Designing of forced degradation studies and development of validated stability indicating method for simultaneous estimation of desloratadine and montelukast odium in their formulation

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ABSTRACT

A simple, rapid, accurate, precise and validated high performance liquid chromatographic method for simultaneous estimation of Desloratadine(DES) and Montelukast sodium(MKT) in bulk and pharmaceutical formulation. The RP-HPLC was performed on YMC C18 column (250, 4.6I'd, 5μm particle size) column with mobile phase Orthophosporic acid (pH-2.1): Methanol (40:60v/v) and column temperature $30^{o}C$ The flow rate of the mobile phase was adjusted to 1ml/min and the injection volume was 10μl.Detection was performed at 278nm.The Retention time for DES and MKT were 3.504 and 4.724 min respectively. The method was validated and shown to be linear for DES and MKT in 5-15 μl and 10-30 μl respectively. Both the drugs have the regression 0.999.DES was highly susceptible to Oxidation and least susceptible to Basic condition MKT was susceptible to acidic condition. The relative standard deviation of DES and MKT for intra-day was 0.24% and 0.11%respectively,inter day was found 0.25% and 0.12% respectively. The Developed RP-HPLC method is suitable for estimation of Desloratadine and Montelukast sodium in tablet formulation. Hence this method can be used in quality control for routine analysis of the finished product.

Key words: Montelukast sodium, Desloratadine, RP-HPLC

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INTRODUCTION

Desloratadine (DES) is chemically 8-chloro-6, 11-dihydro-11-(4-piperdinylidene) - 5*H* benzo [5, 6] cyclohepta [1, 2- b] pyridine. It is used for the relief of perennial allergic rhinitis and for the symptomatic treatment of pruritus and urticarial associated with chronic idiopathic urticaria [8]. Desloratadine is not official in any pharmacopoeia. Molecular weight is 310.82g/mole.it is non-sedating peripheral histamine H1 Receptor antagonist, the active metabolite of loratadine.

Montelukast sodium is a selective and orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene CysLT1receptor.Montelukast sodium is describe chemically as [R (E)]-1-[[[1-[3-[2-(7-chloro-2quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy -1-methylethyl) phenyl] propyl] thio] methyl]cyclopropaneacetic acid, monosodium salt. Montelukast binds with high affinity and selectivity to the CysLT1 receptor(in preference to other pharmacologically important airway receptors, such as the prostanoid, cholinergic, or βadrenergicreceptor). Montelukast inhibits physiologic actions of LTD4 at the CysLT1 receptor without any agonist activity. Literature survey reveals that various HPLC LC-MS, Electro kineticchromatographic, electrophoresis method and spectrophotometric methodshave been reported for the estimation ofmontelukast sodium and Desloratadine. The present studyillustrates development and validation of asimple, accurate and precise procedure for "Designing of forced degradation studies and development of validated stability indicatin methods for simultaneous estimation of desloratadine and montelukast odium in their formulation".
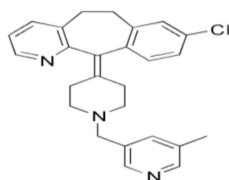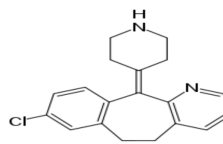
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Fig: 1 Structure of Montelukast sodium   **Fig: 2 Structure of Desloratadine**

MATERIALS AND METHODS

Experimental Instruments and apparatus:
All HPLC experiments were carried out on a Waters Alliance 2695 separation module, with waters 2996 photodiode array detector in isocratic mode using Auto sampler. Data collection and processing was done using EMPOWER PDA 2 software. The analytical column used for the separation was YMC C18, 250× 4.6 mm I.D., 5μm particle size, Other equipment's used were ultra-sonicator (model 3210, Branson Ultrasonic's Corporation, Connecticut, USA), Analytical balance (contech balance).

Reagents and materials
Drug samples: Montelukst and Desloratadine(working standard 99.12 and 99.75) were obtained as gift sample from sun pharmaceuticals India. Pharmaceutical tablet formulation of MONDESLOR 10/5mg was purchased from local pharmacy.

Chemicals and solvents:
Methanol (HPLC Grade; MERCK), Orthophosphoric acid (HPLC grade, MERCK), Hydrochloric acid(AR), sodium hydroxide(AR),hydrogen peroxide (AR) and HPLC grade water were used for the entire study.

Preparation of working standard:
Standard solution
Standard solutions of montelukast sodium and Desloratadine were prepared by dissolving 5mg of Desloratadine and 10mg of montelukast in two separate 100mL. Volumetric flasks containing 10mL. Of HPLC grade water,sonicate for 5min and final volume ware made up to the mark with HPLC grade waterseparately. From these stock solutions take 5mL. From each flask and transfer into two separate 25mL. Volumetric flasks, the final volumes weremade up to the mark with HPLC grade water to get the concentrations of 10μg/mL of MKT and 20μg/ mL of DES respectively.

Preparation of sample solution:
For the estimation of drugs, 20 tablets (MONDESLOR-10/5) were weighed and triturated in a glass mortar and quantity of powder equivalent to 5mg of Desloratadine was transferred to 100mL. Volumetric flask and dissolved in sufficient quantity of HPLC grade water. It was sonicated for 5mins and volume was made up to 100mL. HPLC grade water. It was filtered through 0.45μ membrane filter. From this solution transfer 5mLinto 25mL. Volumetric flask and the final volume was made up to the mark with HPLC grade water to get the concentrations of 10μg/mL of DES and 20μg/ mL of MKT respectively. The test concentration is injected 6 times into chromatographic system. Each time peak area and retention time was recorded and the results obtained were shown in the table.

Preparation of Mobile phase:
Mobile phase was prepared by mixing OPA (pH-2.1, 0.1%) and Methanol (40/60). It was filtered through 0.45μ membrane filter to remove the impurities which may interfere in the final chromatogram.

Chromatographic condition
YMC C18 (250mm×4.6mm I.D; 5 μm) column is used for detection at a wavelength of 278nm, using Orthophosphoric acid (0.1% pH 2.1) and Methanol (40/60) in a isocratic elution mode as a mobile phase. The contents of the mobile phase was degassed, with a helium sparge for 15 min and filtered through vaccum filtration pumped from the respective solvent reservoirs to the column at a flow rate of 1 mL. /min. The column temperature was maintained at 30°c and run time was 15mins. The injection volume of sample was 10 μl. The retention times for Desloratadine and montelukast sodium were found to be 3.504(DES) and 4.724(MKT),mins respectively. Shows the trails and optimized chromatogram and the conditions are tabulated.

Calibration curve of standard solution:
Two separate standard calibration curves were constructed for each drug. A series of aliquots were prepared from the above stock solutions using HPLC grade water to get the concentrations 5-15μg/mL. DES and 10-30μg/mL.

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MKT. Each concentration was injected 6times into chromatographic system. Each time peak area and retention time were recorded separately for both the drugs. Calibration curves were constructed by taking average peak area on Y-axis and concentration on X-axis separately for both the drugs as shown in the figure no 3, 4. From the calibration curves regression equations were calculated and this regression equations were used to calculate drug content in formulation.
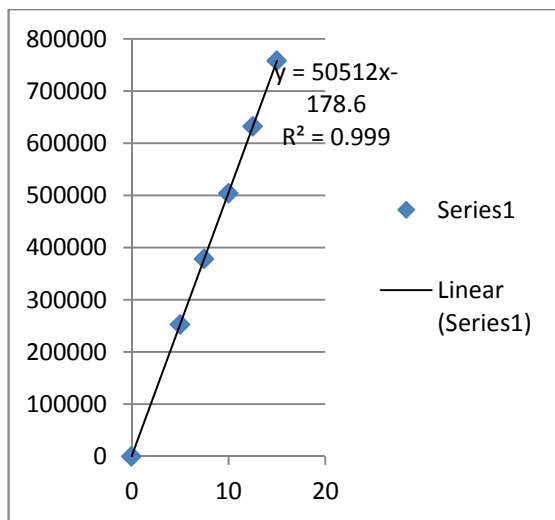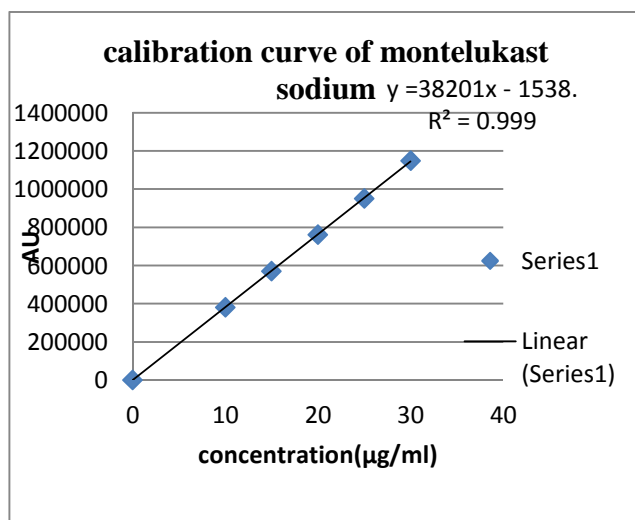

Fig: 3 Calibration curve of DES   **Fig: 4 Calibration curve of Montelukast sodium**

Validation of the method:
Validation of the respective method for the following parameter's was carried as per the ICH guidelines

Linearity
The linearity of the method was determined in concentration range of 5-15 μg/mL for DES and 10-30 μg/mL for MKT. Each solution was injected in triplicate. The average peak area Vs concentration data of both drugs was treated by least squares linear regression analysis and the obtained results were shown in table no: 1

Table no: 1 Summary of the proposed RP-HPLC method

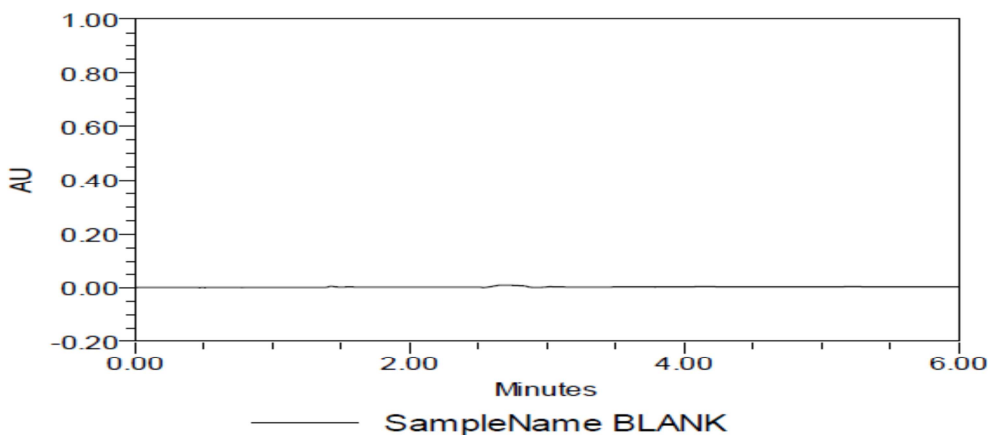| Parameters	DES	MKT
Linearity range (μg/mL.)	5-15	10-30
Regression line equation	Y=50512X-178.6	y = 38201X-1538
Correlation coefficient (r^2)	1.0	1.0
No of data points	5	5
LOD (μg/mL.)	0.429	0.6593
LOQ (μg/mL.)	1.429	2.1978

Fig: 4 Chromatogram of Blank

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Specificity and Selectivity

Specificity is the degree to which the procedure applies to a single analyte and is checked in each analysis by examining blank matrix samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of any other excipients. Two different samples were injected and studied with respective excipients. The HPLC chromatograms recorded for the drug matrix (mixture of the drug and excipients) showed almost no interfering peaks with in retention time ranges. Fig.5 & 6 show the respective chromatograms for DES and MKT with Blank and Standard. The figures shows that the selected drugs were cleanly separated. Thus, the HPLC method proposed in this study was selective.
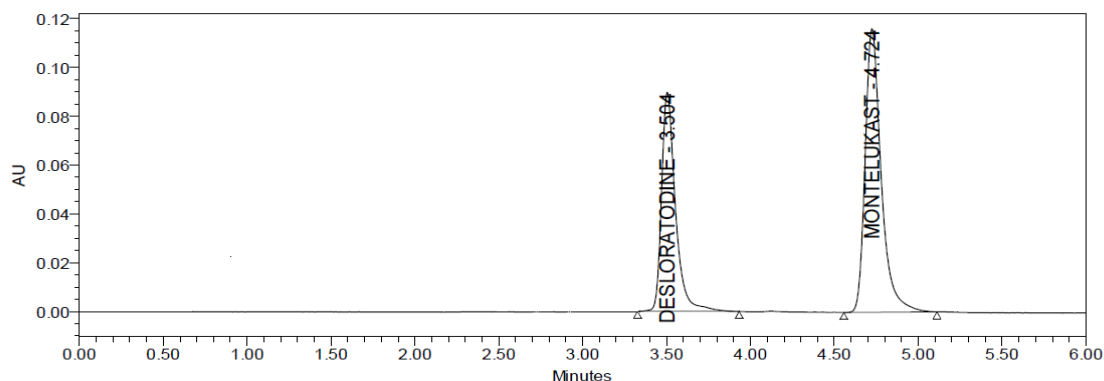

Fig: 6 Chromatogram of Standard

Accuracy, as Recovery

Accuracy was evaluated in triplicate, at three different concentration levels equivalent to 50, 100, and150% of the target concentration of active ingredient, by adding a known amount of each of the working Standards to a preanalysed concentration of both drugs and calculated the % of recovery, And the results obtained were shown in table no:2

Table no: 2 Recovery studies

Drug name	Preanalysed concentration taken (µg/mL.)	Recovery level	Amt of drug added (µg/mL.)	Amt of drug found (µg/mL.)(n=3)	% recovery	Acceptance criteria
Desloratadine	5	50	2.5	7.4	99.33	98-103%
		100	5	9.9	99.06	
		150	7.5	12.46	99.71	
Montelukast	10	50	5	14.76	98.45	98-102% (IP)
		100	10	19.94	99.73	
		150	15	24.93	99.736	

Table no: 3 Data of repeatability of DES and MKT:

Day of Analysis	%Recovery±SD;(n=3)			%RSD
Intraday Precision				
DES (µg/mL.)	5	7.5	10	
Day0	99.01±0.17	99.33±0.77	98.01±0.03	
Day1	98.13±0.09	99.56±1.46	98.13±0.09	0.24
Day2	99±0.2	98.2±0.9	99±0.04	
MKT (µg/mL.)	10	15	20	
Day0	99.33±0.71	98.33±0.78	97.33±0.29	
Day1	100.56±1.53	98.56±1.53	98.56±1.53	0.11
Day2	99.2±0.83	99.2±0.83	99.2±0.83	
Interday Precision				
DES (µg/mL.)	5	7.5	10	
Day0,1,2	99.13±0.09	98.2±0.9	97.13±0.9	0.25
MKT (µg/mL.)	10	15	20	
Day0,1,2	98.56±1.53	99.56±1.53	99.2±0.83	0.12

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was determined with the standard and test sample. The precision of the method was verified by repeatability (intraday) and the intermediate precision studies. Repeatability studies were performed by analysis of the concentrations of working standard for DES and MKT. Method repeatability was achieved by repeating the same

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procedure of preparation of solution for six times and injecting into chromatographic system. Intermediate precision was examined by performing the same procedure on the same day for intra-day precision. The inter day precision of the method was checked by performing same procedure on different days under same experimental conditions. The repeatability of sample application and measurement of peak area were expressed in terms of Relative standard deviation (%RSD) and results obtained from as shown in table no:3

LOD and LOQ
LOD: It is lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value under the stated experimental conclusions. The detection limit is usually expressed as the concentration of analyte. The standard deviation and response of the slope

LOD = 3.3*standard deviation (ɓ)/s

LOQ: The quantitation limit of an analytical procedure is the lowest amount of an analyte of a sample which can be quantitatively determined with suitable precision and accuracy. The standard deviation and response of the slope and the results obtained from as shown the table no. 8.13

LOQ= 10* standard deviation (ɓ)/s

Robustness
To evaluate the robustness of the method, the chromatographic conditions were deliberately

Altered and degree of reproducibility was evaluated. During robustness testing each condition

was varied separately, all other conditions being held constant at the optimized values. Robustness of the proposed method was assessed with respect to small alterations in the flow rate (1.0 ± 0.2mL./min), and Temperature (30^0c $\pm$ 2^0c) and the results obtained from as shown the table.

System suitability parameters: For assessing system suitability, six replicates of working standards samples of DES and MKT were injected and studied the parameters like plate number (N), tailing factor (K), resolution, relative retention time and peak asymmetry of samples. The results were tabulated in table no4

Table no: 4 System suitability parameters of the Proposed RP-HPLC method:

Parameters	Values obtained (n=6)	
	DES	**MKT**
Plate count	9618	12924
Tailing Factor	1.43	1.41
R_t(min)	3.504	4.724
Resolution	0	7.62
Asymmetry	0.9	1.1

Assay of tablet dosage form:
Six replicates of sample solution (5µg/ml of each were injected).from the peak area of the DES and MKT amount of drugs in samples were computed. % assay is given in the table no: 5

Table no: 5 Assay of marketed formulation

Drug name	Labelled claim(mg)	Test concentration (µg/mL.)	Mean Amount found (µg/mL.)n=6	%Estimated Amt	%RSD
Desloratadine	5	10	9.96	99.6	0.07
Montelukast	10	20	19.98	99.9	0.04

STABILITY STUDY
Acid degradation
DES and MKT degradation were carried out in acidic condition. This study indicate that there is 2 degradation products peak of DES for 24 hrs ,both the drugs found to be equally degraded in acidic condition, it is concluded that MKT is very stable in Acidic condition.
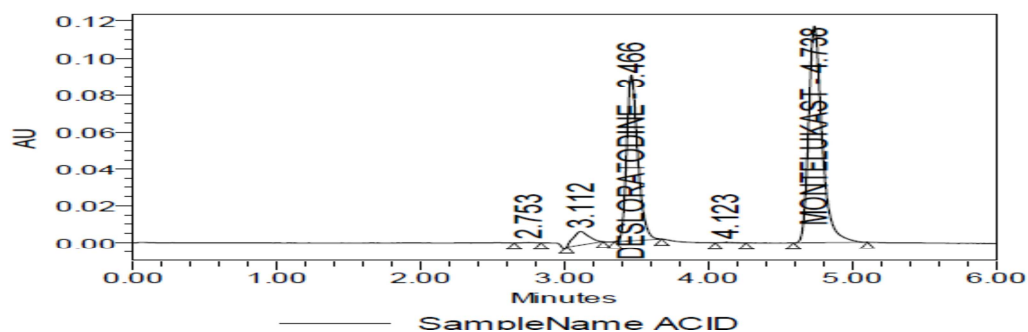
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Fig: 7 Acid degraded chromatogram

Basic degradation

DES and MKT degradation were carried out in acidic condition. This study indicate that there is 2 degradation products peak of DES for 24 hrs ,DES is highly suspectable to basic condition ,when compared to MKT it is concluded that MKT is very stable in Basic condition.
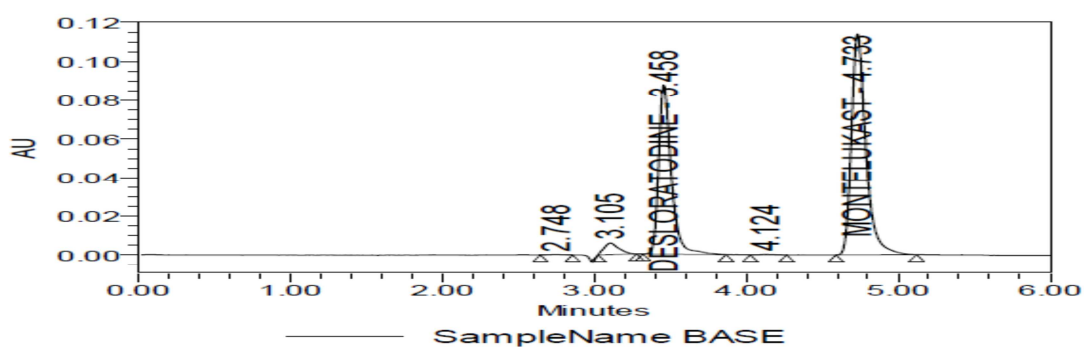

Fig: 8 Base degraded chromatogram

Peroxide condition:

DES and MKT degradation were carried out in acidic condition. This study indicate that there is 2 degradation products peak of DES for 24 hrs ,DES is highly suspectable to oxidation condition ,when compared to MKT it is concluded that MKT is very stable in Peroxide condition.
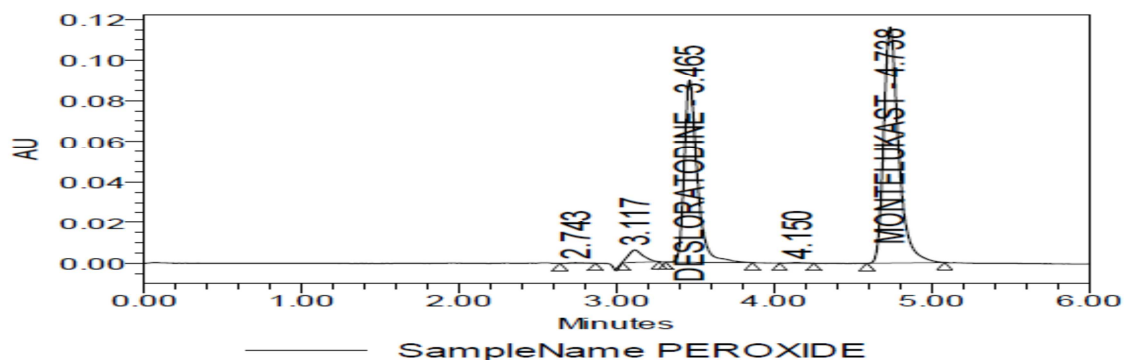

Fig: 9 Peroxide chromatogram

Thermal condition:

DES and MKT degradation were carried out in acidic condition. This study indicate that there is 2 degradation products peak of DES for 24 hrs ,DES is highly suspectable to thermal condition ,when compared to MKT it is concluded that MKT is very stable in Thermal condition.
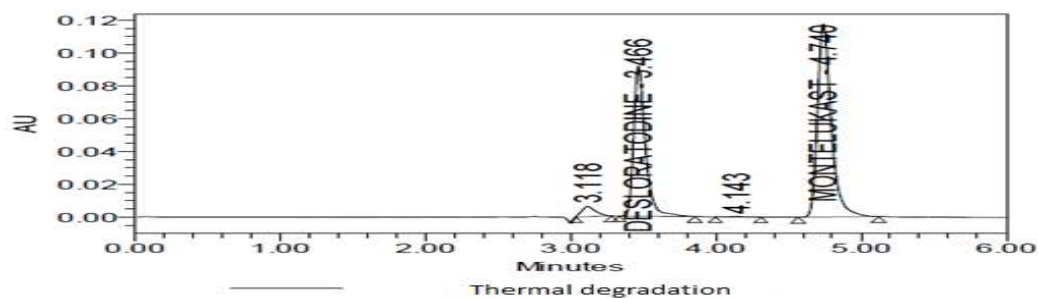
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Fig: 10 Thermal degraded chromatogram

Photolytic condition:
DES and MKT degradation were carried out in acidic condition. This study indicate that there is 2 degradation products peak of DES for 24 hrs ,DES is highly suspectable to Photolysis condition ,when compared to MKT,it is concluded that MKT is very stable in Photolytic condition.
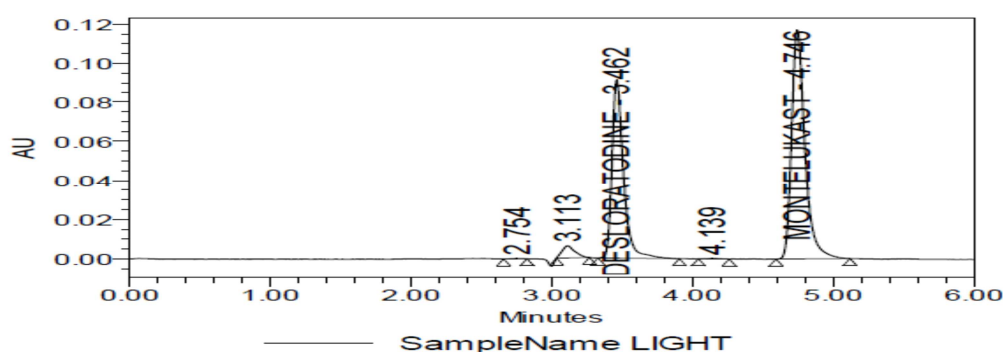

Fig: 11 Photolytic chromatogram

Degradation studies
 Results are tabulated in table no.6 and Representative Chromatograms obtained from forced degradation studies are shown in Fig no. (7, 8, 9, 10, 11)

Table no: 6 Results of forced degradation studies

Stress conditions	DESLORATADINE	MONTELUKST
	% degradation	% degradation
Acidic/0.1 M HCl/60°C reflux/30min	5.0	5
Basic/0.1 M NaOH/60°C reflux/30min	3.0	1
Oxidizing/3% H2O2/cool at RT/30min	6.0	2
Thermal/105°C/6hr	5.0	1
Photolysis/UV light	5.0	1

RESULTS AND DISCUSSION

Optimized chromatographic conditions: Attempts made towards development of a Simple and better method on commonly used C18 column with good resolution were successful. Different logical modifications were tried to get good separation among the drugs and the degraded products. These changes include change in mobile phase composition in isocratic elution as well as gradient modes on different C18 columns. The optimized chromatographic conditions (figure 8.6).The best peak shape and maximum separation was achieved with mobile phase composition of 0.1% OPA (pH 2.1) and methanol (a: b, 40/60v/v). Peak symmetry and reproducibility were obtained on YMC C18 250mm×4.6mm I.D; 5 μm).The optimum wavelength for detecting the analytes was found to be 278nm, a flow rate of 1mL. /min

Linearity, LOD and LOQ
The calibration plot was linear over the concentration range investigated (5-15μg/mL.; *n* = 3) and (10-30μg/mL.; *n* = 3) for DES and MKT respectively. Average correlation coefficient r²=0.9999 for both drug candidates with %RSD values <2.0 across the concentration ranges studied, were obtained from regression analysis. The LOD for DSE and

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MKT were found to be 0.429μg/mL and 0.659μg/mL respectively .The LOQ that produced the requisite precision and accuracy was found to be 1.429μg/for DES and 2.1978μg/mL for MKT. The resultant % RSD values were <1.00 %(table no.8.9.1). The regression results indicate that method was linear in the concentration range studied and can be used for detection and quantification of DES and MKT in a very wide concentration range.

Specificity and Selectivity:
Specificity is checked in each analysis by examining blank and placebo samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of any other excipients.

Accuracy and Precision
Accuracy as recovery was evaluated by spiking previously analyzed test solution with additional Standard drug at three different concentration levels. Recovery of working standard drugs added was found to be 99.32±0.47% for Desloratadine and 99.93±0.46% for montelukast sodium with the value of RSD less than 2% indicating that the proposed method is accurate for the simultaneous estimation of both drugs from their combination drug products in presence of their degradation products. The low RSD values indicate the repeatability and reproducibility of the Method.

Robustness
The elution order and resolution for both components were not significantly affected. RSD of peak areas were found to be well within the limit of 2.0%.

System suitability
The system suitability parameters were found to be within acceptance criteria. Good peak with resolution between two drugs is >1.5, asymmetric factor is <2 show that the two drugs were better separated.

Degradation studies
Results are tabulated in table no. 6

Acid hydrolysis (fig. 7)
Upon performance of acid degradation studies 5% of Desloratadine and 5% of Montelukast sodium was degraded.

Base hydrolysis (fig.8)
Upon performance of base degradation studies 3% of Desloratadine and 1% of Montelukast sodium was degraded.

Peroxide hydrolysis (fig.9)
Upon performance of base degradation studies 6% of Desloratadine and 2% of Montelukast sodium was degraded

Thermal degradation (fig.10)
Upon performance of base degradation studies 5% of Desloratadine and 1% of Montelukast sodium was degraded

Photolytic degradation (fig.11)
Upon performance of base degradation studies 5% of Desloratadine and 1% of Montelukast sodium was degraded

CONCLUSION

Developed RP-HPLC method can resolve all degradant peaks of both the drugs.so this method can give analysis of both the drugs in presence of its degradant products. Hence this method is Stability indicating in nature. The developed RP-HPLC method was found to be simple, specific and accurate. Therefore this method can be applied for routine analysis of drugs in formulation of drugs in formulation and in bulk drug.

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