250 x 4.6 mm, 5µm particle size by using 0.1% Phosphoric acid buffer as mobile phase-A and acetonitrile as Mobile phase B with gradient elution as mentioned in the above gradient programming and optimized the method and here enclosed Optimized chromatogram (Fig. 2).

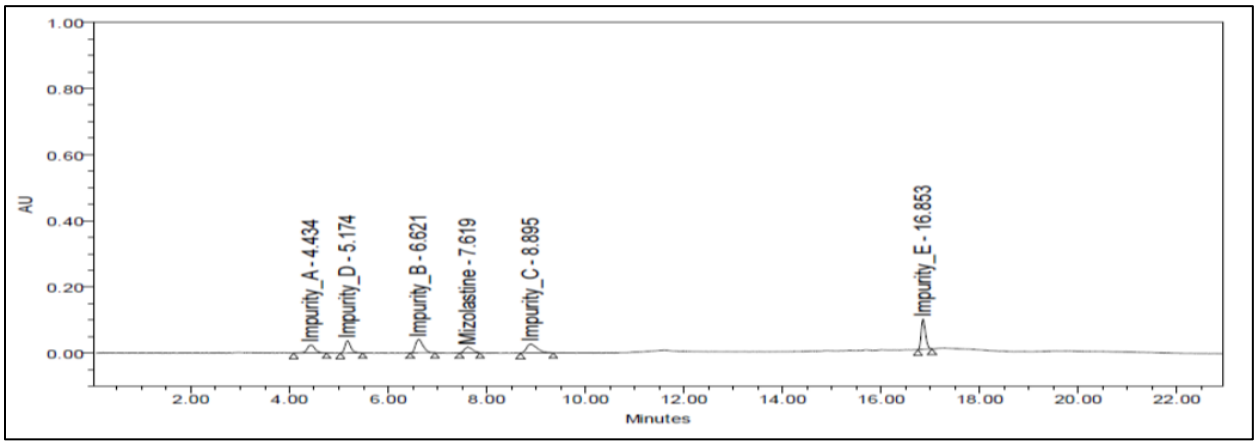

Fig. 2. Optimized chromatogram

Analytical Method Validation

The developed method was validated in accordance with ICH guidelines[19,20,21,22] considering System Suitability, Specificity, Forced degradation, Linearity, accuracy, precision and LOD/LOQ.

System suitability

System suitability is an integral part of many analytical procedures, System suitability was verified to check the performance of the system. Tailing factor, plate count and percentage relative standard deviation (%RSD) of six replicates found within the limits.

Specificity

Specificity is assessed by checking the blank, placebo, known and degradant impurities interference with the principle peak. All known impurity peaks must be separated with each other without interference of Mizolastine peak.

Accuracy or Recovery

Accuracy is the exactness of an analytical method or the closeness of agreement between the values. For quantitation of impurities, accuracy is determined by analysis samples spiked with known amount of impurities. The accuracy was

evaluated by measurement (n=3) applying the method to the sample spiked with known amounts of known impurities corresponding to 50 %, 100 % and 150 % of specification.

Precision

Precision is the measure of how close the data values to each other for a number of measurements under the same analytical conditions. The degree of agreement among individual test results is the precision of analytical process. System precision, Method precision and Solution stability were evaluated as a part of precision. Spiked sample solution was found stable up to 48 hours at room temperature with the difference in 10% individual known impurity from initial to time intervals.

Linearity

Linearity of an analytical method is its ability to produce test results that are directly proportional to the concentration of analyte in samples within a given range and was evaluated by plotting a graph and determining slope. The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares is calculated and correlation coefficient should be 0.99 for all the impurities.

Determination of LOD and LOQ:

Limit of Detection (LOD) is the point at which a measured value is larger than the uncertainty associated with it, the lowest concentration of the analyte in a sample that can be detected. Limit of qualification (LOQ) is the lowest level of analyte that can be accurately and precisely measured and this limit is required only for impurity methods.
LOD and LOQ can be determined by three methods

1. Signal to noise ratio.
2. Standard deviation of the response and slope
3. Visual evaluation

LOD and LOQ were determined by standard deviation of the response and slope from the linearity graph of Mizolastine and its impurities.

Robustness

Robustness is the capacity of a method to remain unaffected by small, deliberate variations in the method parameters and this can be evaluated by varying method parameters such as flow rate, pH of buffer/mobile phase, composition of mobile phase, temperature, wavelength etc. Deliberate variations in method should not change the results by 10 percent to that of the optimized method.

Forced degradation

Stress degradation should be no interference between the peaks obtained for a chromatogram of preparations. According to ICH guidelines, stress degradation studies were conducted. The peaks of degradation should be well apart from each other and Forced degradation experiments were conducted to obtain the degradation of about 20 percent by various types of stress conditions.

Results and Discussion

System suitability

Prepared the standard solution as per methodology and injected six times into the chromatographic system and obtained % RSD from six replicate injections was 0.6. The observed tailing factor for Mizolastine peak from the first injection of standard solution is 1.29 and that of Plate count system is 8598, suitability results are given in Table 2.

Table 2. System suitability and system precision results

Injection	Mizolastine RT	Peak area of Mizolastine

1	8.214	500441
2	8.216	507726
3	8.234	505769
4	8.262	503208
5	8.274	508308
6	8.341	503205
Mean	8.26	504776
SD	0.05	3029.3
%RSD	0.6	0.6

Specificity

Prepared blank, placebo, standard solution, sample solution, Impurities spiked sample solution and individual impurities solutions as per method and injected into HPLC system to evaluate the peak purity and interference of any peak with Mizolastine and known impurities. Blank and placebo peaks were well separated from known impurities and Mizolastine peak. All known peaks were separated with each other and Mizolastine peak. Specificity results are addressed in Table 5 and specificity chromatograms and peak purity plots are shown from (fig. 3,4 & 5). Hence the above method found to be specific.

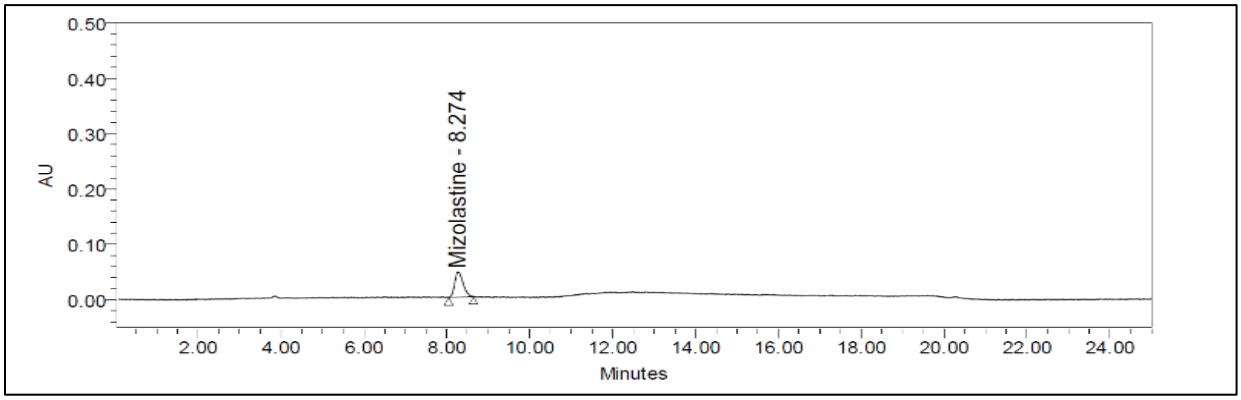

Fig. 3. Standard Chromatogram

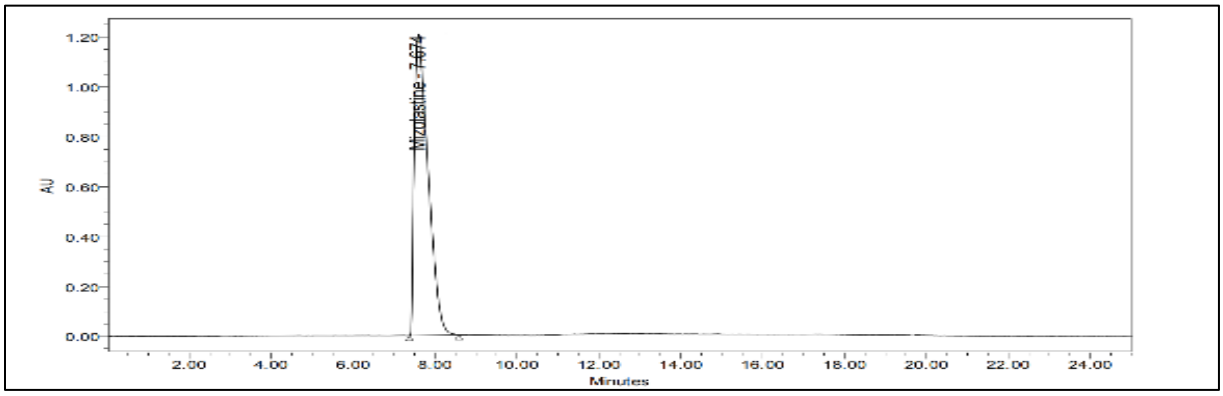

Fig. 4. Chromatogram of control sample

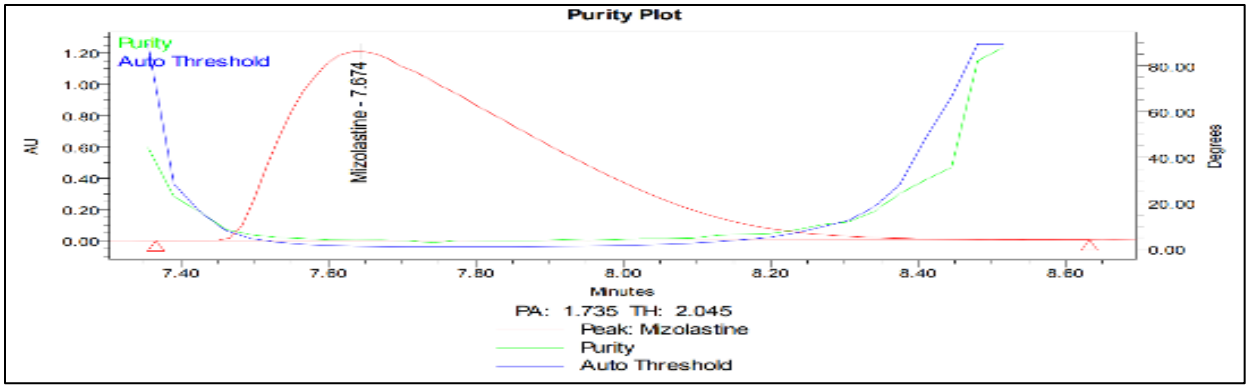

Fig. 5. Peak Purity of Control Sample

Accuracy

The test sample were prepared at each % level and tested against standard according to the description of the methodology. The total average recovery for Impurity A is 100.0% with 1.4 % RSD, Impurity B is 98.8 % with 1.5 % RSD, Impurity D is 98.6 % with 1.2 % RSD, Impurity C is 99.2 % with 0.8 % RSD, Impurity E is 100.4% with 1.8% RSD.The accuracy results are addressed in Table 3.

Table 3. Accuracy results (% Recovery)

Level	Imp. A	Imp. B	Imp. D	Imp. C	Imp. E
50 % Mean % Recovery	99.08	100.11	98.58	100.04	102.57
50 % % RSD	0.2	1.3	0.5	1.4	0.7
100 % Mean % Recovery	101.58	99.15	97.5	99.02	99.55
100 % % RSD	0.7	0.6	0.5	0.5	0.3
150 % Mean % Recovery	99.4	97.28	99.85	98.53	99.2
150 % % RSD	0.1	1.2	2.6	0.3	0.9
Overall Mean % Recovery	100.0	98.8	98.6	99.2	100.4
RSD of overall % Recovery	1.4	1.5	1.2	0.8	1.8

Precision

System precision

It is demonstrated by calculating %RSD for retention time and peak areas of Mizolastine peak from six replicate injections of standard solution preparation. The system precision results are enclosed in Table 2.

Method precision

Method precision was evaluated by injecting spiked known impurities on drug product at specification level. % RSD values for Retention time (RT)and peak area responses of individual impurities should not be more than 10%. %RSD of Impurity A of RT found 0.6 and that of Peak area is 0.8, Impurity D found 0.6 and 0.3, Impurity B found 0.9 and 0.4, Impurity C found 0.4 and 0.7 and that of Impurity E found 0.2 and 0.5. The data demonstrated that the values are met the acceptance criteria. Hence the method was found Precise and the results are addressed from Table 4 & 5.

Table 4. Method precision for Impurity A, Impurity D, and Impurity B

Injection	Imp. A RT	Peak Area of Imp. A	Imp. D RT	Peak Area of Imp. D	Imp. B RT	Peak area of Imp. B
1	4.190	294693	5.210	695435	6.960	553157

2	4.199	298459	5.227	696256	7.015	554526
3	4.214	294216	5.263	697153	7.019	551984
4	4.242	292263	5.265	691453	7.020	554748
5	4.244	291894	5.289	696157	7.115	552365
6	4.257	294286	5.298	695953	7.118	558425
Mean	4.224	294301.83	5.259	695385	7.041	554201
SD	0.03	2340.82	0.03	2008.1	0.06	2350.4
%RSD	0.64	0.80	0.65	0.30	0.89	0.40

Table 5. Method precision for Impurity C and Impurity E

Injection	Imp. C RT	Peak area of Imp. C	Imp. E RT	Peak area of Imp. E
1	9.230	391459	17.535	812539
2	9.232	392922	17.57	808965
3	9.238	391580	17.574	804785
4	9.244	394856	17.586	805369
5	9.298	392963	17.616	801251
6	9.314	398874	17.636	804158
Mean	9.259	393776	17.586	806178
SD	0.04	2783.4	0.04	3979.5
%RSD	0.40	0.7	0.20	0.5

Solution Stability

Spiked sample solution was found stable up to 48 hours at room temperature with the difference in 10% individual known impurity from initial to time intervals. Solution stability results are addressed in Table 6.

Table 6. Spiked Sample solution stability at Room temperature

	At Room temperature (25°C) %Difference					
Hours	**Imp. A**	**Imp. D**	**Imp. B**	**Imp. C**	**Imp. E**	**Mizolastine**
Initial	NA	NA	NA	NA	NA	NA
24 Hours	1.94	0.05	0.52	1.42	3.01	0.91
48 Hours	0.62	1.20	0.98	0.64	0.54	1.73

Mobile Phase Stability

The mobile phase was found stable for 4 days at bench top condition, no haziness of mobile phase was observed.

Linearity and RRF Calculation

A series of known impurity and Mizolastine from LOQ to 150% of specification level were injected into HPLC system as per method. Linearity was conducted by preparing the five levels of linearity solutions and Plot a graph of concentration(Conc.) versus Area response for impurity solutions and standard solutions. Relative response factors for all individual impurities established based on slope method and calculated the RRF values from the linearity data. Calculate the relative response factor for all the known impurities using following formula.

$$\textbf{Factor (RRF) of impurity} = \frac{\text{Slope of impurity solution}}{\text{Slope of standard solution}}$$

The obtained all known impurities and Mizolastine correlation coefficient were not less than 0.99. All the linearity data and RRF values were addressed in table 7 & 8 and the linearity graphs are shown in (Fig. 6).

Table 7. Linearity results of Mizolastine, Impurity A, and Impurity D

Linearity levels	**Mizolastine**		**IMP. A**		**IMP. D**	
	Conc. (ppm)	**Area response**	**Conc. (ppm)**	**Area response**	**Conc. (ppm)**	**Area response**
25%	0.25	133657	0.25	70320	0.25	172195
50 %	0.50	253232	0.50	143030	0.50	337151
75%	0.75	391293	0.75	215721	0.75	514216
100 %	1.00	491736	1.00	287333	1.00	688720
125%	1.25	616460	1.25	354023	1.25	860305
150 %	1.50	734758	1.50	428126	1.50	1009203
CC (r)	**0.99**		**0.99**		**0.99**	
SCC (r^2)	0.999		0.999		0.999	
Slope	479501		284986		677601	
Y-Intercept	17293		396.44		4064.5	
RRF	NA		0.59		1.4	

Table 8. Linearity results of Impurity B, Impurity C, and Impurity E

Linearity levels	**IMP. B**		**IMP. C**		**IMP. E**	
	Conc. (ppm)	**Area response**	**Conc. (ppm)**	**Area response**	**Conc. (ppm)**	**Area response**
25%	0.25	132733	0.25	96371	0.25	197099
50 %	0.50	268179	0.50	196670	0.50	377017
75%	0.75	396221	0.75	284349	0.75	592027
100 %	1.00	536142	1.00	383756	1.00	782582
125%	1.25	661533	1.25	484722	1.25	967874
150 %	1.50	797370	1.50	569733	1.50	1160509
CC (r)	**0.99**		**0.99**		**0.99**	
SCC (r^2)	0.999		0.999		0.999	
Slope	530647		380614		774877	
Y-Intercept	1046.8		2896.6		1500.2	
RRF	1.1		0.79		1.62	

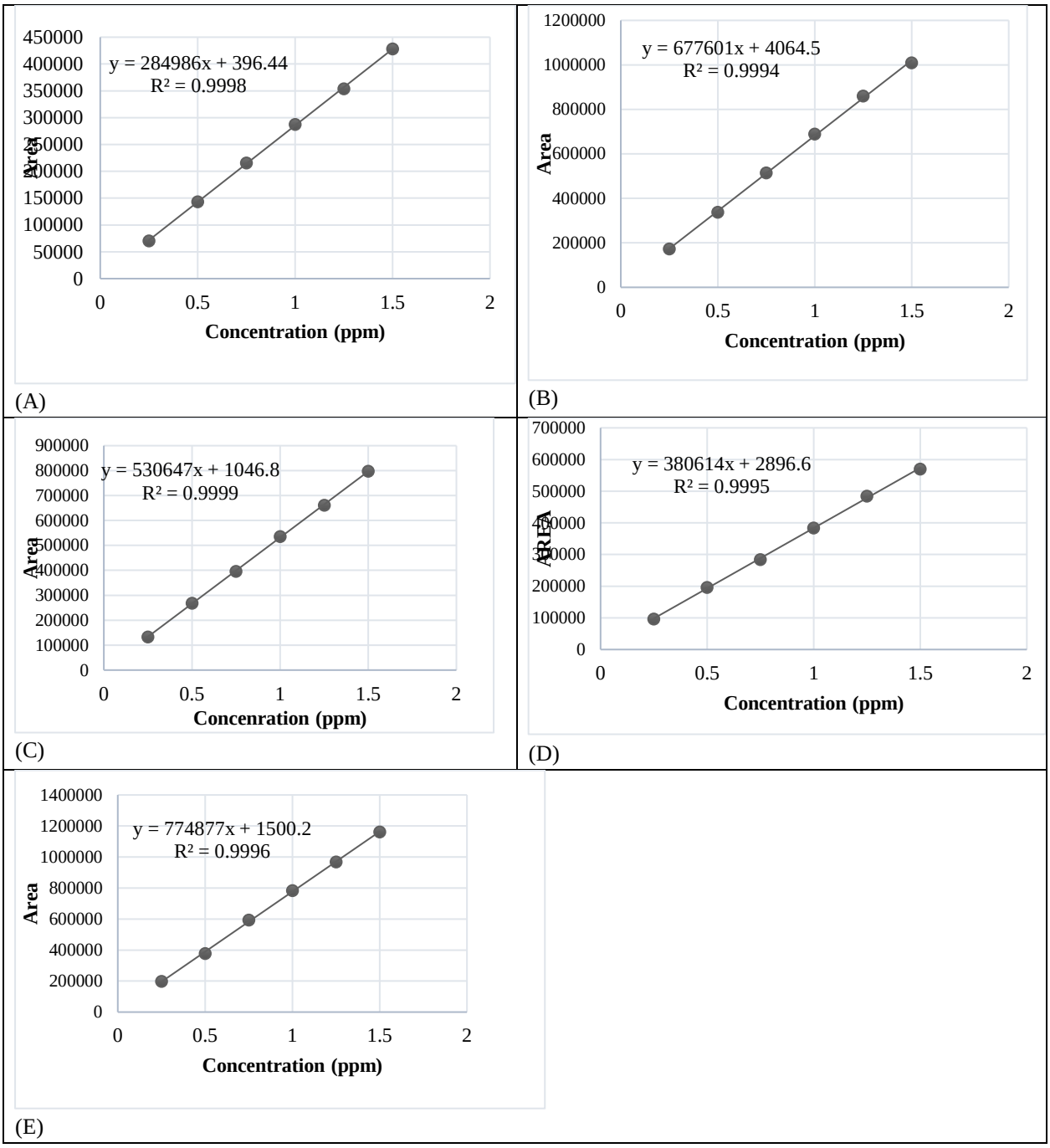

Fig. 6. Linearity graphs of impurities

Determination of LOD and LOQ

Limit of Detection (LOD) and Limit of Quantification (LOQ) values of all known impurities and Mizolastine were determined by using Standard deviation of the response and Slope. From the above linearity curve, standard deviation on response and slope of known impurities and main peak were calculated at different concentrations and the LOD & LOQ were calculated as below:

LOD= (10Xstandard deviation on Y-intercept)/slope

LOQ= (3XStandard deviation on Y-intercept)/slope

The LOD and LOQ values and concentrations are addressed in Table 9.

Table 9. LOD/LOQ values and Specificity results

Name of the Active/Impurity	LOD (Conc. in ppm)	LOQ (Conc. in ppm)	Retention Time	Peak Purity	
				Peak angle	Peak threshold
Mizolastine	0.245	0.742	8.274	1.735	2.045
Impurity A(IMP. A)	0.153	0.463	4.262	1.480	1.855
Impurity B(IMP. B)	0.015	0.045	6.491	1.530	1.631
Impurity C(IMP. C)	0.190	0.574	8.916	1.476	1.895
Impurity D(IMP. D)	0.038	0.115	5.162	2.521	3.010
Impurity E(IMP. E)	0.157	0.475	16.970	1.405	1.788
Control sample	--	--	7.674	0.152	0.366

Robustness

Robustness of the method was assessed by varying the instrumental conditions such as column temperature (± 5°C) , flow rate ((±0.1mL) and Organic variation of mobile phase(±5%). The deliberate changes in the method have no significant changes in retention time and no distorted chromatography was observed for Mizolastine and its known impurities. This indicates that the method is robust. Results for robustness studies are addressed in the Table 10.

Table 10. Robustness studies for spiked sample

RRT of Impurities in spiked sample						
parameter	**Variation**	**Imp. A**	**Imp. D**	**Imp. B**	**Imp. C**	**Imp. E**
Original conditions	None	0.55	0.67	0.89	1.18	2.25
Mobile phase variation	Organic 5% minus	0.50	0.57	0.84	1.13	1.74
	Organic 5% plus	0.61	0.76	0.89	1.11	2.69
Flow Rate mL/min	0.9mL/min	0.55	0.66	0.87	1.14	2.07
	1.1mL/min	0.55	0.67	0.87	1.14	2.36
Column oven temperature	25 ^{o}C	0.60	0.74	0.88	1.11	2.57
	35 ^{o}C	0.54	0.65	0.86	1.14	2.08

Forced degradation

Mizolastine tablets was forcefully stressed by exposure to 0.01N Hydrochloric acid (HCL), 0.01N Sodium Hydroxide (NAOH), 3.0% Hydrogen peroxide (H2O2), Hydrolysis, UV and thermal. Control and stressed samples were injected into the HPLC system and evaluated the Peak purity, interference of degradants and mass balance. Forced degradation chromatograms and peak purity plots are shown and the results for forced degradation studies were addressed in the Table 11 and N.D implies Not Detected.

Table 11. Forced Degradation Studies results

Sample Name	Imp. A	Imp. B	Imp. C	Imp. D	Imp. E	Purity angle	Purity threshold	Assay
Control Sample	N.D.	N.D.	N.D.	N.D.	N.D.	0.152	0.366	100.0
0.01N HCl for 24 hrs.	N.D.	N.D.	N.D.	N.D.	N.D.	2.251	5.183	99.52
0.01N NaOH for 24 hrs.	N.D.	N.D.	N.D.	N.D.	N.D.	0.327	0.879	99.80
3.0% H_2O_2 for 24 hrs.	N.D.	N.D.	N.D.	N.D.	N.D.	0.400	0.841	99.50
Water 40 ^{0}C for 24 hrs.	N.D.	N.D.	N.D.	N.D.	N.D.	0.166	0.404	99.85
UV for 24 hrs.	N.D.	N.D.	N.D.	N.D.	N.D.	0.400	0.850	99.86

Thermal 105 ^{0}C for 6 hrs.	N.D.	N.D.	N.D.	N.D.	N.D.	0.192	0.439	99.82

System suitability parameters were achieved and found within the acceptance limits. There was no interference of blank, placebo with that of Mizolastine and Known inherent impurities. Hence the above method is specific. Based on the impurities recovery results, it is concluded that there is no interference from excipients present in the formulation and the method is accurate when established between 50% to 150%. System precision, method precision were found to be within the specified limits and spiked sample solution was found stable up to 48 hours at room temperature. The Correlation Coefficient for all known impurities and Mizolastine found to be 0.99. Rrobustness study results found within the acceptance criteria, even deliberate changes were made in the chromatographic conditions. Hence developed method is Robust. In force degradation studies all generated impurities have not interfered with the Mizolastine peak, known impurities peaks and also with each other. The purity angle of Mizolastine and its known impurities is less than the purity threshold. This is the first reported RP-HPLC method for the determination of five inherent impurities in Mizolastine tablets. The present study provides new information and supporting data for assessment of related substances in Mizolastine drug substance and dosage forms.

Conclusion

Developed a simple RP-HPLC method for determining five inherent impurities in Mizolastine pharmaceutical bulk and dosage form which is specific, linear, accurate, simple, precise and robust method.This method is suitable for routine analysis in pharma industries for determination of five inherent impurities in Mizolastine drug substances and Pharmaceutical dosage forms.

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Nil

Authors Contribution

This work is carried out in coordination with all the authors.

Conflict of Interest

The authors declare no conflict of interest.

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