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Analytical Method Development and Validation of Levocetirizine Hydrochloride and Montelukast Sodium in Combined Tablet Dosage Form by RP-HPLC.

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ABSTRACT

Montelukast, chemically known as (S,E)-2-(1-((1-(3-(2-(7-chloroquinolin-2-yl) vinyl) phenyl) -3- (2- (2–hydroxypropan - 2 -yl) phenyl) propylthio) methyl) cyclopropyl) acetic acid, is a leukotriene receptor antagonist used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. Levocetrizine, chemically known as 2-[2-[4-[(*R*)-(4-chlorophenyl)-phenyl-methyl] piperazin-1- yl] ethoxy] acetic acid, is a third-generation non-sedative antihistamine. The present work deals with the development and validation of a simple, sensitive, rapid, selective, precise and accurate isocratic high performance liquid chromatography method for the simultaneous determination of levocetrizine hydrochloride and montelukast sodium in combined tablet dosage form. HPLC separation was carried out by reversed phase chromatography on an XTerra C_8 (4.6 x 150 mm, 3.5 µm) analytical column, held at ambient temperature. The mobile phase consisted of phosphate buffer (pH 4): acetonitrile (60:40 *v/v*), run at a flow rate of 0.8 ml/min and with UV detection at 230 nm. Under the optimized conditions the retention times of the levocetirizine hydrochloride and montelukast are 2.432 min and 6.218 min, respectively.The method was found to be linear over an analytical range of 30-70 µg/ml for both the drugs. The LOD & LOQ values are 3.36 & 9.90 and 3.20 & 9.86 µg/ml for levocetirizine hydrochloride and montelukast, respectively. The low % RSD values (<1) and excellent recovery values indicated the high precicison and accuracy of the proposed method respectively. The % RSD values for parameters like method robustness and method ruggedness showed the method to be robust and rugged. The developed method was successfully applied to the simultaneous determination of levocetrizine hydrochloride and montelukast sodium in combined tablet dosage form. The percent recovery was 99.1 % for levocitrizine and 98.0 % for montelukast. No interference was observed from the coformulated substances. Hence, the proposed method could be useful and fit for the quantification of levocetirizine hydrochloride and montelukast sodium in combined tablet dosage form.

Keywords: HPLC, Levocetirizine Hydrochloride, Montelukast Sodium, Precicision, Ruggedness.

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INTRODUCTION

Levocetirizine Hydrochloride is chemically 2-[2-[4-[(*R*)-(4-chlorophenyl)-phenyl-methyl] piperazin- 1-yl] ethoxy] acetic acid. The structure of Levocetrizine is as shown in the fig 1 Levocetirizine (as levocetirizine dihydrochloride) is a third-generation non-sedative antihistamine, developed from the second-generation antihistamine cetirizine. Chemically, levocetirizine is the active enantiomer of cetirizine. Montelukast Sodium is chemically (S, E)-2-(1-((1-(3-(2-(7-chloroquinolin-2-yl) vinyl) phenyl)-3-(2- (2 hydroxy propan-2-yl) phenyl) propylthio) methyl) cyclopropyl) Acetic acid. The structure of Montelukast is as shown in the fig 2. Montelukast is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. It is usually administered orally. Literature review deals some few Analytical methods have been reported for the determination of Levocetrizine Hydrochloride and Montelukast Sodium in individual and with other combinations. For the estimation of Levocetirizine in individual and in combination some analytical methods such as UV Spectroscopy [1,2], HPLC [3-7], estimation of Montelukast in individual and with other combination such as electro kinetic capillary chromatography [8], Volta metric method [9], HPLC [10-14], LC-ESI-MS/MS [15],' in combination of Levocetrizine and Montelukast Spectroscopy methods [16,17] and HPTLC method [18] have been reported but as per our knowledge there is no HPLC method was reported for both combination.So an attempt was made to report a simple, reliable and reproducible RP-HPLC method which was duly validated by statistical parameters precision, accuracy, linearity, LOD, LOQ, Robustness, and Ruggedness. The method has been satisfactorily applied to the determination of Levocetirizine Hydrochloride and Montelukast Sodium in pharmaceutical preparations.

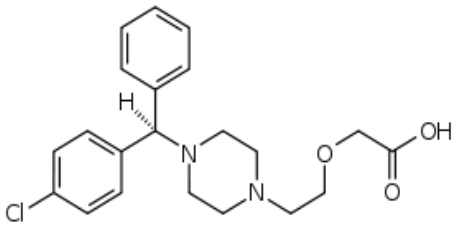

Figure 1: Structure of Levocetrizine

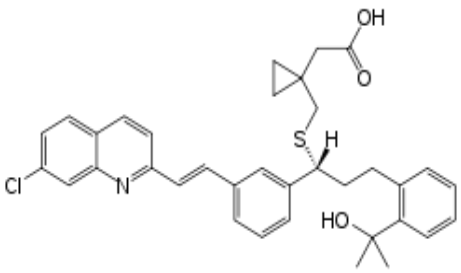

Figure2: Structure of Montelukast

MATERIALS AND METHODS

Equipment and apparatus

Different kinds of equipments viz analytical weighing balance (shimadzu AUX 200), High performance liquid chromatography (waters, separation module 2695) equipped with Auto Sampler and DAD (Dual Absorbance Detector) detector. Column Symmetry C8 (4.6 x 150mm, 3.5µm, Make: XTerra), pH meter, Vacuum filter pump (model XI 5522050 of Millipore), Millipore filtration kit, mobile phase reservoir, Sample filtration assembly and glasswares were used throughout the experiment.

Chemicals and solvents

Potassium di hydrogen ortho phosphate and Orthophosphoric acid (AR grade, Qualigens) were used for preparing the buffer. HPLC grade acetonitrile (Qualigens) was used for diluent preparation. Pure sample of Levocetirizine Hydrochloride and Montelukast Sodium was a gift sample from a local pharmaceutical industry.

Commercial samples of tablets (Montek-LC) containing the drug Levocetirizine Hydrochloride and Montelukast Sodium were purchased from the local pharmacy.

Chromatographic Parameters

Equipment	: High performance liquid chromatography equipped with Auto Sampler and DAD (Dual Absorbance Detector) detector
Column	: Symmetry C8 (4.6 x 150mm, 3.5µm, Make: XTerra)
Flow rate	: 0.8 ml per min
Wavelength	: 230 nm
Injection volume	: 20 µl
Column oven	: Ambient
Run time	: 8min

Preparation of mobile phase

Mix a mixture of above buffer 600 ml (60%) and 400 ml of Acetonitrile HPLC (40%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation

Use the Mobile phase as Diluent.

Preparation of standard solution

Accurately weigh and transfer 10 mg of Levocetirizine Hydrochloride and Montelukast Sodium working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 5ml of Levocetirizine Hydrochloride and Montelukast Sodium from the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent.

Further pipette 5ml of Levocetirizine Hydrochloride and Montelukast Sodium from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of sample solution

Accurately weigh and transfer equivalent to 10 mg of Levocetirizine Hydrochloride and Montelukast Sodium sample into a 10ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 5ml of of Levocetirizine Hydrochloride and Montelukast Sodium from the above stock solution into a 50ml volumetric flask and dilute up to the mark with diluent.

Further pipette 5ml of Levocetirizine Hydrochloride and Montelukast Sodium from above stock solution into a10ml volumetric flask and dilute up to the mark with diluent.

Method Validation

The proposed method was validated as per ICH guidelines. The drug solutions were prepared as per the earlier adopted procedure given in the experiment.

Linearity study

Linearity was performed by taking from stock solution aliquots of 3, 4, 5, 6 and 7 ml were taken in 10ml volumetric flasks and diluted upto the mark with diluent such that the final concentration of Levocetirizine Hydrochloride and Montelukast Sodium in the range of 30 to 70 µg/ml. Volume of 20 µl of each sample was injected and calibration curve was constructed by plotting the peak area versus the drug concentration. The observations and calibration curve is shown in Table 1, 2, 3 and figure 3, 4

Assay

The assay performed by the marketed formulation of Levocetirizine Hydrochloride and Montelukast Sodium (Montek-LC) by taking equivalent weight of tablet and diluted and injected in HPLC. Results are shown in Table 4, 5, 6 and figure 5,6.

Accuracy as recovery

It was done by recovery study. Sample solutions were prepared by spiking at about 50 %, 100% and 150 % of specification limit to Placebo and analyzed by the proposed HPLC method. Results are shown in Table 7, 8 and figure 7, 8, 9.

System precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. Standard solution of (50 ppm) were prepared as per test method and injected for 5 times. Results are shown in Table 9.

Limit of Detection and Limit of Quantification

The parameters LOD and LOQ were determined on the basis of Signal to Noise ratio(S/N).
Results are shown in figure 10,11,12,13.

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. Results are shown in Table 10, 11, 12, 13.

Ruggedness

To evaluate the Ruggedness of the method, ruggedness was performed on different day by using different make column of same dimensions. Results are shown in Table 14.

RESULTS AND DISCUSSIONS

Levocetirizine Hydrochloride is third-generation non-sedative antihistamine. Montelukast is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. A simple reverse phase HPLC method was developed for the determination of Levocetrizine and Montelukast. Symmetry C8 (4.6 x 150mm, 3.5µm, Make: XTerra) in an isocratic mode with mobile phase Acetonitrile:Phosphate buffer P^H3 (40:60) was used. The flow rate was 0.8 ml/ min and effluent was monitored at 230 nm. The retention time for Levocetrizine and Montelukast was found to be 2.461 and 6.231 min respectively.

From the linearity Table 1, 2 it was found that the drug obeys linearity within the concentration range of 30-70µg/ml for Levocetrizine and Montelukast. From the results shown in accuracy Table 6, 7 it was found that the percentage recovery values of pure drug were in between 99.0 to 101.0, which indicates that the method was accurate and also reveals that the commonly used excipients and additives present in the

pharmaceutical formulations were not interfering the proposed method. From the results shown in precision Tables 8, it was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility. The system suitability parameters also reveal that the values were within the specified limits for the proposed method. The results of robustness were shown in tables 9, 10, 11, 12 it was found that the results are within the limits. The results of ruggedness were shown in tables 13. It was found that the results are within the limits, the proposed method is found to be rugged.

Linearity:-

Table-1: Linearity data of Levocetirizine

S.No	Linearity Level	Concentration	Area
1	I	30ppm	1713320
2	II	40ppm	2275094
3	III	50ppm	2837868
4	IV	60ppm	3436641
5	V	70ppm	3974415
Correlation Coefficient			0.9997

Table-2: Linearity data of Montelukast

S.No	Linearity Level	Concentration	Area
1	I	30ppm	2328702
2	II	40ppm	3090603
3	III	50ppm	3867504
4	IV	60ppm	4627404
5	V	70ppm	5455305
Correlation Coefficient			0.9994

Table-3: Linearity parameters

Parmeters	Results observed Levocetrizine	Results observed Montelukast
Slope	56910	77509
Intercept	5599.1	21099
Correlation	0.9997	0.9994

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Table-4: Assay of Tablet formulation

S.no	Levocetrizine		Montelukast	
	Standard area	Sample area	Standard area	Sample area
01	2855793	2846873	3840441	3856346
02	2879702	2855793	3834363	3840441
AVG	2867747	2851333	3837402	3848393.5
STDEV	16906.2	6307.3	4297.2	11246.5
%RSD	0.58	0.22	0.11	0.29

Table-5: Table showing the percentage purity of the tablet formulation

Drug	Lable claim (mg/tab)	Amount estimated (mg/tab)	% Purity
Levocetirizine	10	9.88	98.8
	10	9.94	99.4
Montelukast	5	4.91	98.2
	5	4.89	97.8

Table-6: Assay result of the Developed Method

Drug	Lable claim (mg/tab)	Amount estimated (mg/tab)	%amount estimated	%RSD
Levocetirizine	10	9.91	99.1	0.63
Montelukast	5	4.90	98.0	1.42

Table-7:The accuracy results for Levocetrizine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1416052	5.0	4.95	99.0%	100.2%
100%	2835342	10.0	9.98	99.8	
150%	4251503	15.0	15.28	101.8%	

Table-8: The accuracy results for Montelukast

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1922292	5.0	4.94	98.8%	99.7%
100%	3861847	10.0	9.97	99.7%	
150%	5791330	15.0	15.10	100.6%	

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Table-9: The Precision results for Levocetrizine and Montelukast

Sl.No	Conc. Taken in µg/ml	Retention Time of Levocetrizine	Retention Time of Montelukast	Area of Levocetrizine	Area of Montelukast
1	50	2.476	6.166	2855793	3840441
2	50	2.432	6.218	2879702	3824363
3	50	2.401	5.784	2838886	3909846
4	50	2.474	6.031	2846873	3856346
5	50	2.433	6.216	2874483	3864504
	AVRG			2859147.4	3859100
	STDEV			17536.4	32275.2
	%RSD			0.61	0.83

Robustness:

Table-10:System suitability results for Levocetrizine

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	2071.2	1.3
2	0.8*	2123.4	1.3
3	1.0	2142.7	1.3

* Results for actual flow (0.8 ml/min) have been considered from Assay standard.

Table-11:System suitability results for Montelukast

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	4001.1	1.0
2	0.8*	3935.2	1.0
3	1.0	4032.4	1.0

***** Results for actual flow (0.8ml/min) have been considered from Assay standard.

Table-12: System suitability results for Levocetrizine

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2261.2	1.3
2	Actual*	2142.7	1.3
3	10% more	2318.5	1.3

*****Results for actual Mobile phase composition (40:60 Acetonitrile: Buffer) have been Considered from Assay standard.

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Table-13:System suitability results for Montelukast

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4957.3	1.0
2	Actual*	3935.2	1.0
3	10% more	4963.2	1.0

* Results for actual Mobile phase composition (40:60Acetonitrile: Buffer) have been considered from Assay standard.

Table-14: Results of Ruggedness for developed method

	Retention Time of Levocetrizine	Area of Levocetrizine	Retention Time of Montelukast	Area of Montelukast
Standard(50mcg)	2.432	2855793	6.218	3840441
Analyst(1)(50mcg)	2.506	2879702	6.271	3834363
Analyst(2)(50mcg)	2.476	2838886	6.166	3909846
Analyst(3)(50mcg)	2.433	2866873	6.216	3856346
Analyst(4)(50mcg)	2.474	2874483	6.031	3864504
AVRG	2.464	2863147.4	6.180	3861100
STDEV	0.031	16272.2	0.091	29799.4
%RSD	1.28	0.56	1.47	0.77

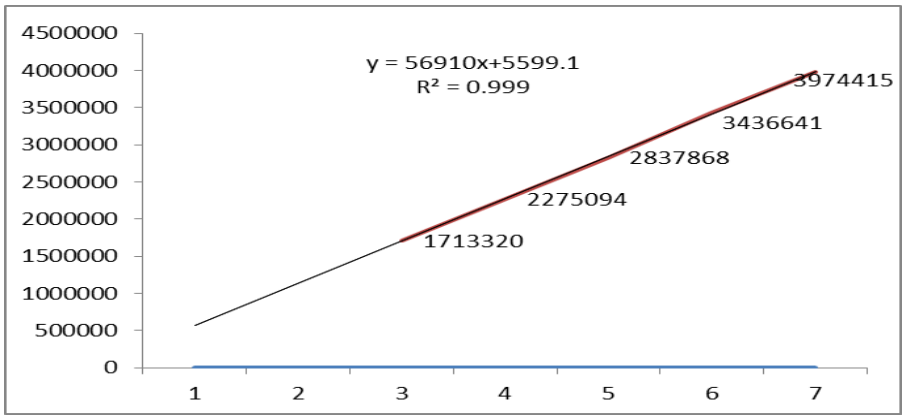

Figure-3: Linearity graph of Levocetrizine

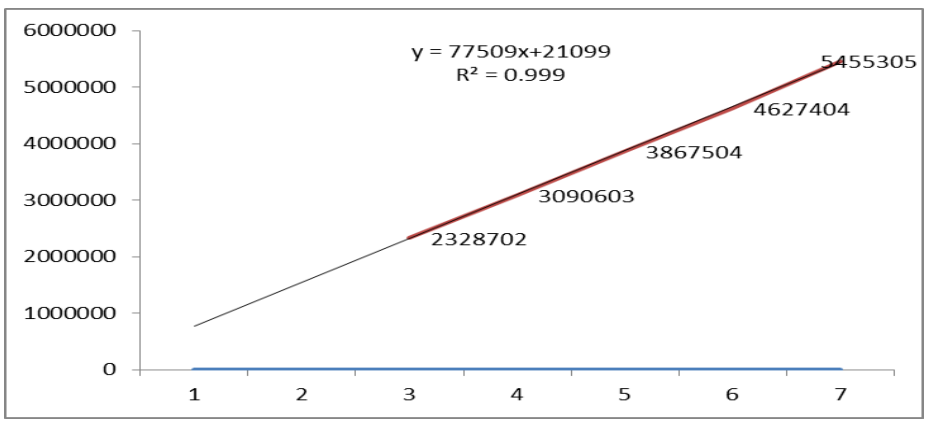

Figure-4: Linearity graph of Montelukast

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Assay Chromatogram:

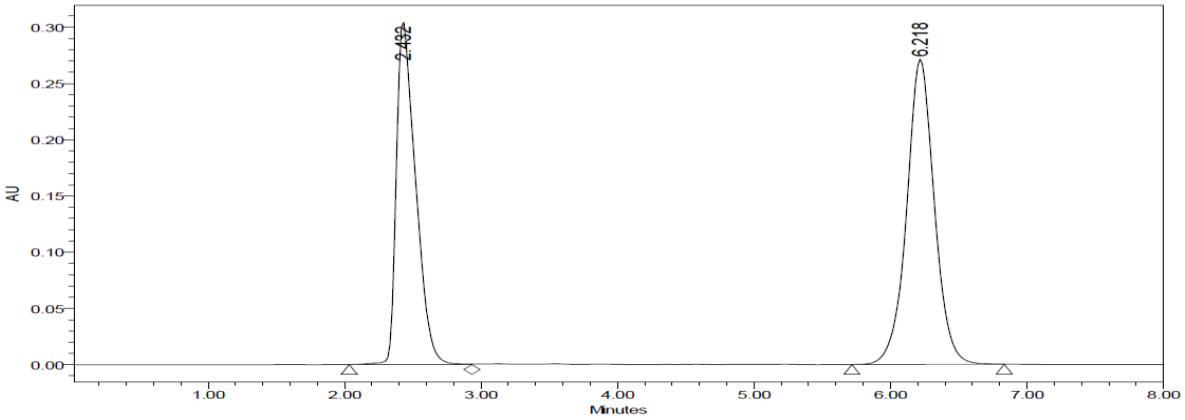

Figure-5: Chromatogram of Levocetrizine and Montelukast (Standard)

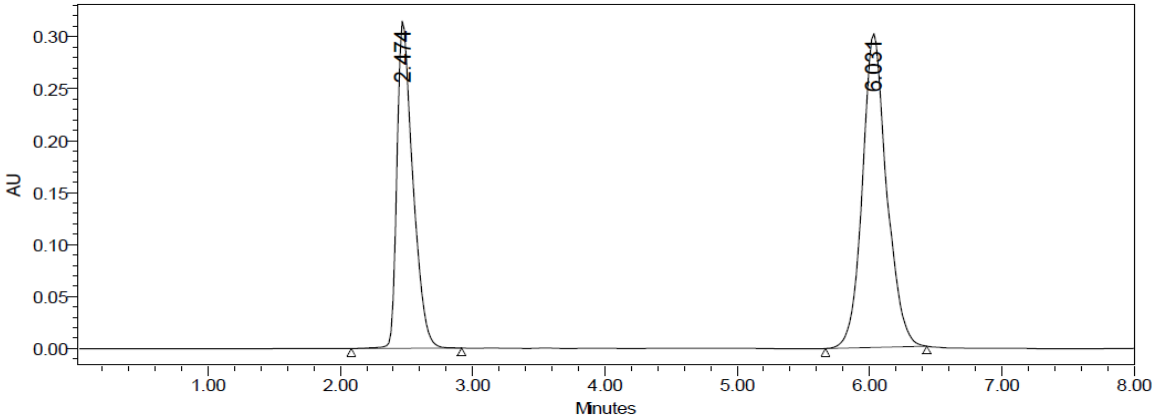

Figure 6: Chromatogram of Levocetrizine and Montelukast (Sample)

Accuracy chromatogram:

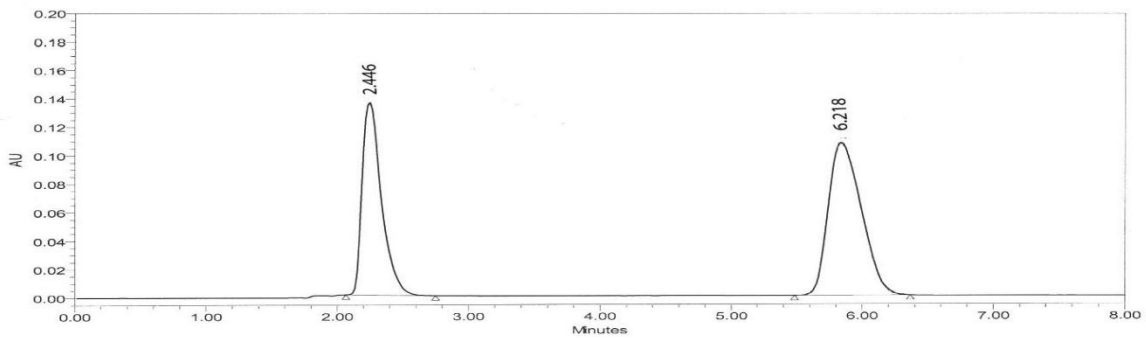

Figure-7: Chromatogram of Accuracy 50%

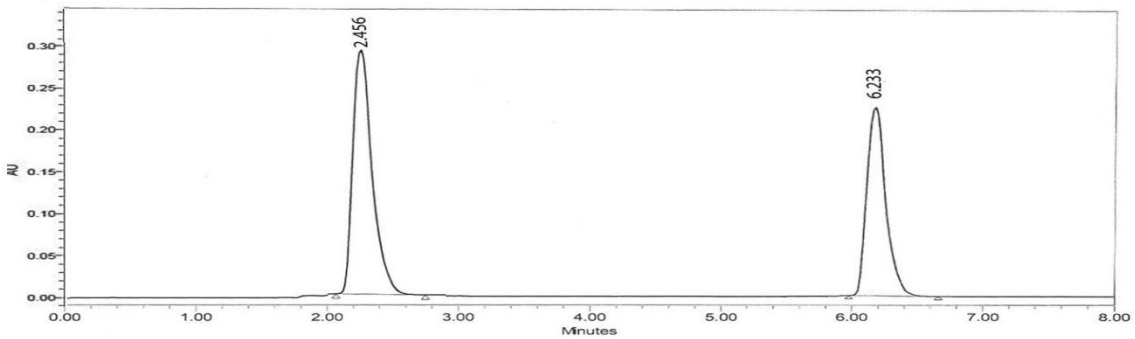

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Figure-8: Chromatogram of Accuracy 100%

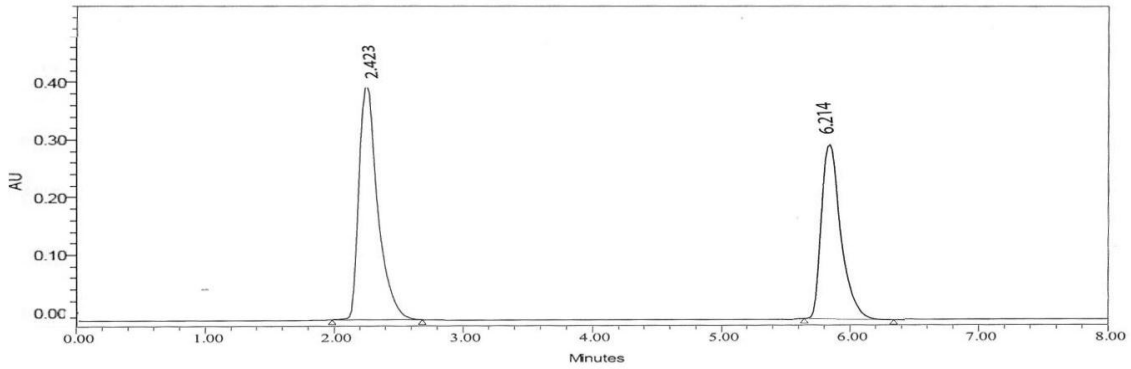

Figure-9: Chromatogram of Accuracy 150%

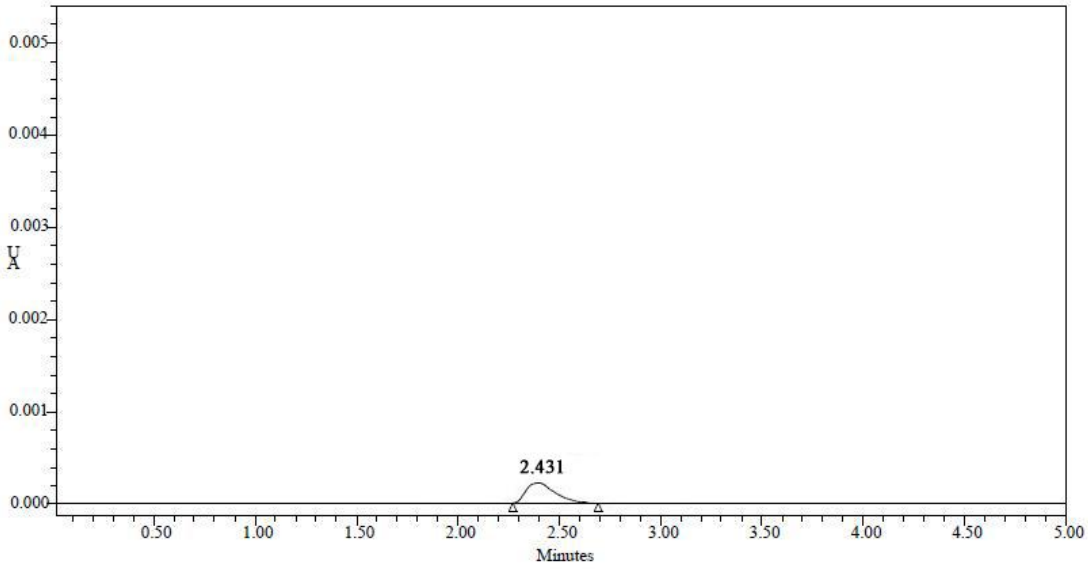

Figure-10:chromatogram of Limit of Detection of Levocetrizine

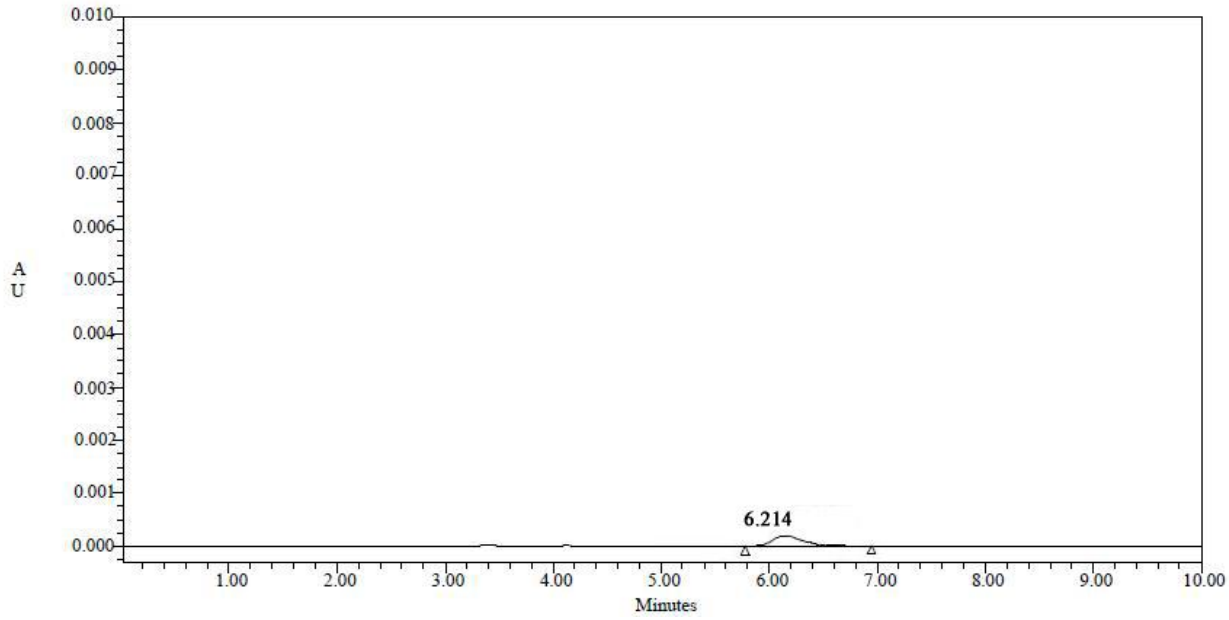

Figure-11: Chromatogram of Limit of Detection of Montelukast:

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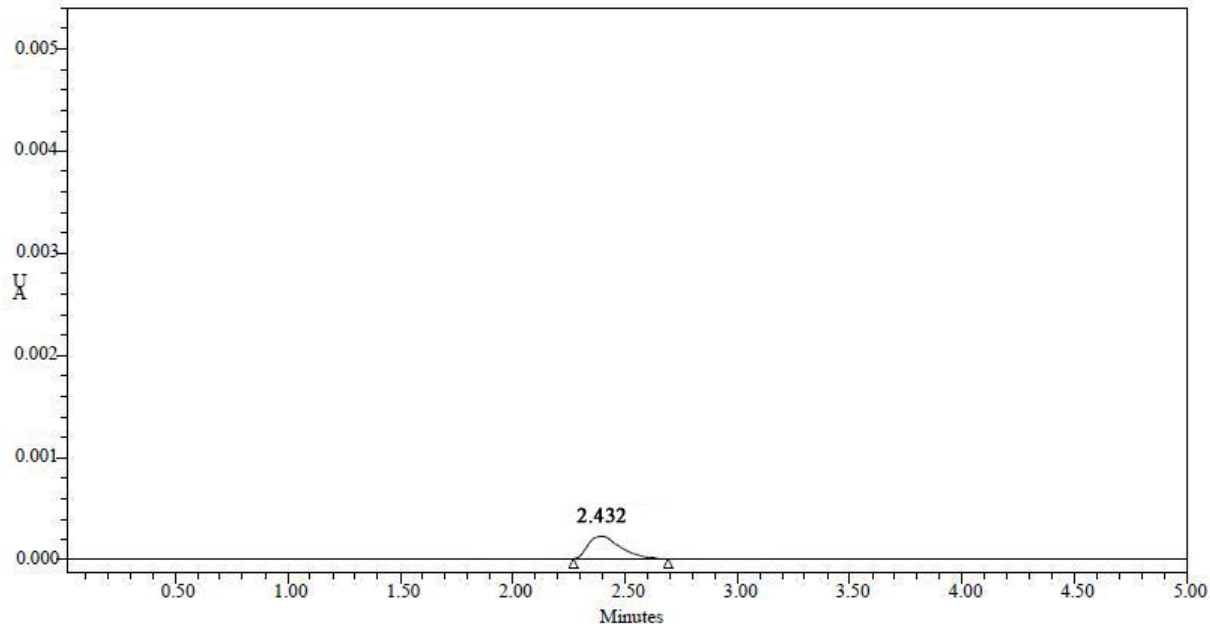

Figure-12: Chromatogram of Limit of Quantification of Levocetrizine

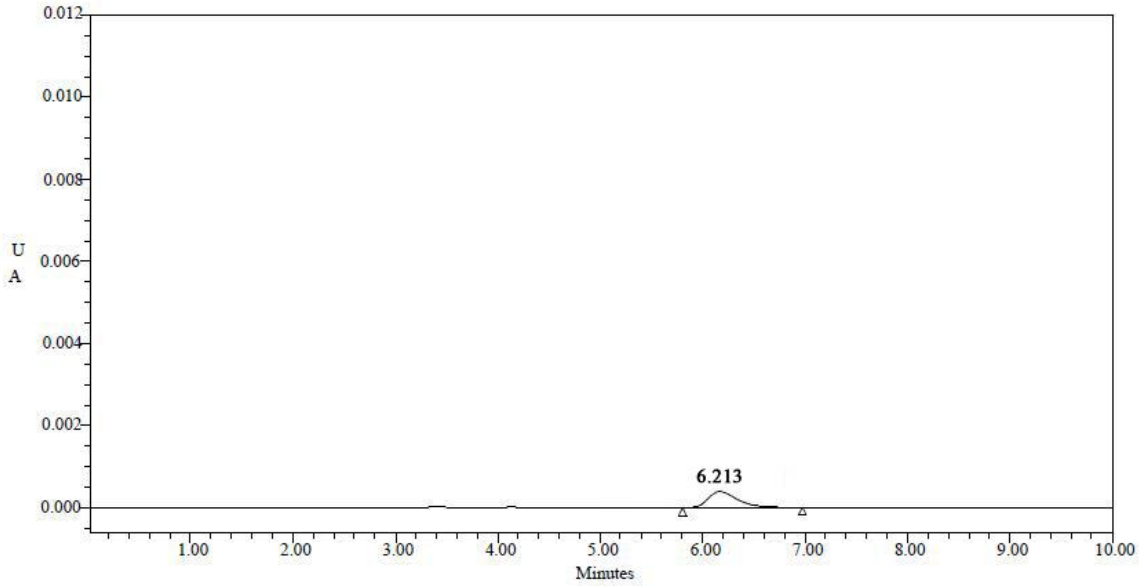

Figure-13: chromatogram of Limit of Quantification of Montelukast

SUMMARY AND CONCLUSION

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost HPLC method. It is successfully applied for the determination of Levocetrizine and Montelukast in pharmaceutical preparations without the interferences of other constituents in the formulations.

In HPLC method, HPLC conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried to get optimum results. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with Buffer: acetonitrile (60:40 v/v) with 0.8 ml/min flow rate is quite robust.

The optimum wavelength for detection was 230 nm at which better detector response for drug was obtained. The average retention time for Levocetrizine and Montelukast was found to be 2.461 and 6.231 min respectively. System suitability tests are an integral part of chroma-tographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were

carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 30 – 70 μg/ml with regression 0.9997 and 0.9994, intercept 5599.1 and 21099 and slope 56910 and 77509 for Levocetrizine and Montelukast respectively. The low values of % R.S.D. indicate that method is precise and accurate. The mean recoveries were found in the range of 99.0 – 101.0 %.

Sample to sample precision and accuracy were evaluated using five samples of same concentration and three samples each of three different concentrations respectively, which were prepared and analyzed on same day. These results show the accuracy and reproducibility of the assay.

Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions; the % R.S.D. reported was found to be less than 2 %.The proposed method was validated and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. Therefore, there is no significant difference in the results achieved by the proposed method.

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