Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Suppl 2, 2014

Research Article

METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC FOR SIMULTANEOUS ESTIMATION OF RUPATADINE FUMARATE AND MONTELUKAST SODIUM IN COMBINED TABLET DOSAGE FORM

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Received: 09 Oct 2013, Revised and Accepted: 19 Jan 2014

ABSTRACT

Objective: A simple, precise, cost effective stability indicating RP-HPLC method has been developed and validated for the determination of Rupatadine and Montelukast in pharmaceutical compositions. This method can give analysis of both drugs in presence of its degredent products under variety of stress condition. Montelukast was highly susceptible to acidic condition and photo degradation; while Rupatadine was moderately degrade under alkaline condition.

Methods: The chromatographic separation was achieved on hibar^R 250-4, C-18 columns (250mm ×4.6mm,5um) using a mobile phase consisting of Methanol:Water (90:10v/v) with 0.1% Triethyllamine pH 3.41 adjusted with ortho phosphoric acid at a flow rate of 1ml/min. Detection wavelength was found 260 nm.

Results: The Retention times of Rupatadine and Montelukast were found 4.31 and 11.59 minute respectively. The method was found to be linear over the range of 15-40 µg/ml for both the drugs with correlation co-efficient (r^2) 0.996 & 0.999 for Rupatadine and Montelukast respectively. Percentage recoveries obtained for both the drugs were 99.49-100.25% and 99.52-100.53% for Rupatadine and Montelukast respectively. The %RSD for precision and accuracy of the method was found to be less than 2%.

Conclusion: The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. Developed HPLC method can resolve all degredent peak of both drug. So this method is stability indicating in nature. The method developed can be used for the routine analysis of Rupatadine and Montelukast from their combined dosage form.

Keywords: Rupatadine Fumarate, Montelukast Sodium**,** Degradent Products, Stability indicating assay method

INTRODUCTION

The international conference on harmonization (ICH) guide-lines emphasizes that the purity and assay of drug susceptible to change during storage, must be determined by using validated stability-indicating methods, which can selectively determine the drug in presence of its process and degradation impurities.

Rupatadine is chemically 8-chloro-6, 11-dihydro-11-[1-[(5-methyl3-pyridinyl) methyl] -4-Piperidinylidene]-5H-benzo [5, 6]-cyclo hepta [1,2b] pyridine (fig.1). 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 non-immunological 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 H_1 receptor.[2] It is not official in any Pharmacopeia. It is light pinkish powder and freely soluble in Very soluble in methanol, ethanol and propylene glycol and DMSO and slightly soluble in water. Molecular weight of Rupatadine is 532 gm/mol and formula is $C_{30}H_{30}CIN_3O_4$.

Montelukast Sodium (1-(1R)-1-[3-[(1E)-2-(7-chloro-2- quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl] -propyl] thio] methyl] cyclo propaneacetic acid,(Fig.2). it is Cysteinyl leukotriene receptor antagonist and used for Treatment of Asthma [3]. it is official in Indian pharmacopeia 2010. Monosodium salt is a white colored powder and it is freely soluble in ethanol, methanol and practically insoluble in acetonitrile. Molecular weight of Montelukast Sodium is 608.2 gm/mol and formula is $C_{35}H_{35}ClNO_3S.Na$.

Literature Review revels that there was no reported Stability Indicating RP-HPLC method for Simultaneous Estimation of Rupatadine and Montelukast in combined dosage form [9-13]. 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 Rupatadine Fumarate in bulk and Pharmaceutical Dosage form.

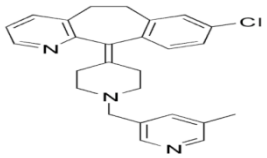

Fig1:Chemical Structure of Montelukast

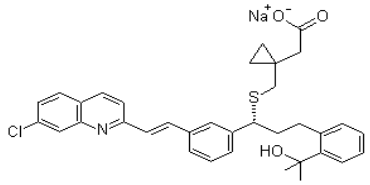

Fig. 2: Chemical Structure of Rupatadine Sodium Fumarate

MATERIALS AND METHODS

Experimental Instruments and Apparatus

The chromatography was performed on Shimadzu instrument equipped with PDA detector and LC Solution software, hibar^R 250-4 C-18 columns (250mm ×4.6mm, 5um) was used as stationary phase. Shimadzu analytical balance and ultra sonicater were used during the research work.

Reagents and materials

Standard samples of MNKT and RTN were obtained from FDC Pvt. Ltd, (Mumbai, Maharashtra).Combination tablet formulation containing Montelukast 10 mg and Rupatadine 10 mg was procured from local pharmacy. Triple distilled water, Methanol, and 0.45

membrane filters (Millipore) used were of HPLC grade. Triehyllamine and Orthophosphoric Acid used were AR grade.

Chromatographic Conditions

The mobile phase containing Methanol: Water (90:10%v/v) with 0.1% Triethyllamine pH 3.41 adjusted with ortho phosphoric acid at a flow rate of 1ml/min..Detection wavelength was found 260 nm. The Retention times of Rupatadine and Montelukast were found 4.31 and 11.59 minutes respectively.

Preparation of working standard

10mg of each of RTN and MNKT were weighed separately and transferred in two different 10 ml volumetric flasks. Both the drugs were dissolved in 10 ml of Methanol by ultra sonication and then volume was made up to the mark with Methanol to obtain final concentration 1000µg/ml of each component. 1ml of stock solution was withdrawn & transferred in 10 ml volumetric flask and volume was made up with methanol to achieve 100 µg/ml of MNKT and 100 µg/ml of RTN.

Preparation of Sample Solution

Powder of tablet formulation equivalent to 10mg RTN and 10mg MNKT were transferred to a 100ml volumetric flask and volume was made up with methanol. The mixture was mixed and sonicated for 10 min and made up to the mark with methanol, to get final concentration of 100µg/ml and 100µg/ml of RTN and MNKT respectively.

After that filter with whatman filter paper to remove unwanted particle. Then the sample solution was filtered through 0.45µm cellulose acetate filter paper (0.45µm).

Preparation of Mobile Phase

Methanol was sonicated for 10 min and filter through 0.2µm syringe filter and kept in bottle **A**. then 0.31 ml of triethylamine dissolved in 100ml Water, then pH was adjusted to 3.41 with ortho phosphoric acid filter through 0.2µm syringe filter and kept in bottle B. and these components were used as mobile phase in 90:10 v/v respectively in gradient elution mode.

Calibration curve of standard solution

Calibration curve of Rupatadine fumarate and Montelukast sodium were plotted over concentration range of 15-40µg/ml respectively. 1.5, 2, 2.5, 3, 3.5,4 ml of stock solution (100µg/ml) were transferred in 10ml volumetric flask and volume were made up with methanol up to 10ml. Each solution was injected in to column to get chromatogram.

Validation of the method

Validation of the optimized RP-HPLC method was carried out with respect to the following parameters.

Linearity

The linear response of was determined by analyzing six independent levels of the calibration curve in the range of 15-40 µg/ml for MNKT & RTN both. Result should be expressed in terms of Correlation co-efficient.

Precision

A) Repeatability

Precision of method was determined by analyzing 6 different solution of same concentration at same time and relative standard deviation is shown in table1.

B) Intra-day precision

Variation of results within same day is called Intra-day precision. The Intra-day precision was determined by analyzing 3 different solution of same concentration 30 µg/ml of both drug at variant times of same day on Relative standard deviation (%RSD) is given in table 2.

C) Inter-day precision

Variation of results among days called Inter-day precision. The Inter-day precision was determined analyzing 3 different solution of same concentration 30 µg/ml of both drug at different days (n=3) it is given in table 3.

Accuracy The accuracy of the methods was determined by calculating recoveries of MNKT and RTN by the standard addition method.

LOD&LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) of the MNKT and RTN were calculated using the standard deviation of responses (N) and slopes (S) of respective calibration curves using signal-to-noise ratio.

LOD = 3.3 × N/S LOQ = 10 × N/S

Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. Robustness of method is determine by change in Saturation Time, Analytical Wavelength & mobile phase composition there is no change in peak area & shape data show in table 4.

Assay of the tablet dosage form

The proposed validated method was successfully applied to determine RTN and MNKT in tablets dosage form. Three replicates of sample solution (100µg/ml of each) were injected. From the peak area of the RTN and MNKT, amounts of drugs in samples were computed. %Assay is given in to table 5.

STABILITY STUDY

Acid degradation

RTN and MNKT degradation study were carried out in acidic condition. This study indicate that there is 5 degradation product peak of MNKT at 12hrs it concluded that MNKT is very labile to acidic condition, it highly susceptible to acidic condition. While RTN is slightly degrade to acidic condition so it is more acid stable compare to MNKT.(fig.11)

Alkaline degradation RTN and MNKT degradation study were carried out in various Alkaline condition. This study indicates that both drugs moderately degrade under alkaline condition. Two degradent products of MNKT and one degredent product of RTN was found. (fig.12) **Peroxide degradation** RTN and MNKT degradation study were carried out in 3% H_2O_2. This study indicate that MNKT degrade moderately in oxidative condition, Two degredent peaks were found of MNKT while RTN slightly degrade in oxidative condition.(fig.13)

Thermal degradation

Thermal degradation of MNKT and RTN were carried out in hot air oven at 60°C for 8hr. There were two degradation peaks were found for MNKT in drug product and no degradation peak was found for RTN. This study indicates that the MTKT was degraded moderately in higher thermal condition and it was practically unstable to thermal stress condition, while RTN is Thermally stable in nature.(fig.14)

Photo degradation

Photo degradation of MNKT and RTN were carried out by direct sun light exposure there were two degradation peaks found for MNKT in drug product. And no degradation peak found for RTN at 0.5hrs exposure of sun light.(fig.15) while at 6 hrs exposure of sun light to drug product MNKT was completely degrade about 97% degradation observed and RTN is slightly degrade about 1% so it indicates that MNKT is very photo sensitive compound and it is more prone to the photo the photo degrade conditions and RTN is

photo stable compound so it is stable under photo degrade conditions.(fig.16)

RESULT AND DISCUSSION

RP-HPLC method was optimized with a view to develop a simple, accurate method for estimation of drug in pharmaceutical formulation and in bulk drug. UV scanning at 200-450 nm for both MNKT and RTN show that 260 nm is the suitable wavelength for detection of drugs.(Fig.3) The mobile phase of Methanol :Water (90:10v/v) with 0.1% Triethyllamine pH 3.41 adjusted with ortho-phosphoric acid at a flow rate of 1ml/min was selected because it gave highest resolution, minimum tailing and Rt values of 4.31 and 11.59 min for RTN and MNKT respectively (Fig.5). RTN and MNKT both showed linearity in the concentration range of 15-40 µg/ml (r^2 =0.996) & (r^2 =0.999) for RTN and MNKT respectively (Fig.6). The LOD and LOQ were found to be 1.49 and 4.51 µg/ml and 1.21 and 3.66 µg/ml, respectively for RTN and MNKT. Repeatability of measurement of peak area was determined by six replicate and six time measurement of working standard of MNKT and RTN and %RSD was found to be 1.055 and 1.12% for RTN and MNKT respectively (Table.1). Intraday & Inter day Precision was found below 2%. (Table 2&3). Recovery studies of the drugs were carried out for the accuracy parameter. These studies were carried out at three levels (80%, 100%, and 120%) by standard addition method. Recovery was found to be 99.49-100.25%% and 99.52-100.53% for RTN and MNKT respectively (Table 4).

Table 1: Data of Repeatability of Rupatadine and Montelukast by HPLC (n=6)

S. No.	Concentration(µg/ml)		Area	
	RTN	MNKT	RTN	MNKT
1	30	30	884715	1081035
2	30	30	863657	1058725
3	30	30	856842	1049874
4	30	30	882511	1073337
5	30	30	876217	1080287
6	30	30	891659	1062417
MEAN			876890	1067613
SD			14042.1	12603.27
% RSD			**1.16**	**1.18**

Table 2: Intraday Precision of Rupatadine and Montelukast by HPLC

Conc. (µg/ml)	Conc. (µg/ml)	Mean Area ± SD (n=3)%	%RSD	Mean Area ± SD (n=3)%	%RSD
RTN	MNKT	RTN	RTN	MNKT	MNKT
30	30	876890±8542	0.97	1070207±7025.9	0.65
30	30	823258±8848.7	1.07	1059588±5982.3	0.56
30	30	839572±6890	0.72	1048962±5818	0.5546

Table 3: Interday Precision of Rupatadine and Montelukast by HPLC

Conc. (µg/ml)	Conc. (µg/ml)	Mean Area ± SD (n=3)%	%RSD	Mean Area ± SD (n=3)%	%RSD
RTN	MNKT	RTN	RTN	MNKT	MNKT
30	30	832093±8811.35	1.05	1042366±9999.92	0.95
30	30	840635±7048.23	0.84	1064011±6390.65	0.6
30	30	817095±7523.88	0.92	1038322±8925.54	0.85

To confirm the specificity. The standard solution with varying amount of Lactose was added. There is no interference of peak MNKT and RTN in presence of Lactose (Excipient) was noticed, indicating the specificity of the method. The assay values for MNKT and RTN are presented in (Table 5). The result of dosage form analysis by developed method was compatible with the labelled amount of each component of tablet. There was no interference of excipient for analysis of dosage form.

Table 4: Recovery studies of Rupatadine and Montelukast

Level of Recovery	Mean of % Recovery	
	RTN	MNKT
80%	100.25	99.52
100%	99.49	100.53
120%	100.13	99.34

Table 5: Assay Results for Tablets Using the Proposed Method

Formulation Amount of drug taken (mg)			Amount of drug found (mg)% Amount found (n=3) ±SD			
Tablets	RTN	MNKT	RTN	MNKT	RTN	MNKT
1	**10**	**10**	10.083±0.035	9.97±0.0561	100.83±0.3511	99.68± 1.1229

Table 6: System suitability parameters by HPLC

S. No	System suitability parameters	Criteria	Results	
			RTN	MNKT
1	Theoretical plates	More than 2000	4958	4825
2	Resolution	More than 2	17.087	
3	Tailing factor	≤2	1.5	1.2

Table 7: Summary of validation parameter for the proposed method

Parameter	RP-HPLC method	
	RTN	MNKT
Linearity Range	15-40 µg/ml	15-40 µg/ml
Linearity equation	Y=31170X+3786	y=34446X+44477
Correlation co efficient	0.996	0.999
Repeatability (%RSD)	1.055 %	1.12 %
Intraday (%RSD)	0.72-1.07%	0.55-65%
Interday (%RSD)	0.84-1.05%	0.6-0.95%
% Recovery	99.49-100.25%	99.52-100.53%
Robustness	0.41-1.05%	0.80-1.2%
Specificity (%RSD)	0.70-0.984%	0.80-1.068%
LOD	1.49 µg/ml	1.21µg/ml
LOQ	4.51 µg/ml	3.66 µg/ml
%Assay	99.48%	100.02%

Table 8: Results of Forced Degradation studies

Stress Condition	Time (Hrs)	%Degradation	
		RTN	MNKT
Acidic(1N HCl)	12	9.14	38.5
Basic (1N NaOH)	12	9.9	14.6
Peroxide (3%H_2O_2)	12	2.54	10.8
Thermal (60°C)	8	1.82	10
SunLight (0.5 Hrs)	0.5	0.82	14.2
SunLight (6 Hrs)	6	12.23	97.6

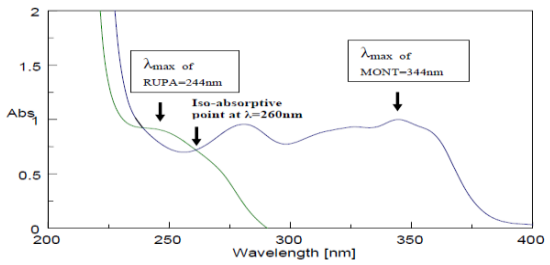

Fig. 3: Overlay spectra of standard MNKT and RTN

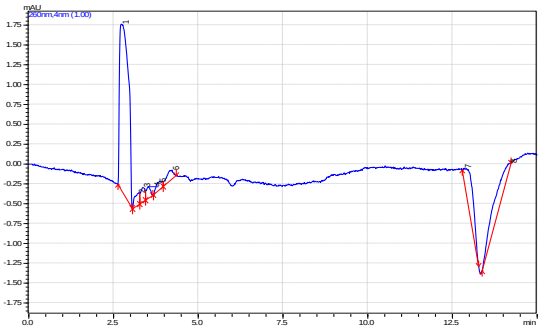

Fig. 4: Chromatogram of Blank Mobile Phase

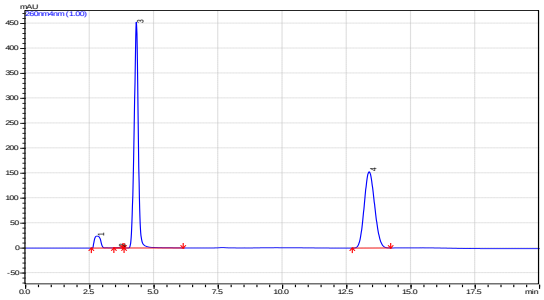

Fig. 5: Chromatogram of RTN & MNKT Standard Solution(100ppm)

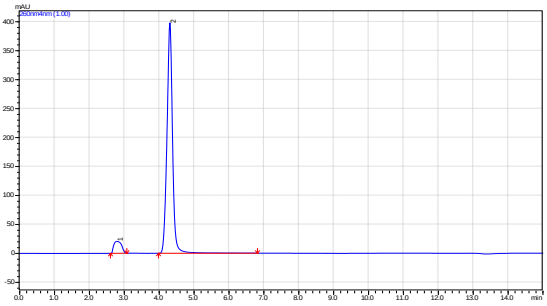

Fig. 6: Chromatogram of RTN Standard solution (100 PPM)

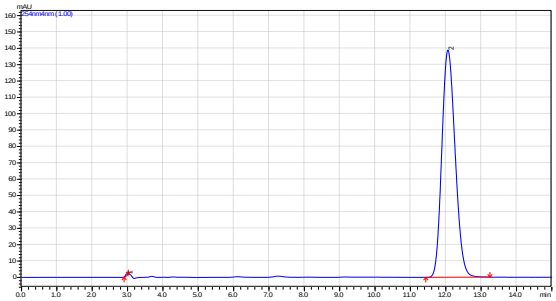

Fig. 7: Chromatogram of MNKT Standard solution (100 PPM)

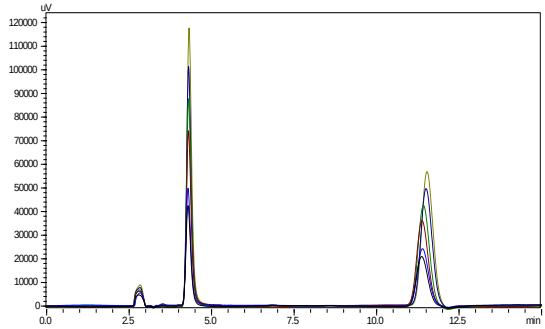

Fig. 8: Chromatogram of Linearity of RTN and MNKT Solution

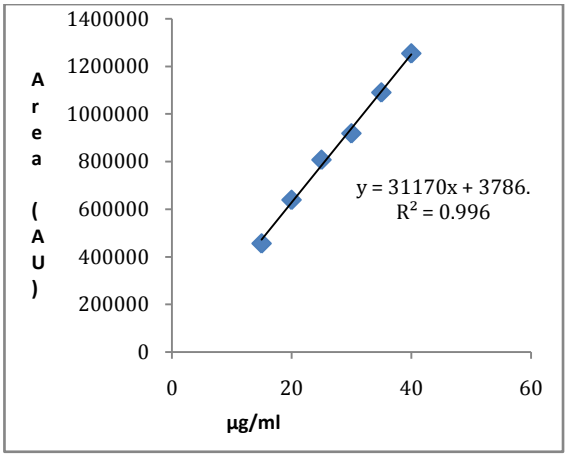

Fig. 9: Calibration graph for the RTN (Concentration Vs peak area)

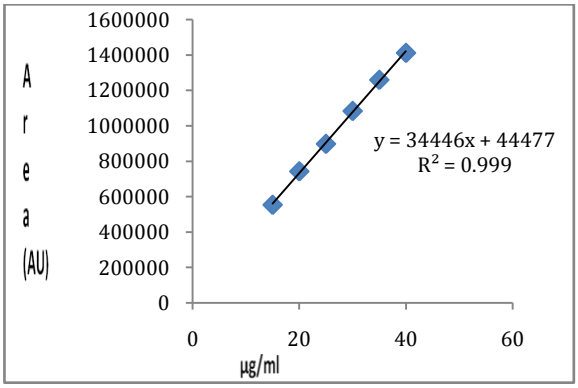

Fig. 10: Calibration graph for the MNKT (Concentration Vs peak area)

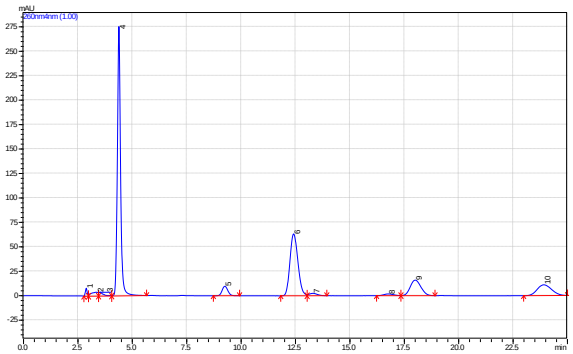

Fig. 11: Degradation of Rupatadine and Montelukast under 1N HCl, 12 Hrs

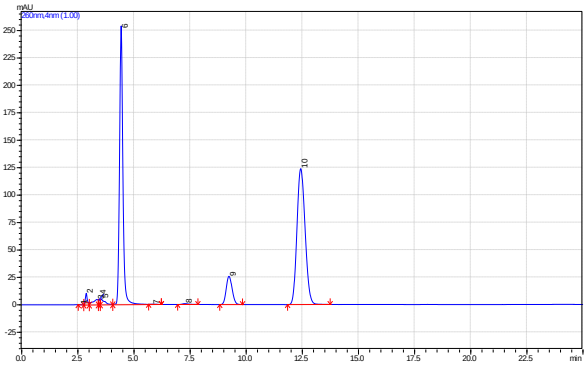

Fig. 12: Degradation of Rupatadine and Montelukast under 1N NaOH, 12Hrs

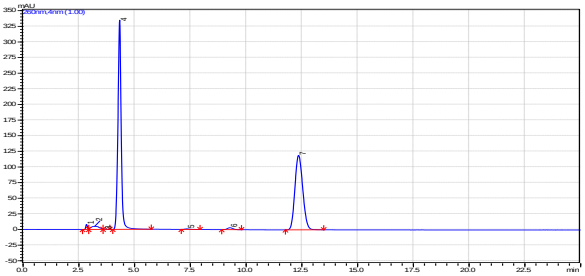

Fig. 13: Degradation of Rupatadine and Montelukast under3% H_2O_2, 12 Hrs

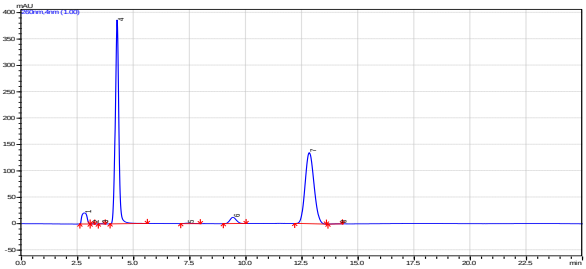

Fig. 14: Degradation of Rupatadine and Montelukast under Thermal condition 60°C, 8Hrs

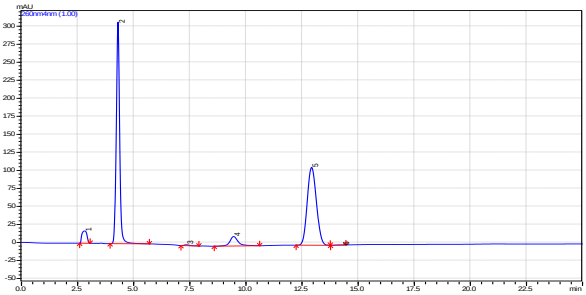

Fig. 15: Degradation of Rupatadine and Montelukast under Sun light, 0.5Hrs

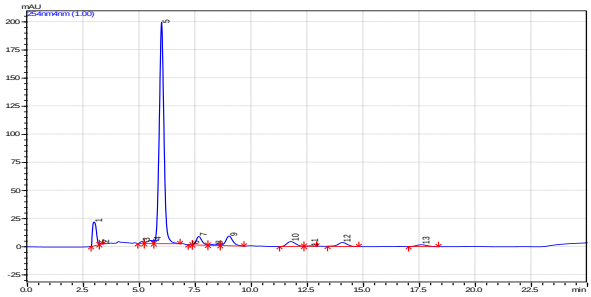

Fig. 16: Degradation of Rupatadine and Montelukast under Sun light, 6 Hrs

CONCLUSION

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

ACKNOWLEDGEMENTS

The authors would like to thank, FDC Pharmaceutical Ltd. Mumbai, India for providing a gift sample of standard Rupatadine and Montelukast. The authors would like to thank to Department of Pharmaceutical Scinces, Rajkot, India for providing necessary facilities to carry out the work.

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