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Development and Validation of Stability Indicating RP-HPLC Method for Montelukast Sodium and Desloratadine in Pharmaceutical Dosage Form

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ABSTRACT:

A simple, rapid, precise and accurate stability-indicating RP-HPLC method was developed and validated for the simultaneous determination of Montelukast Sodium and Desloratadinein pharmaceutical dosage form. Method was carried out by using Sheisedo C18 (250 * 4.6 mm, 5µm) column and Acetonitrile : Methanol : water (35:40:25 % v/v/v) as mobile phase at 1.0 ml/min flow rate. Detection was carried out at 256 nm. Rt was found to be 2.224 min for Montelukast Sodium and 3.004min for Desloratadine. For stability study drugs were subjected to acid hydrolysis, alkaline hydrolysis, oxidative degradation and thermal degradation. Montelukast Sodium was highly susceptible to acidic and thermal condition. Pharmaceutical dosage form was more stable than Active pharmaceutical ingredient.The linearity of the proposed method was investigated in the range of 100-600µg/ml (r2= 0.998) for Montelukast Sodium and 50-300 µg/ml(r2= 0.991) for Desloratadine. The limit of detection were 15.62µg/ml and 9.78 µg/ml and the limit of quantification were 46.88 µg/ml and 29.66µg/ml for Montelukast Sodium and Desloratadine respectively.

KEY WORDS: Montelukast Sodium, Desloratadine, Stability indicating RP-HPLC Method, Validation

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1. INTRODUCTION

Montelukast Sodium (1-(1R)-1-[3-[(1E)-2-(7-chloro-2- quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl] -propyl] thio] methyl] cyclopropaneacetic acid. It is Cysteinyl leukotriene receptor antagonist and used for Treatment of Asthma [3]. It is official in Indian pharmacopeia 2010. It is freely soluble in ethanol,methanol and water. Molecular weight of Montelukast Sodium is 608.17 gm/mol and formula is $C_{35}H_{35}ClNO_3S.Na$.

Desloratadine is chemically 8-chloro-6,11dihydro-11-(4-piperdinylidene)-5H benzo [5,6] cyclohepta [1,2-b] pyridine. It is the second generation Non Sedating, Long Acting Anti Histaminic Drug which is dual inhibitor of histaminic receptor and PAF[1]. It Act by inhibition of the degranulation of mast cells induced by immunological and nonimmunological stimuli, and inhibition of the release of cytokines, particularly of the TNF in human mast cells and monocytes by antagonist the activity of PAF and H1 receptor.[2] It is not official in any Pharmacopeia. It is slightly soluble in water, very soluble in ethanol and propylene glycol. Molecular weight of Desloratadine is 310.82 gm/mol and formula is $C_{19}H_{19}ClN_2$.

Literature Review revels that there was no reported Stability Indicating RP-HPLC method for Simultaneous Estimation of Montelukast Sodium and Desloratadine in combined dosage form [7-12].So the present work is aimed for To develop an accurate, specific, repeatable Stability Indicating RP-HPLC method for Simultaneous estimation of Montelukast Sodium and Desloratadine in bulk and Pharmaceutical Dosage form.

2. MATERIALS AND METHODS

2.1. Method development:

2.1.1 Materials:

Montelukast Sodium was obtained as gift sample fromSkant Healthcare Ltd. and Maceods Pharmaceutical.Desloratadine was obtained as gift sample from Sun pharmaceutical Industries Ltd. Methanol, Acetonitrile, Water and Ortho phosphoric acid – HPLC grade were purchased from Ran Kem Lab.

2.1.2 Instrumentation and Chromatographic method:

The analysis of the drug was carried out on a Peak HPLC system equipped with a reverse phase ShisedoC_{18} column, peak pump with auto samplerand a detector running on Peak LC Solution Software. The mobile phase consists of Acetonitrile:Methanol: Water (35:40: 25% v/v/v) and the flow rate were maintained at 1.0 ml/min. The mobile phase was freshly prepared and passed through nylon membrane filter of pore size of 0.45µm and it was degassed by sonicating for 10min. before it was used. The elution was monitored at wavelength of 256 nm with UV detector, and the injection volume was 10µl.

2.1.3 Determination of maximum absorbance:

The standard solutions of Montelukast Sodium and Desloratadine were scanned in the range of 200-400 nm against mobile phase as blank.Isobestic point of Montelukast Sodium and Desloratadine at256nm. Thus the wavelength selected for the determination of Montelukast Sodium and Desloratadine was 256nm.

2.1.4 Preparation of stock and standard solutions:

Accurately weighed 100mg of Montelukast Sodium and 50mg of Desloratadine were dissolved in 100 ml volumetric flask containing 100 ml of Methanol which is considered as stock solution. Working standard solution of Montelukast Sodium and Desloratdine were prepared by making various dilutions of the drug solution from the stock solution. Six sets of the drug solution were prepared in the mobile phase containing 100-600µg/ml of Montelukast Sodium and 50-300 µg/ml of Desloratadine. Each of this drug solution was injected into the column and the peak area and retention time was recorded.

2.1.5 Assay of marketed formulation (Brand name of tablet – Ventidox - DL):

Twenty tabletwere weighed and average weight of a single tablet was calculated. Tablets were crushed and mixed using a mortar and pestle. Then drug sample equivalent to 100mg of Montelukast Sodium and 50mg of Desloratadine were accurately weighed and transferred into a 100ml volumetric flask and mixed with known amount of methanol and the active pharmaceutical ingredients were extracted into the methanol followed by ultra-sonication and then filtered through a nylon membrane of pore size 0.45µm. The drug sample was diluted by adding methanol to obtain a stock solution of 100µg/ml of Montelukast Sodium and 50 µg/ml of Desloratadine.

2.2 Method validation

The Proposed method was validated according to ICH guidelines. The parameters assessed were linearity, precision, accuracy, LOD and LOQ.

2.2.1 System Suitability

System suitability tests are an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. System Suitability was performed on standard solution and system suitability parameters were calculated at the start of study for each parameter.

2.2.2 Linearity and Range.

The linearity was determined at Three levels over the range of 100 - 600 µg/ml Montelukast Sodium and 50 - 100 µg/ml Desloratadine. Peak area of above linearity solution preparations were taken at each concentration three times.

2.2.3 Accuracy

Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are Calculated.

2.2.4 Precision

A. Repeatability Study:

Standard solutions of 200, 300, 400 µg/ml Montelukast Sodium and 100, 150, 200 µg/ml Desloratadine were prepared and chromatograms were recorded. Area was measured of the same concentration solution three times and %RSD was calculated.

B. Intra-day precision

Mixed solutions containing 200, 300, 400 µg/ml Montelukast Sodium and 100, 150, 200 µg/ml Desloratadine were analyzed three times on the same day ,% R.S.D was calculated.

C. Inter-day precision

Mixed solutions containing 200, 300, 400 µg/ml Eplerenone and 80, 120, 160 µg/ml Torsemide were analyzed on three different days and % R.S.D was calculated.

2.2.5 Limit of Detection and Limits of Quantitation

Limit of Detection (LOD)

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. The limit of detection (LOD) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline:

$$LOD = 3.3\times \text{Intercept} / \text{Slope}$$

Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline:

$$LOQ = 10 \times \text{Intercept} / \text{Slope}$$

2.2.6 Robustness

The robustness of the method was established by making deliberate minor variations in the following method parameters

a) pH of mobile phase: ± 0.2
b) Flow rate : ± 0.2 ml/min
c) Change in the ratio of component in the mobile phase: ± 2%.

2.3 Stability studies

Stability Studies was carried out on the drug in order to check the stability of the drug by providing various stress conditions like acid, base, oxidation and thermal degradation compared with normal conditions. The purpose of force degradation method is to provide evidence that the analytical method is efficient in determination of drug substances in commercial drug product in the presence of its degradation products.

2.3.1 Acidic hydrolysis

Take 2 ml solution of Montelukast 1000 µg/ml and Desloratadine 500 µg/ml, 2 ml of 0.1M HCl was added. The solution was heated for 1 hr at 60^{o}C and transferred to a 10ml volumetric flask, cooled, neutralized by 0.1M NaOH and diluted up to mark with methanol to get final concentration 100 µg/ml of MontelukastSodiuum and 50 µg/ml of Desloratadine.

2.3.2 Alkaline hydrolysis

Take 2 ml solution of Montelukast 1000 µg/ml and Desloratadine 500 µg/ml, 2 ml of 0.1M NaOH was added. The solution was heated for 1 hr at 60^{o}C and transferred to a 10ml volumetric flask, cooled, neutralized by 0.1M HCl and diluted up to mark with methanol to get final concentration 100 µg/ml of MontelukastSodiuum and 50 µg/ml of Desloratadine.

2.3.3 Oxidative degradation

Take 2 ml solution of Montelukast 1000 µg/ml and Desloratadine 500 µg/ml, 2 ml 6% H_2O_2 was added at room temperature for 4 hours at 60^{o}C and transferred to a 10ml volumetric flask, cooled diluted up to mark with methanol to get final concentration 100 µg/ml of MontelukastSodiuum and 50 µg/ml of Desloratadine.

2.3.4 Thermal degradation

Take 2 ml solution of Montelukast 1000 µg/ml and Desloratadine 500 µg/ml, heat the solution for 2 hr at 80°C and transferred to a 10ml volumetric flask, cooled diluted up to mark with methanol to get final concentration 100 µg/ml of MontelukastSodiuum and 50 µg/ml of Desloratadine.

3. RESULTS AND DISCUSSION

3.1 Linearity:

The calibration curve showed (Fig.7) good linearity in the range of 100-600µg/ml for Montelukast Sodium and 50-100µg/ml for Desloratadine with correlation coefficient (r^2) of 0.998 and 0.991 for Montelukast Sodium and Desloratadine respectively. Results are given in Table 6.

3.2 Recovery:

Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples.And % recovery values found to be in the range of 99.98% - 100.4% for Montelukast Sodium and 99.96 – 100.9% for. The result are given in table 7.

3.3 Precision:

Intraday precision was carried out using test samples prepared and analyzed on the same day. Interday precision was assessed by analysis of the same solutions on consecutive days. The % RSD value were below 2 indicate that the method is precise. The results are given in table 8, 9 and 10.

3.4 Robustness:

Small deliberate changes in chromatographic conditions such as change in mobile phase ratio (± 2 %), change in pH (±2 units) and flow rate (± 2 units) were studied to determine the robustness of the method. The results were in favor of (% RSD< 2%) the developed RP-HPLC method for the analysis of Montelukast Sodium and Desloratadine. The results are given in table 12 and 13**.**

3.5 Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD was found to be 15.62µg/ml and the LOQ 46.88 µg/ml for Montelukast Sodium and the LOD was found to be 9.78µg/ml and the LOQ 29.66 µg/ml for Desloratadine estimated by using the standard formulas.

3.6 Stability studies:

Stability indicating RP – HPLC method was performed in different stress condition using the Acetonitrile : Methanol : water (35:40:25 % v/v/v) as mobile phase suggested the following degradation behavior.

The chromatograms obtained on stress degradation, like photolytic degradation and similarly other conditions were shown in figure 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 and 19.

3.7 DISCUSSION

RP-HPLC method was found to be linear over the range of 100 - 600 µg/ml for Montelukast Sodium and 50 - 300 µg/ml for Desloratadine. The method has been validated by studying accuracy and precision, LOD, LOQ and system suitability according to ICH guideline.

The Stability study of Montelukast Sodium and Desloratadine indicates that the drug significantly degrade under acidic and thermal conditions.

4. CONCLUSION:

A simple, economic, accurate and robust RP-HPLC method has been developed and validated for the estimation of Montelukast Sodium and Desloratadine in bulk and pharmaceutical dosage form. The reverse phase liquid chromatography was performed using Sheisedo-C_{18} (250mm x 4.6mm, 5 µm) column. The mobile phase used was Acetonitrile: Methanol: Water (pH-3.2) (35:40:25 %v/v/v) with flow rate 1 ml/min. The detection was carried out at 256nm. The retention time were found be 2.224 ± 0.01 min and 3.004 ± 0.01 min for Montelukast Sodium and Desloratadine respectively. There was no interference from any excipients in the determination of drugs in dosage form which indicates the method is specific. All method validation parameters lie within its acceptance criteria as per ICH Q2 (R1) guideline so we can conclude that method is simple, linear, accurate and precise. Hence, it can be successfully used for the routine analysis of Montelukast Sodium and Desloratadine in pharmaceutical dosage forms.

Stability indicating RP – HPLC method has been developed for Montelukast Sodium and Desloratadine in pharmaceutical dosage form. Montelukast Sodium is easily degrade in acidic and thermal condition while Desloratadine was slightly degrade. Desloratadine is more stable than Montelukast Sodium in various stress

condition. From the result, We can conclude that Pharmaceutical dosage form was more stable than Active pharmaceutical ingredient.

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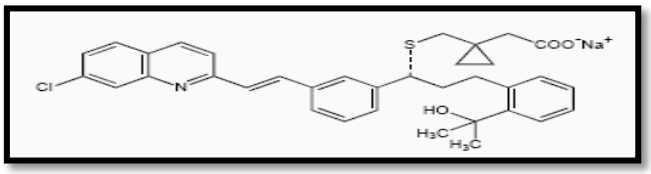


Figure: 1 Structure of Montelukast Sodium

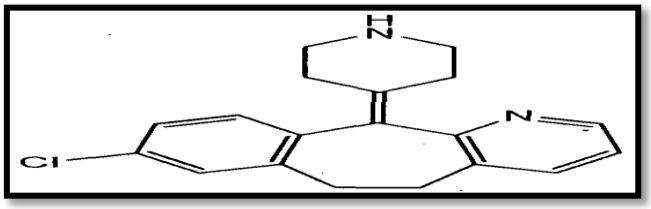


Figure: 2 Structure of Desloratadine

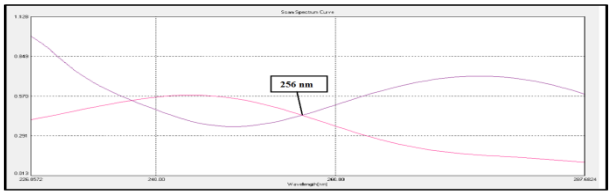


Figure 3 Selection of detection wavelength for HPLC

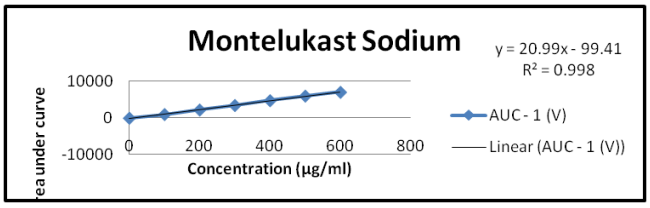
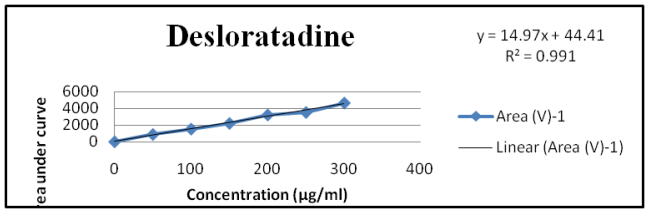


Figure 4 Calibration curve of Montelukast Sodium



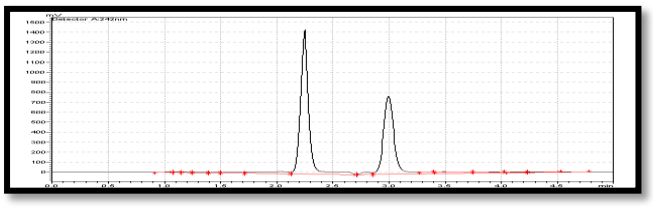


Figure 6 Chromatogram of Tablet Formulation of Montelukast sodium and Desloratadine

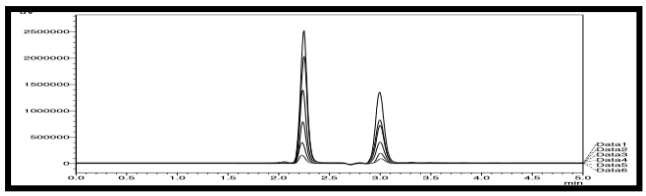


Figure 7 Linearity data of Montelukast sodium and Desloratadine

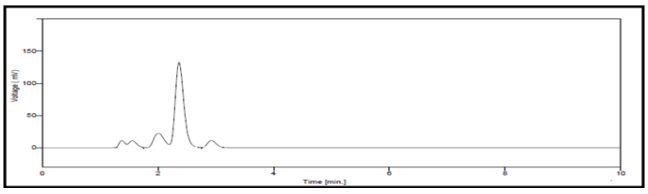


Figure 8 Acid hydrolysis peak of Montelukast Sodium

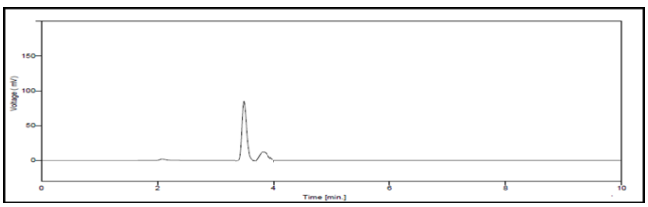


Figure 9 Acid hydrolysis peak of Desloratadine

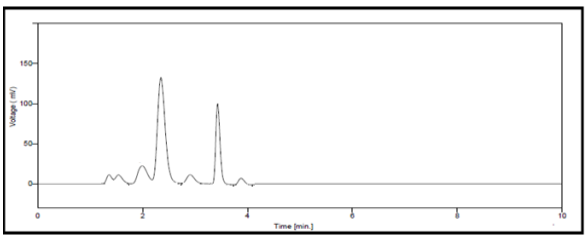


Figure 10 Acid hydrolysis peak of Pharmaceutical dosage form

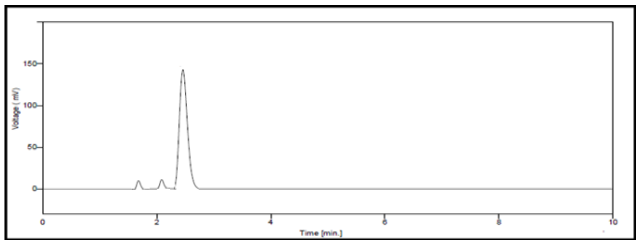


Figure 11 Alkali hydrolysis peak of Montelukast Sodium

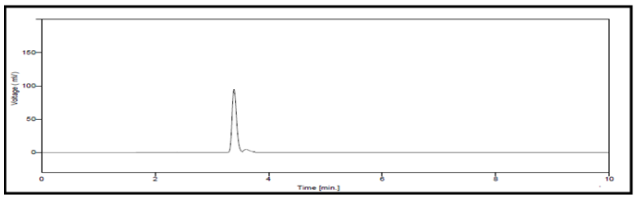


Figure 12 Alkali hydrolysis peak of Desloratadine

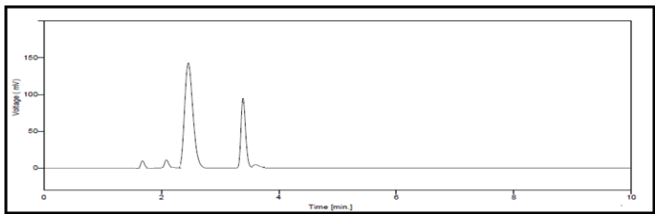


Figure 13 Alkali hydrolysis peak of Pharmaceutical dosage form

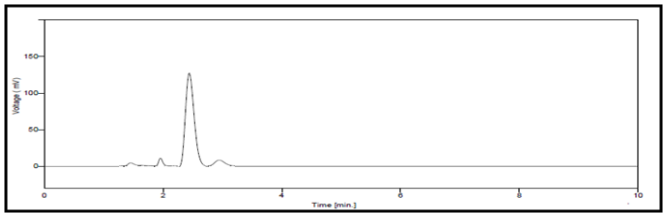


Figure 14 Oxidative degradation peak of Montelukast Sodium

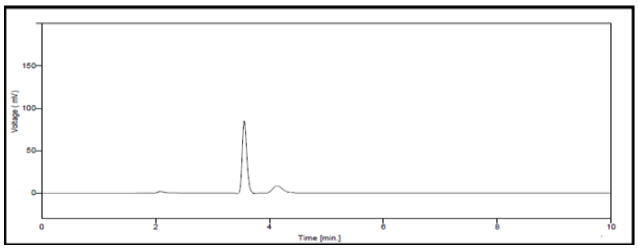


Figure 15 Oxidative degradation peak of Desloratadine

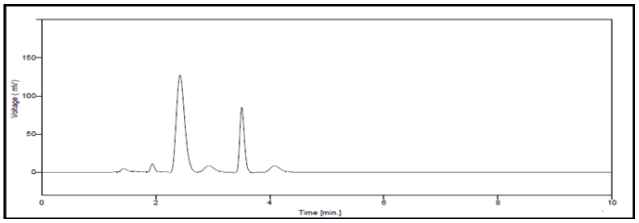


Figure 16 Oxidative degradation peak of Pharmaceutical dosage form

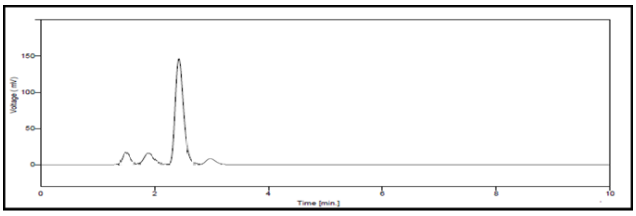


Figure 17 Thermal degradation peak of Montelukast Sodium

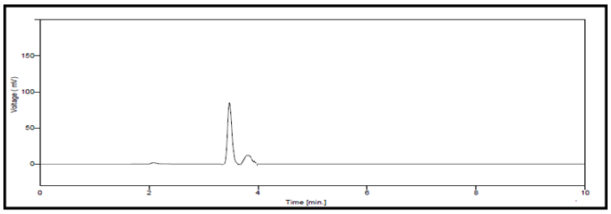


Figure 18 Thermal degradation peak of Desloratadine

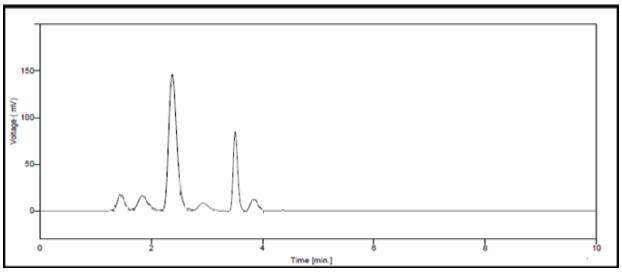


Figure 19 Thermal degradation peak of Pharmaceutical dosage form

Parameters	Specifications
Column	Sheisedo C_{18} (250mm*4.6mm, 5µm)
Mobile phase	Acetonitrile : Methanol :Water (35 : 40 : 25 % V/V/V) pH 3.2
Flow rate	1 ml/min
Run time	10 min
Detection wavelength	256 nm
Retention time	2.224 min for Montalukast sodium and 3.004 min for Desloratadine

Table 1 Finalization of Chromatographic condition

Montelukast sodium		Desloratadine	
Conc. (µg/ml)	**Mean. Area±S.D**	**Conc. (µg/ml)**	**Mean.Area±S.D**
0	0	0	0
100	1010.834 ± 2.2887	50	882.512 ± 2.5231
200	2236.295 ± 3.1223	100	1526.799 ± 1.8780
300	3455.928 ± 2.8980	150	2214.946 ± 2.7941
400	4756.749 ± 2.1787	200	3225.325 ± 3.2051
500	5999.576 ± 2.3203	250	3525.921 ± 3.0464
600	7025.394 ± 3.5253	300	4365.496 ± 3.2918

Table 2 Calibration curve for Montelukast Sodium and Desloratadine

Parameters	Result	
	Montelukast Sodium	**Desloratadine**
Linearity Range (µg/ml)	100 – 600 µg/ml	50 - 300 µg/ml
Slope	20.99	14.97
Intercept	99.41	44.41
Retention Time (min)	2.224	3.004
Correlation Coefficient	0.998	0.991

Table 3 Statistical Data of Montelukast sodium and Desloratadine

Drug	Actual conc. of Drug (µg/ml)	Conc. of Drug Found (µg/ml)	% of Drug found	Avg. of % Drug found	SD	%RSD
Montelukast sodium	100	99.89	99.89	100.49	0.67	0.67
	100	101.23	101.23			
	100	100.36	100.36			
Desloratadine	50	50.79	100.58	99.99	0.52	0.52
	50	49.92	99.84			
	50	49.98	99.56			

Table 4 Assay Result of Marketed Formulation

Sr. No.	System suitability parameter	Montelukast Sodium	Desloratadine	Specification as per IP and USP 34 NF 29
1	Retention time (min)	2.224	3.004	-
2	Resolution (R)	2.268		More than 1.5
3	Theoretical plate number (N)	4301.832	4769.076	Not less than 2000
4	Tailing factor (T)	1.272	1.114	Not greater than 2

Table 5 System suitablility parameters for Montelukast Sodium and Desloradadine

Montelukast Sodium			Desloratadine		
Conc. (µg/ml)	Area Mean ± S.D.	%RSD	Conc. (µg/ml)	Area Mean ± S.D.	%RSD
100	1010.791 ± 1.0850	0.1073	50	882.204 ± 2.0047	0.2272
200	2236.550 ± 1.9020	0.0850	100	1526.821 ± 1.1439	0.0749
300	3456.156 ± 2.3875	0.0690	150	2214.695 ± 2.0630	0.0931
400	4756.413 ± 1.2700	0.0267	200	3225.412 ± 2.7995	0.0925
500	5999.922 ± 2.2391	0.0373	250	3926.503 ± 1.3433	0.0395
600	7025.210 ± 2.1265	0.0302	300	4965.318 ± 2.2332	0.0511

Table 6 Linearity for Montelukast Sodium and Desloratadine

% Recovery	Target Conc., (µg/ml)	Spiked conc., (µg/ml)	Final Conc., (µg/ml)	Conc., Obtained		% Assay	
				MTKT	DES	MTKT	DES
80%	100 + 50	80 + 40	180 + 90	180.22	89.96	100.12	99.96
	100 + 50	80 + 40	180 + 90	179.98	90.55	99.98	100.6
	100 + 50	80 + 40	180 + 90	180.15	90.25	100.08	100.3
100%	100 + 50	100 + 50	200 + 100	200.05	100.5	100.03	100.5
	100 + 50	100 + 50	200 + 100	200.74	100.49	100.37	100.5
	100 + 50	100 + 50	200 + 100	200.25	100.24	100.13	100.2
120%	100 + 50	120 + 60	220 + 110	220.55	110.65	100.25	100.6
	100 + 50	120 + 60	220 + 110	219.99	110.98	99.99	100.9
	100 + 50	120 + 60	220 + 110	220.87	109.99	100.4	99.99

Table 7 Accuracy for Montelukast Sodium and Desloratadine

Montelukast Sodium			Desloratadine		
Conc. (µg/ml)	Area Mean ± S.D. (n=3)	%RSD	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	%RSD
200	2236.216 ± 4.3483	0.1944	100	1527.895 ± 4.1865	0.2740
300	3457.143 ± 4.8858	0.1413	150	2214.816 ± 3.8489	0.1737
400	4756.612 ± 3.2030	0.0673	200	3225.237 ± 4.1726	0.1293

Table 8 Repeatability for Montelukast Sodium and Desloratadine

Montelukast Sodium			Desloratadine		
Conc. (µg/ml)	Area Mean ± S.D. (n=3)	%RSD	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	%RSD
200	2236.478 ± 6.8021	0.3041	100	1528.099 ± 7.1607	0.4686
300	3457.066 ± 5.1505	0.1489	150	2216.036 ± 6.8358	0.3084
400	4754.590 ± 7.4869	0.1574	200	3226.550 ± 6.0361	0.1994

Table 9 Intra-day precision for Montelukast Sodium and Desloratadine

Montelukast Sodium			Desloratadine		
Conc. (µg/ml)	Area Mean ± S.D. (n=3)	%RSD	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	%RSD
200	2239.139±10.1903	0.4551	100	1529.859±10.2557	0.6703
300	3458.206 ± 9.0670	0.2621	150	2217.980±10.3456	0.4664
400	4757.803 ± 9.8184	0.2063	200	3229.064 ± 9.9407	0.3281

Table 10 Inter-day precision for Montelukast Sodium and Desloratadine

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Montelukast Sodium	15.62	46.88
Desloratadine	9.78	29.66

Table 11 LOD and LOQ for Montelukast Sodium and Desloratadine

Sr. no.	Montelukast Sodium (300 µg/ml)					
	pH		Flow rate		Mobile phase	
	+ 0.2 units	-0.2 units	+0.2 units	-0.2 units	+2.0 %	-2.0 %
1	3456.254	3461.954	34410256	3465.259	3455.236	3458.256
2	3459.124	3476.125	3459.265	3475.214	3457.254	3463.254
3	3449.321	3469.125	3456.259	3487.125	3461.253	3465.235
Mean	3454.899	3469.068	3452.260	3475.866	3457.914	3462.248

S.D	5.0398	7.0856	9.6475	10.9475	3.0623	3.5965
% RSD	0.1458	0.2042	0.2794	0.3149	0.0885	0.1038

Table 12 Robustness for Montelukast Sodium

Sr. no.	**Desloratadine (150 µg/ml)**					
	Ph		**Flow rate**		**Mobile phase**	
	+ 0.2 units	**-0.2 units**	**+0.2 units**	**-0.2 units**	**+ 2.0 %**	**- 2.0 %**
1	2214.125	2231.256	2252.213	2275.362	2215.265	2222.256
2	2218.325	2235.125	2258.245	2278.965	2216.958	2225.685
3	2225.362	2246.995	2271.236	2295.125	2210.362	2219.254
Mean	2219.270	2237.792	2260.564	2283.150	2214.195	2222.398
S.D	5.6778	8.2014	9.7213	10.5253	3.4257	3.2178
% R.S.D	0.2558	0.3664	0.4300	0.4610	0.1547	0.1447

Table 13 Robustness for Desloratadine

Sr No.	**Parameter**	**Montelukast Sodium (MTKT)**	**Desloratadine (DES)**
1	**Linearity range**	100 – 600 µg/ml	50 – 300 µg/ml
2	**Equation (y = mx + c)**	Y=20.99x – 99.41	Y=14.97x +44.41
3	**Correlation coefficient**	0.998	0.991
4	**LOD (µg/ml)**	15.62 µg/ml	9.78 µg/ml
5	**LOQ (µg/ml)**	46.88 µg/ml	29.66 µg/ml
6	**Repetability (%RSD)**	0.0673 – 0.1944	0.1293 – 0.2740
7	**Intraday precision (%RSD)**	0.1489 – 0.3041	0.1994 – 0.4686
8	**Interday precision (%RSD)**	0.2063 – 0.4551	0.3281 – 0.6703
9	**Robustness (%RSD)**	0.0885 – 0.3149	0.1447 – 0.4610
10	**% Recovery**	99.98% - 100.4%	99.96% 100.9%
11	**Assay**	100.49%	99.99%

Table 14 Summary of RP – HPLC Method

Stress Condition	**% Degradation of API**		**% Degradation of pharmaceutical dosage form**	
	MTKT	**DES**	**MTKT**	**DES**
Acid Hydrolysis	15.48	7.32	14.25	6.76
Alkaline Hydrolysis	8.15	5.04	8.02	5.01
Oxidative	9.35	5.59	8.98	5.12
Thermal	12.48	6.39	11.84	6.15

Table 14 Summary of Stability indicating RP – HPLC Method