

# Method Development And Validation Of Cetirazine Hcl And Montelukast Sodium

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## **Abstract:**

*The detection, quantification of pharmaceutical products is mainly done by a dominant technique known as HPLC. Chromatographic techniques is used to analyze the parameters and optimize the parameters which are used in the method development and its validation. The present work mainly focuses on the method development and its validation of the Montelukast sodium and Cetirizine hydrochloride. All the parameters optimized has given the précised values.*

**Keywords:** HPLC, validation, Cetirizine hydrochloride, Montelukast sodium.

## **1. INTRODUCTION:**

The wheezing along with shortness of breath due to the asthma is reduced by the Montelukast sodium [1]. Apart from this Montelukast sodium is also used in decreasing the several attacks of asthma. The basic category of Montelukast sodium is a leukotriene inhibitor. The other drug proposed in the present work is cetirizine hydrochloride which is basically act by blocking the histamine receptors and mainly used for treating the allergic rhinitis and runny nose etc [2-5]. Analytical method development is nothing but development of precise method which will full fill all the laboratory requirements by following the standard ICH guidelines [6]. In the proposed research work the development and validation of the Montelukast and cetirizine hydrochloride as been done and the results obtained are precise and according to the laboratory requirements [7].

## **2. MATERIALS AND METHODS**

### *Instrumentation:*

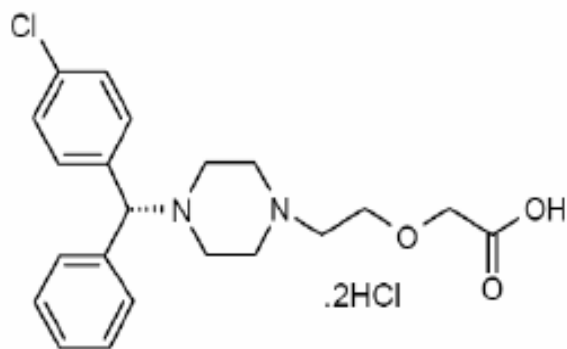
Younglin Acme 9000 HPLC instrument is used for the quantitative analysis. The instrument mainly contains solvent delivery module with UV/Visible-detector, Inertsil extended C18 ODS RP-column (250 X 4.6mm). For its recording, analysis manual injector and window based Autochro 3000 software was used. UV/Vis double beam spectrophotometer SL 160 with "Spectra Treats" software.

### *Chemicals and reagents:*

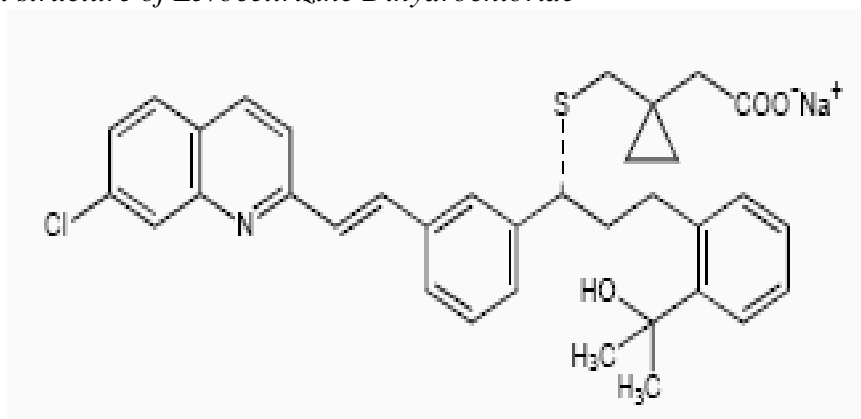
The used glassware for the development of method was of standard quality. The chemicals used were of pharmaceutical grades. The chemicals like methanol and water (milli Q) used for mobile phase preparation was of HPLC grade.

### *Drugs:*

Levocetirizine dihydrochloride and Montelukast sodium reference standards are from Dr. Reddys Laboratories, Hyderabad, India, and Biocon Limited, Bangalore, India respectively as gift samples.



*Chemical structure of Levocetirizine Dihydrochloride*



*Chemical structure of Montelukast sodium*

#### *Wavelength selection:*

Proper wavelength selection will give high compassion of the HPLC method that use the UV detection. Wavelength that gives the best responses for all the drugs is known as ideal wavelength. The Levocetirizine dihydrochloride and Montelukast sodium UV spectrum was detected between the 200-400nm.

#### *Chromatographic method selection:*

The nature of the sample like, molecular weight and stability, ionic/ionisable/neutral molecule helps in selection of proper method. Here reverse phase chromatography is used because the selected drugs are ionic and polar in nature.

#### *Chromatographic condition:*

Equipment used for the development of method was Younglin Acrne 9000 HPLC instrument. The trail was performed by using 0.02M phosphate buffer and Methanol, 20:80 v/v as diluent, C18 Inertsil extended, 250 x 4.6 mm, packed with 5µm as column, 1.5 ml/min as flow rate, 8min run time. Ambient (30°C) column temperature, 20µl injection volume, 7.2 pH, 226nm as wavelength.

#### **VALIDATION:**

The validation of the developed technique was as per the food and drug adultration and ICH guideline and parameters of validation mainly includes, Accuracy, sensitivity ( LOD and LOQ), specificity, range, precision, and robustness.

**Accuracy:** Recovery experiments were performed at 3 concentration levels (80%, 100%, 120%), administration of 3 samples was done for each concentration for determining accuracy of developed method. Percentage recovered of the added Levocetirizine

dihydrochloride and Montelukast sodium and RSD for each replicate sample was quantified [8].

**Precision:** The repeatability of the method and system for methods were resolute by measuring some samples of standard and sample solutions correspondingly. By studying the inter day and intraday variability we determined the precision of the two drugs under study. The response factor of the drug peak and present relative standard deviation were calculated by six repeated injection of the standard sample in HPLC. By this the developed technique was originated to be precise [9].

**Robustness:**

Robustness of process checked by making small and purposeful adjustments to the investigational parameters, like flow rate ( $\pm 0.2$  mL/min), column temp ( $\pm 5^\circ\text{C}$ ), composition of mobile phase, wavelength ( $\pm 3$  nm), composition of organic substance ( $\pm 5\%$ ). Change has been done in assessing the impact on the process. The data obtained for all cases were evaluated by manipulating RSD percentage and recovered percentage [10].

**Sensitivity:**

Detection limit (LOD)/quantitation limit (LOQ) for Levocetirizine HCL and Montelukast.Na was calculated by analyzing different Levocetirizine HCL and Montelukast.Na solutions and calculating the signal-to-noise ratio. The concentration of the LOD that gives a signal-to-noise ratio of roughly 3:1, while the quantification limit (LOQ) is the concentration which gives a signal-to-noise ratio of roughly 10:1 with an RSD < 10% (n=3) [11].

**Linearity:**

Different sample solutions were prepared to test the linearity and specificity of the process by dilute the standard solution with the diluent at various concentrations of Levocetirizine dihydrochloride (10-100  $\mu\text{g/ml}$ ) and Montelukast sodium (20-200  $\mu\text{g/ml}$ ), These diluents was measured at 226nm and chromatograms were recorded and linear relationships were obtain sandwiched between the area of peak and concentration. Obtained regression equations were used for the determinations of the amount of drug [12].

**Specificity:**

Specificity is solitary of the considerable characteristics of Liquid Chromatography and refers to the capability of the analytic technique to differentiate in dynamic mix between analyte and the erstwhile components [13]. Method's specificity was assessed by inject 10  $\mu\text{l}$  normal, sample, blank solutions separately [14-17].

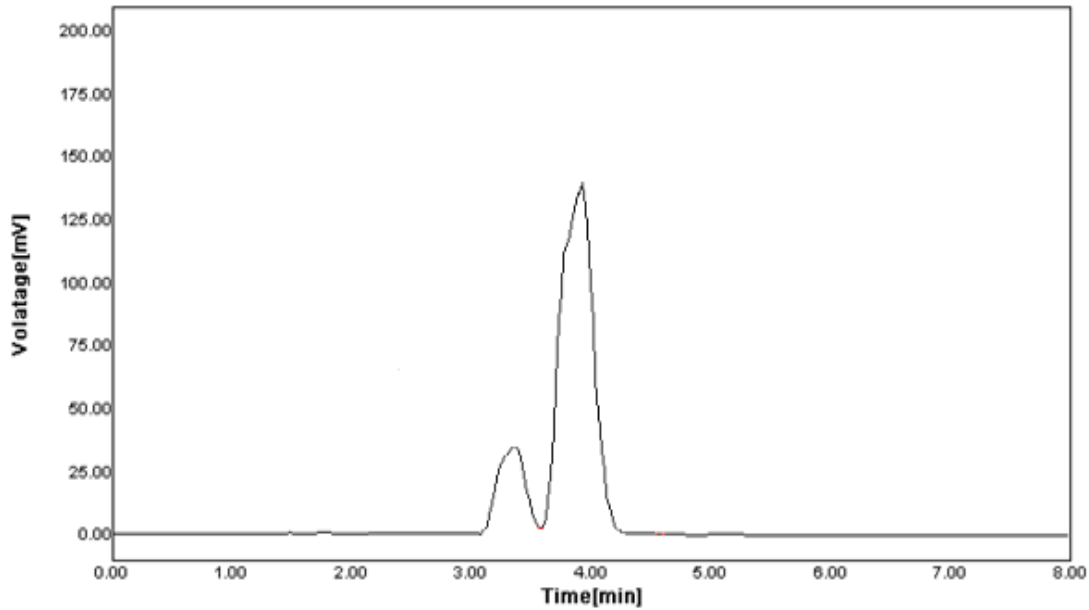
### 3. RESULTS AND DISCUSSION:

*RP-HPLC method development for Levocetirizine dihydrochloride and Montelukast sodium:*

Many trails have been done for obtaining the optimized method development

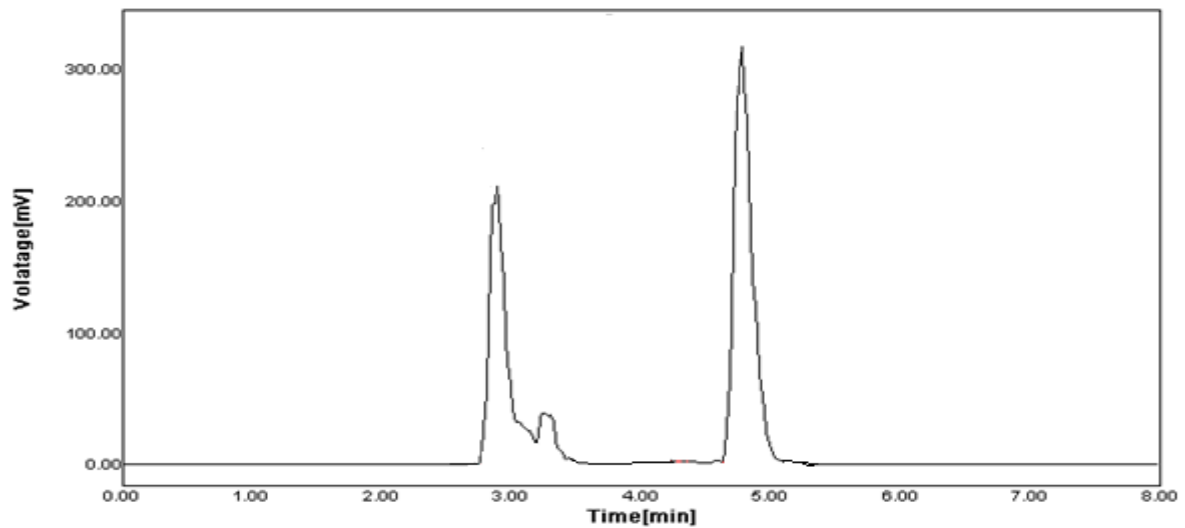
**Trail 1:**

The trail was performed by using 0.02M phosphate buffer and Methanol 20:80 v/v as diluent, C18 Inertsil extended, 250 x 4.6 mm, packed with 5 $\mu\text{m}$  as column, 1 ml/min as flow rate, 8min run time. 30 degree column temperature, 15 $\mu\text{l}$  injection volume, 2.5pH, 232 and 345 as wavelength. The obtained chromatogram by this trail has given merged peaks.



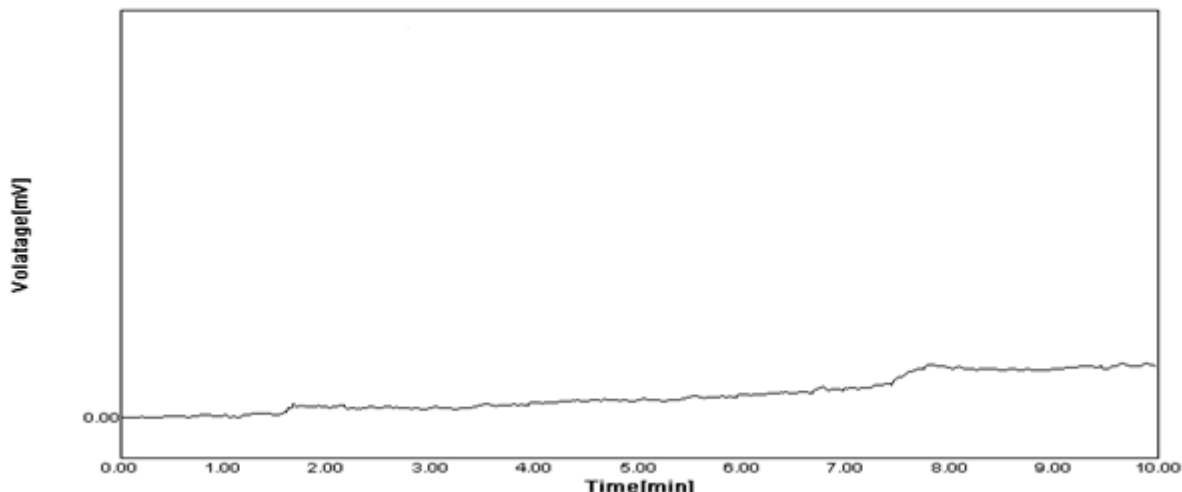
*Trail 2:*

The trail was performed by using 0.02M phosphate buffer :Methanol (20:80 v/v) as diluent, C18 Inertsil extended, 250 x 4.6 mm, packed with 5 $\mu$ m as column, 1.5 ml/min as flow rate, 8min run time. Ambient column temperature, 15 $\mu$ l injection volume, 2.5pH, 228nm as wavelength. The obtained chromatogram by this trail has given merged peaks. The proper tailing and consistency in run time was not obtained by this chromatogram.



*Developed chromatographic conditions:*

The trail was performed by using 0.02M phosphate buffer :Methanol (20:80 v/v) as mobile phase, C18 Inertsil extended, 250 x 4.6 mm, packed with 5 $\mu$ m as column, 1.5 ml/min as flow rate, 8min run time. Ambient column temperature, 20 $\mu$ l injection volume, 7.2 pH, 226nm as wavelength. The obtained chromatogram by this trail has given merged peaks.



Standard Chromatogram

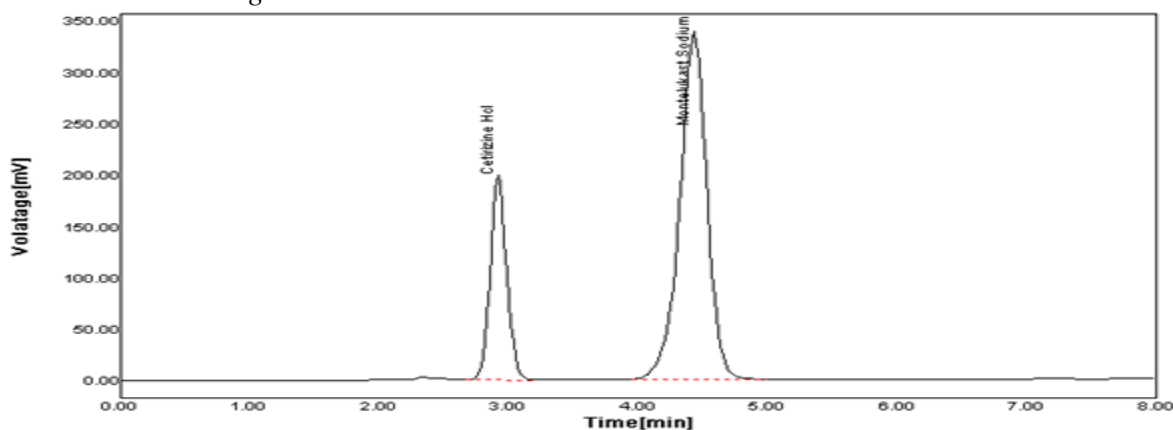


Fig: 4

#### 4. RESULT

The obtained peaks were very sharp and well resolved with good theoretical plate count, with acceptable tailing and short run time. The trail was considered for all the validation parameters were performed. The present optimized chromatographic conditions were used throughout the entire study.

Table 4: Result of Standard Chromatogram

S. No.	Drug	RT(min)	Area(mV*s)	TP	TF	Resolution
1.	Cetirizine Hcl	2.9167	1733112	3096.6	1.0556	0.0000
2.	Montelukast Sodium	4.4333	4900667	5321.6	1.0189	4.8809
Total			6633779			

*Optimization parameters:*

*The selection of pH of phosphate buffer:*

To improve the peak shapes and resolution of drugs, different pH of phosphate buffer were tried. The pH ranging from 2.5 to 7.2 of buffer solution with methanol, the pH was

adjusted by using orthophosphoric acid. This composition is used as mobile phase at different flow rates.

*Effect of flow rate:*

By using above chromatographic conditions the flow rates 1 and 1.5ml were tried.

*Drug estimation in the formulation by using the developed rp-hplc method:*

The main objective of the present study was to determine the assay of Levocetirizine dihydrochloride and Montelukast sodium in the given formulation by using the developed RP-HPLC method.

*Optimized chromatographic parameters:*

Equipment used for the development of method was Younglin Acne 9000 HPLC instrument. The trail was performed by using 0.02M phosphate buffer :Methanol (20:80 v/v) as mobile phase, C18 Inertsil extended, 250 x 4.6 mm, packed with 5 $\mu$ m as column, 1.5 ml/min as flow rate, 8min run time. Ambient (30°C) column temperature, 20 $\mu$ l injection volume, 7.2 pH, 226nm as wavelength.

*Preparation of Phosphate buffer:*

2.73 gms of potassium dihydrogen phosphate was weighed and transferred in to a beaker of 1000ml and dissolve it by adding small amount of water and make up the volume with the remaining amount of water. Adjust the pH by sodium hydroxide.

*Mobile phase preparation:*

Mobile phase was prepared by adding the buffer and methanol in the ratio 20:80 v/v. Degassing is done by sonication for 10min. Filter the prepared mobile phase by passing through 0.45 $\mu$  membrane filter.

*Preparation of standard solution:*

1mg levocetirizine dihydrochloride and 1 mg of Montelukast sodium was weighed accurately in to clean and dry volumetric flask of 10ml separately. 6ml of methanol was added to each volumetric flask and dissolved by applying sonication after that make up the volume with methanol and mark it as standard solution. From the standard solution pipette out 0.5ml of Levocetirizine dihydrochloride and 1.0 ml of Montelukast sodium in to the volumetric flask of 10ml and final volume was made with diluents. The concentration of Levocetirizine dihydrochloride and Montelukast in the resultant solution is 50 $\mu$ g/ml and 100 $\mu$ g/ml respectively.

*Sample solution preparation:*

Calculate mean weight of the tablets containing 5mg of Levocetirizine dihydrochloride and Montelukast sodium by weighing accurately. Transfer the powder equivalent to the 5mg of Montelukast sodium and Levocetirizine dihydrochloride in to the volumetric flask of 10ml which is previously cleaned and dried. Sonicate the resultant mixture for 20min after adding 6ml of methanol. Again add 20ml of methanol and sonicate it for 15min followed by shaking for 10min on a shaker and ensure the complete extraction and filter the solution from 0.45 $\mu$  membrane filter. From above solution pipette 1ml into the volumetric flask of 10ml and dilute it with the diluents up to the mark.

*i) Preparation of 80% solution:*

From the std solution pipette out 0.8ml of Montelukast sodium and 0.4ml of Levocetirizine dihydrochloride in to volumetric flask of 10ml and volume was made with methanol.

*ii) Preparation of 100% solution:*

From the standard solution pipette out 1.0ml of Montelukast sodium and 0.5ml of Levocetirizine dihydrochloride in to volumetric flask of 10ml and volume was made with methanol.

*iii) Preparation of 120% solution:*

From the standard solution pipette out 1.2ml of Montelukast sodium and 0.6ml of Levocetirizine dihydrochloride in to volumetric flask of 10ml and volume was made with methanol.

*Assay procedure:*

The chromatograms were recorded by injecting the 20 $\mu$ l of std and sample solution in to the HPLC. The areas of Levocetirizine dihydrochloride and Montelukast were measured and % assay was calculated.

$$\% \text{ Assay} = \text{AT/AS} \times \text{WS/DS} \times \text{DT/WT} \times \text{P/100} \times \text{Avg.wt/Label claim} \times 100$$

- AT - Average area counts for sample preparation
- AS - Average area counts for standard preparation
- WS - Weight of working standard taken in mg
- WT - Weight of sample taken in mg
- DS - Standard dilution
- DT - Sample dilution
- P - Percentage purity of working standard
- LC - Label claim in mg.

**VALIDATION:**

**1. ACCURACY:**

Sample Name: Cit-Mont-40,80ppm-pH.7.2-2

Accuracy Standard Chromatogram I:

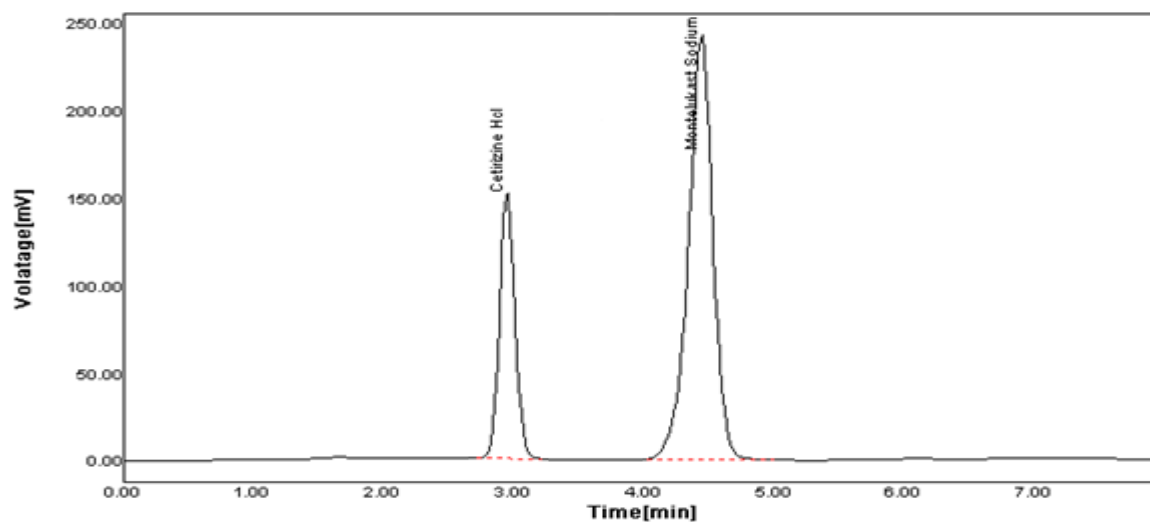


Fig: 5

Sample Name: Cit-Mont-50,100ppm-pH.7.2-2

Accuracy Standard Chromatogram II:

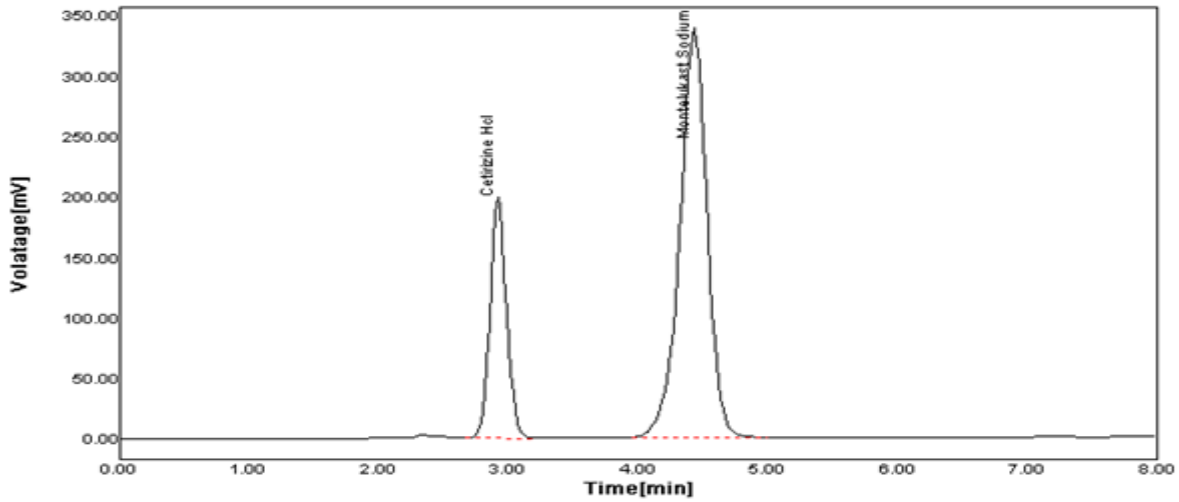


Fig: 6

Sample Name: Cit-Mont-60,120ppm-pH.7.2-2  
 Accuracy Standard Chromatogram III :

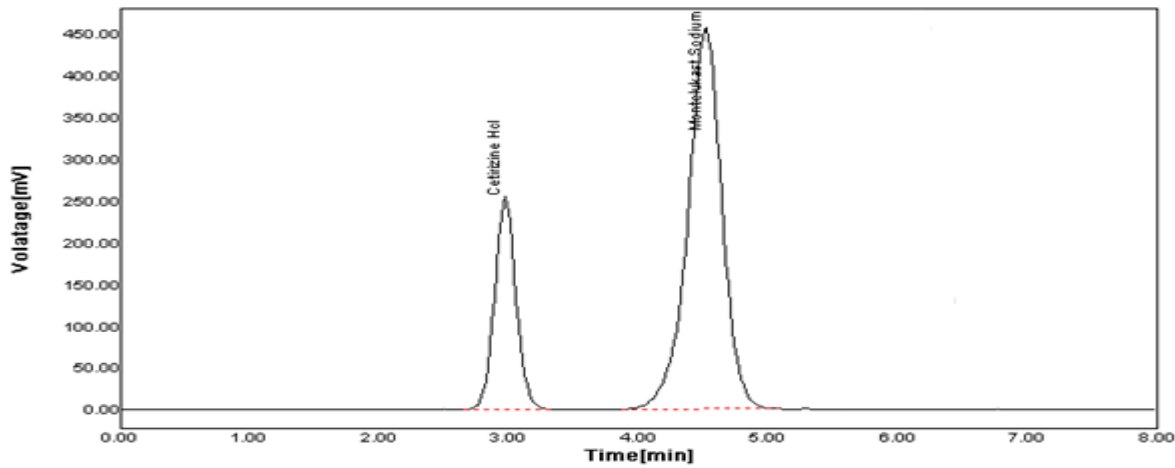


Fig: 7

Cetirizine HCl								
Conc		injec-1	injec-2	injec-3	Avg	Percentage Recovery	STD	Percentage RSD
40pm	80	13714	13725	13736	13725	98.99456	1090.5	0.079452
	%	62	43	43	49		14	
50ppm	100	17338	17315	17331	17328	99.98392	1177.0	0.067924
	%	46	42	12	33		07	
60pm	120	20857	20955	20834	20882	100.4098	6437.0	0.308251
	%	34	73	63	57		73	
Montelukast Sodium								
80ppm	80	38714	38725	38736	38725	98.77608	1090.5	0.02816
	%	62	43	43	49		14	
100ppm	100	49012	49019	49015	49015	100.0186	352.38	0.007189
	%	45	47	42	78		19	
120ppm	120	59357	59455	59234	59349	100.9203	11077.	0.186646
	%	34	73	63	23		27	

Accuracy parameter for Levocetirizine di.HCl and Montelukast Sodium



**Result:** The % Recovery for each level obtained for Levocetirizine dihydrochloride was 99.79609% and Montelukast Sodium was 99.90499%.

**Acceptance criteria:** 98.0% to 102.0%.

**2. PRECISION:**

	injection - 1	injection -2	Avg	mean	Standard deviation	% relative standard deviation
<b>CETIRIZINE HCl</b>						
Method precision-1	1738259	1739825	1739042	1738589.8	3740.8627	0.2151665
Method precision - 2	1747284	1741947	1744615.5			
Method precision - 3	1739264	1738245	1738754.5			
Method precision - 4	1738265	1739542	1738903.5			
Method precision - 5	1735876	1733846	1734861			
Method precision - 6	1732798	1737926	1735362			
<b>MONTELUKAST SODIUM</b>						
	injection - 1	injection -2	Avg	mean	Standard deviation	% Relative standard deviation
Method precision - 1	4918259	4909825	4914042	4909423.1	6108.9075	0.12443229
Method precision - 2	4907284	4901947	4904615.5			
Method precision - 3	4909264	4908245	4908754.5			
Method precision - 4	4918265	4919542	4918903.5			
Method precision - 5	4905876	4903846	4904861			
Method precision - 6	4902798	4907926	4905362			

**Method Precision parameter for Cetirizine HCl and Montelukast Sodium**

**Acceptance criteria:** Should not be more than 2%

**INJECTION PRECISION (IP):** prepare standard solution and from that give six repeatable injections. And calculate the %RSD.

*IP Chromatogram-1*

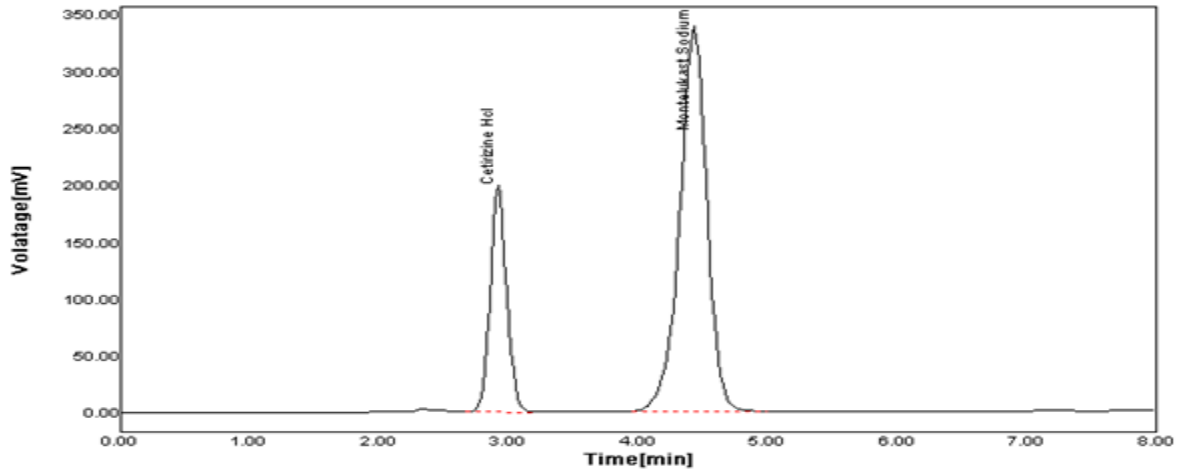


Fig: 8

*IP Chromatogram-2*

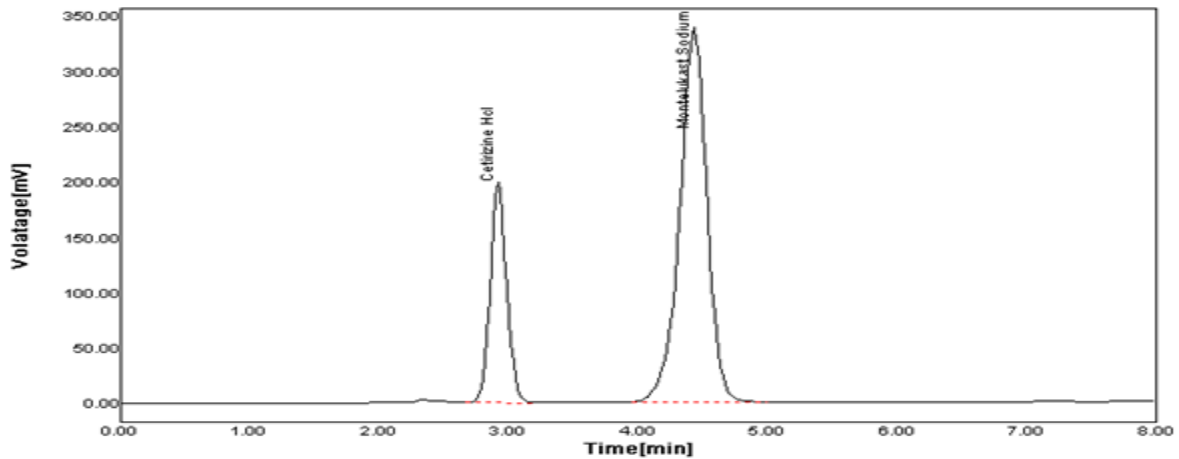


Fig: 9

*IP Chromatogram-3*

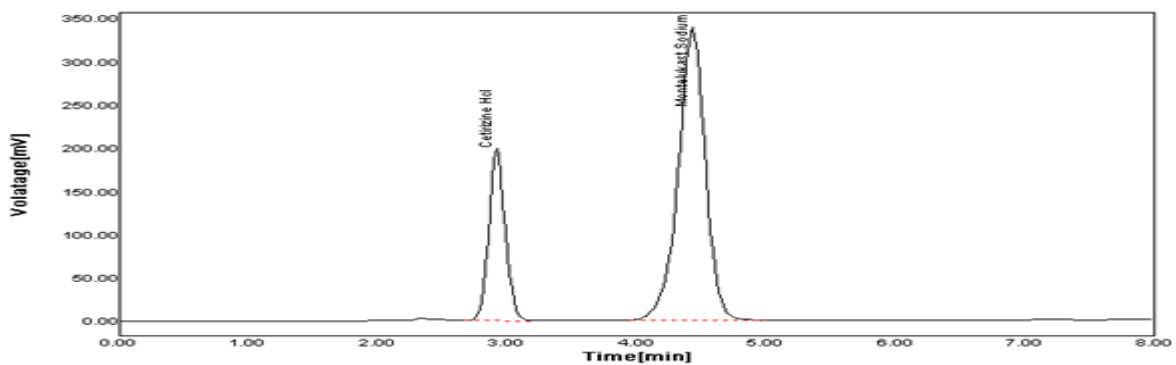


Fig: 10

IP Chromatogram 4

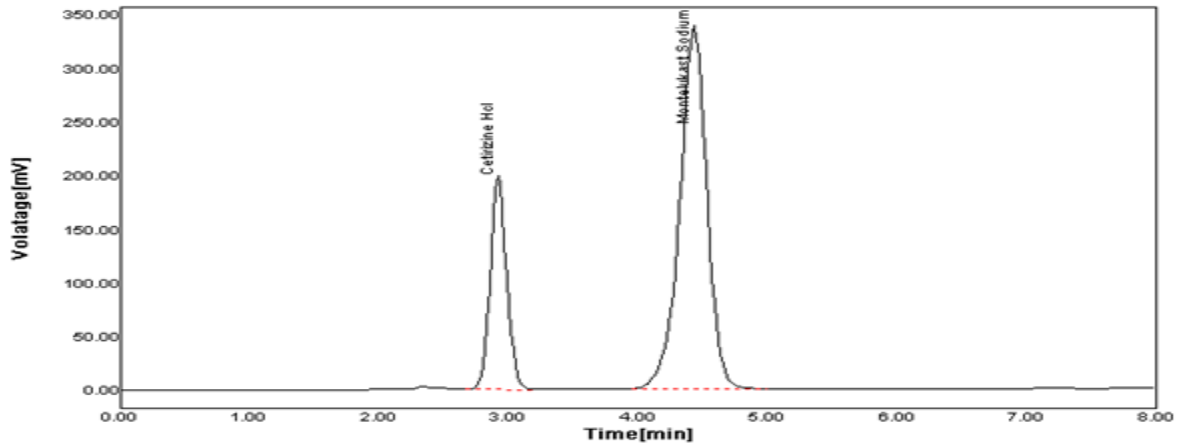


Fig: 11

IP Chromatogram-5

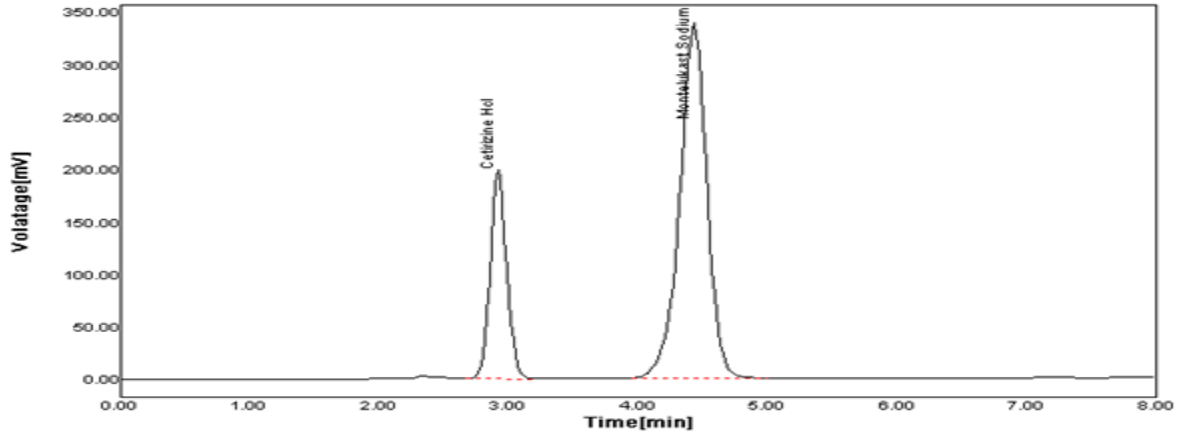


Fig: 12

IP Chromatogram-6

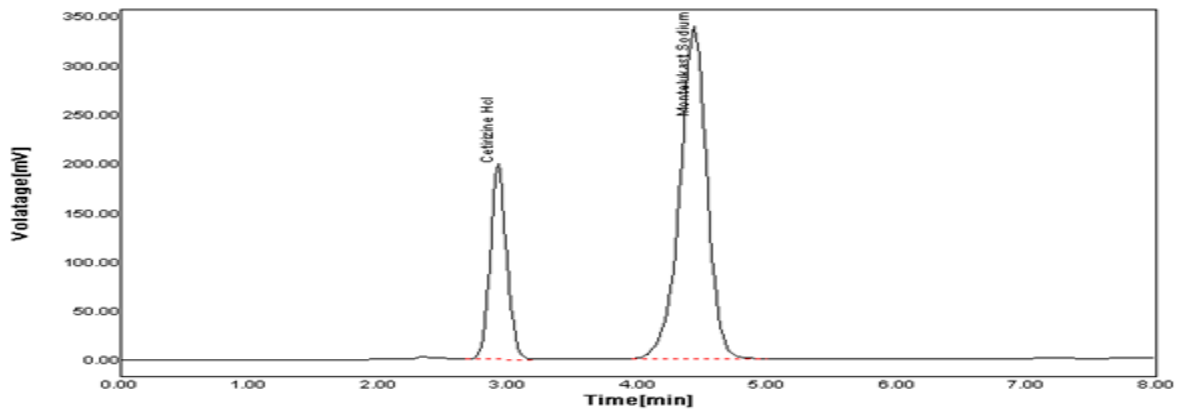


Fig: 13

IP	Cetirizine HCl	Montelukast Sodium
IP-1	1733112	4900667
IP-2	1732798	4902798

<b>IP-3</b>	1738245	4908245
<b>IP-4</b>	1733846	4903846
<b>IP-5</b>	1731947	4901947
<b>IP-6</b>	1731542	4901542
<b>Mean</b>	1733582	4903174
<b>Standard deviation</b>	2428.318	2711.531
<b>% relative standard deviation</b>	0.140075	0.055302

*Injection Precision parameter for Cetirizine HCl and Montelukast Sodium  
Acceptance criteria: not more than 2%.*

**3. LINEARITY:**

<b>Cetirizine Hcl Conc. (ppm)</b>	<b>Average</b>	<b>Montelukast Sodium Conc. (ppm)</b>	<b>Average</b>
<b>1</b>	22075	<b>2</b>	69972
<b>10</b>	363516	<b>20</b>	896851
<b>25</b>	867739	<b>50</b>	2515663
<b>40</b>	1382973	<b>80</b>	3984728
<b>50</b>	1733112	<b>100</b>	4900667
<b>60</b>	2195447	<b>120</b>	6014402
<b>80</b>	2855561	<b>160</b>	7864189
<b>100</b>	3544192	<b>200</b>	9801418

**Linearity parameter for Cetirizine HCl and Montelukast Sodium**

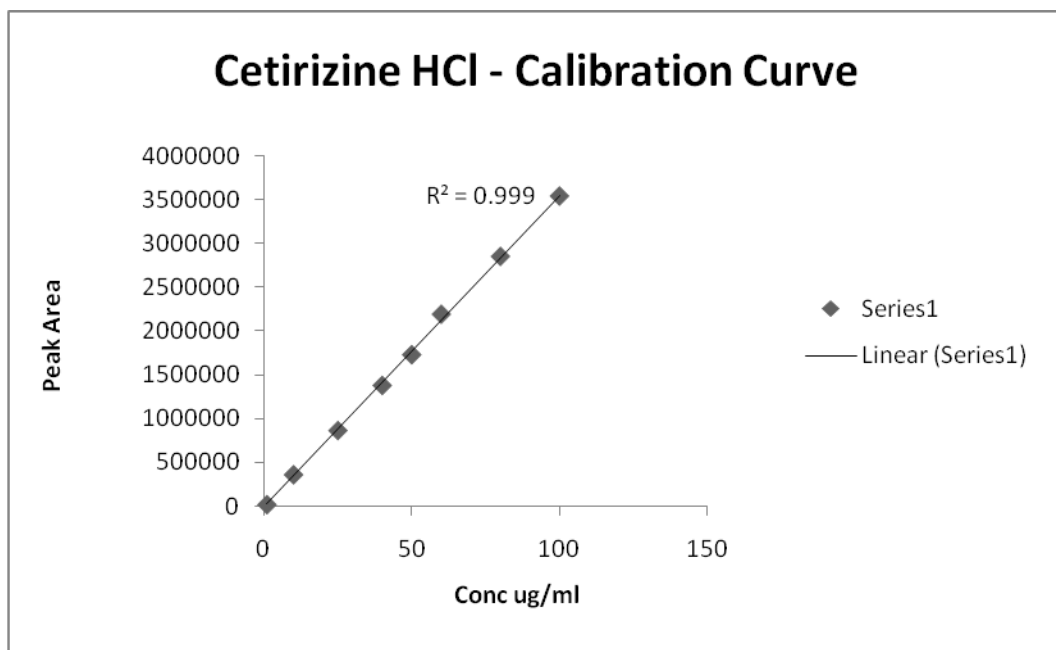


Fig. 14:Cetirizine Hcl linearity curve

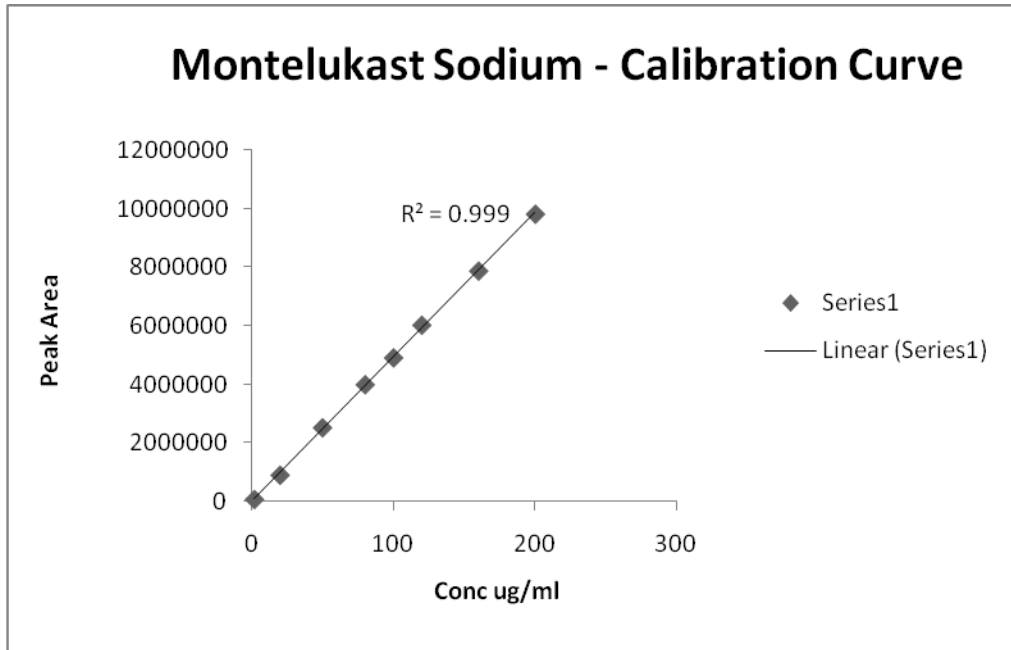


Fig: 15:Montelukast Sodium linearity curve

Sample Name: Cit-Mont-1,2ppm-pH.7.2  
Linearity Chromatogram 1:

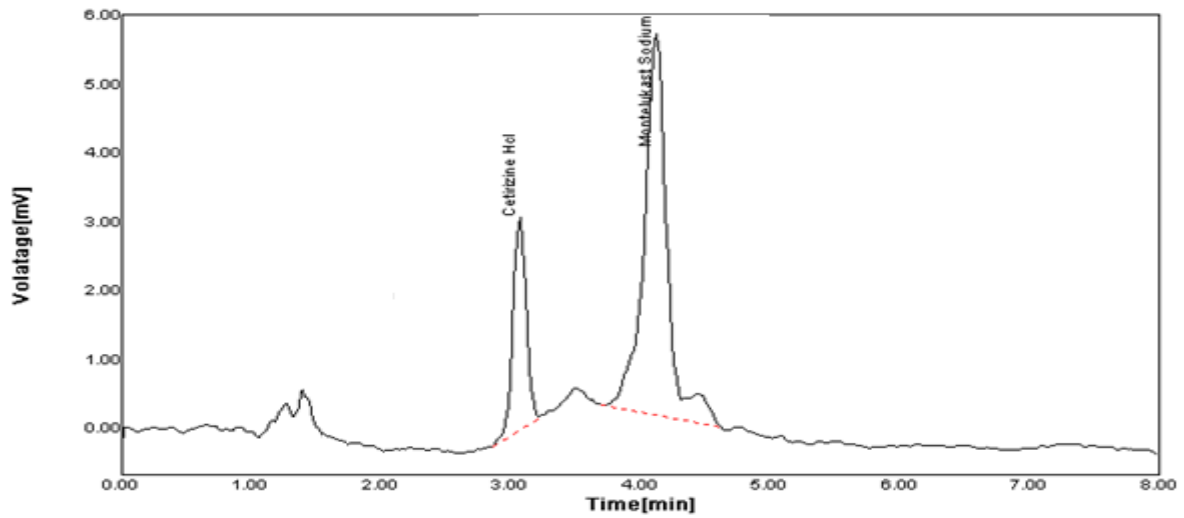


Fig: 16

Sample Name: Cit-Mont-10,20ppm-pH.7.2  
Linearity Chromatogram 2:

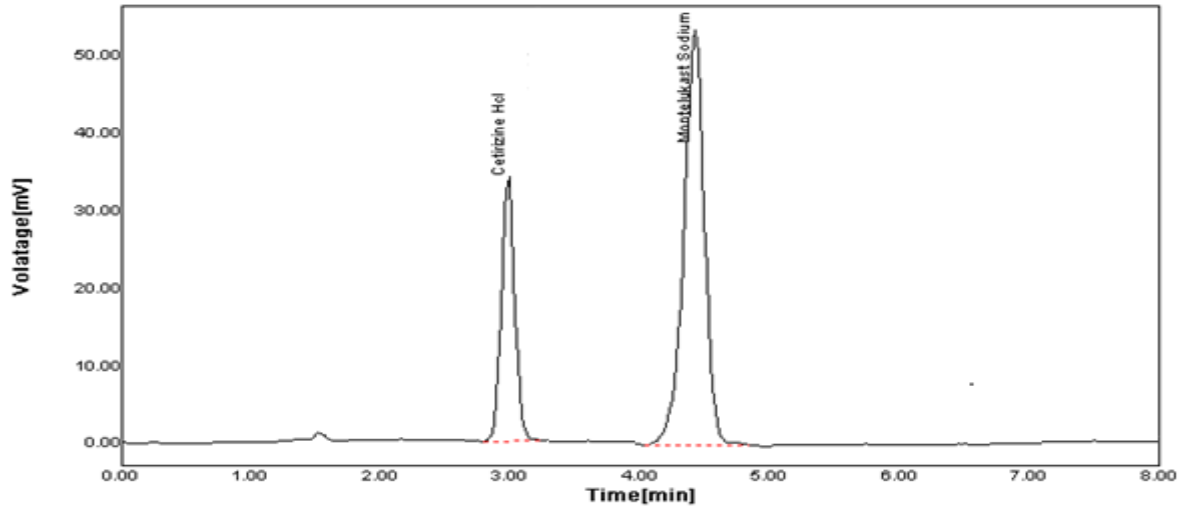


Fig: 17

Sample Name: Cit-Mont-25,50ppm-pH.7.2  
Linearity Chromatogram 3:

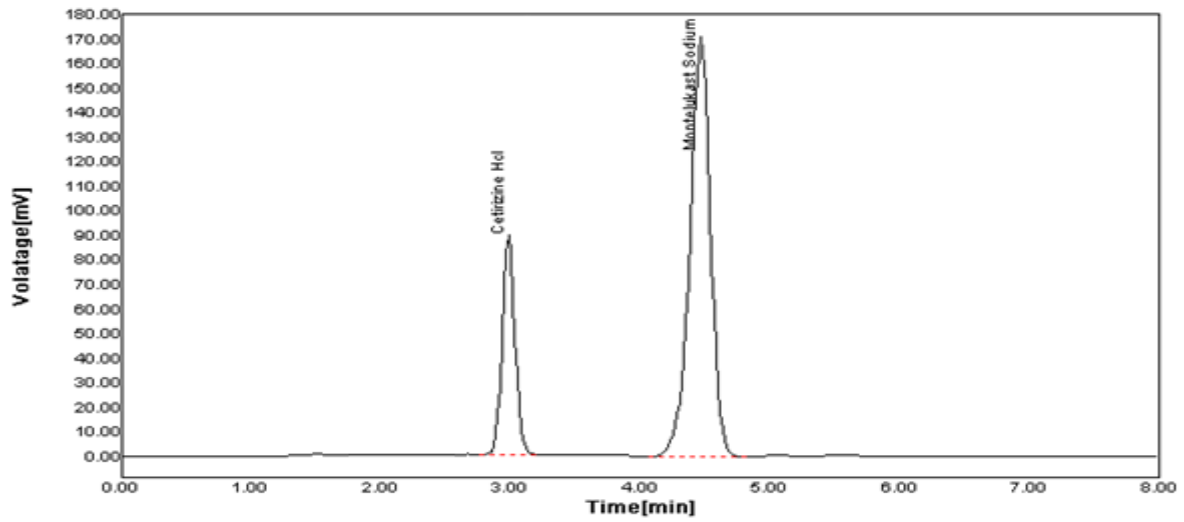


Fig: 18

Sample Name: Cit-Mont-40,80ppm-pH.7.2-2  
Linearity Chromatogram 4:

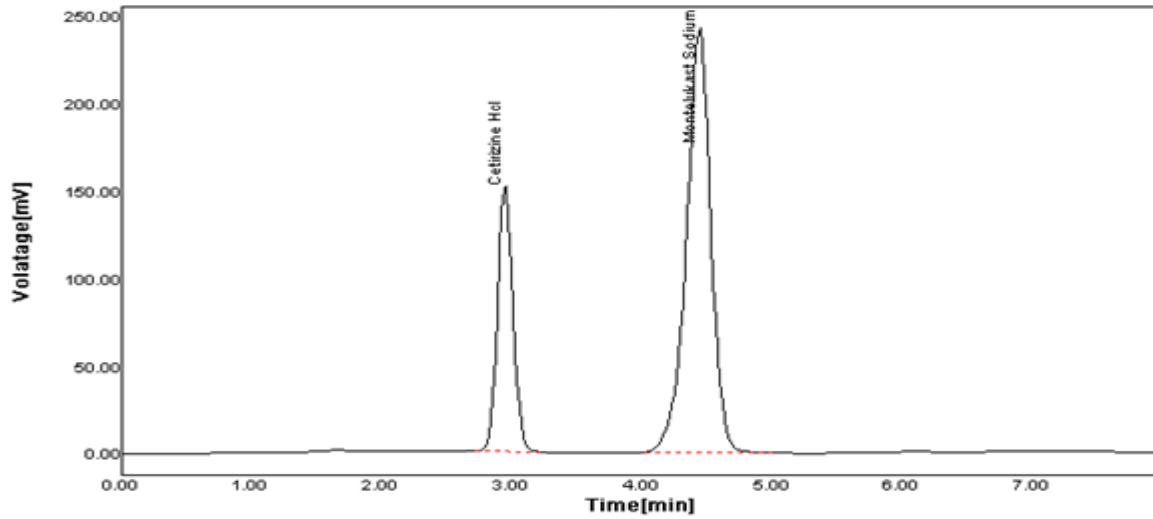


Fig: 19

Sample Name: Cit-Mont-50,100ppm-pH.7.2-2  
Linearity Chromatogram 5:

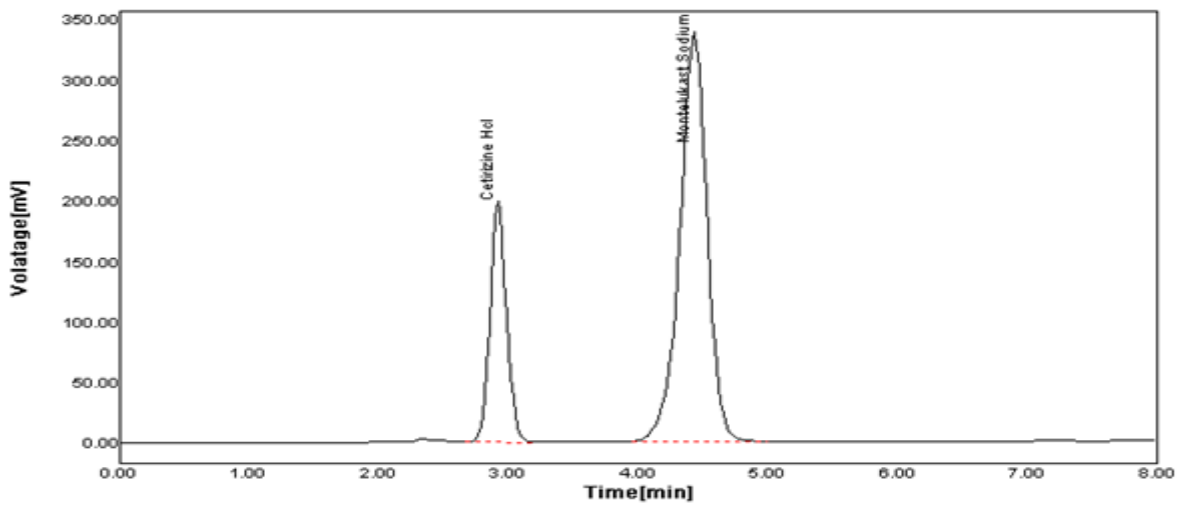


Fig: 20

Sample Name: Cit-Mont-60,120ppm-pH.7.2-2  
Linearity Chromatogram 6:

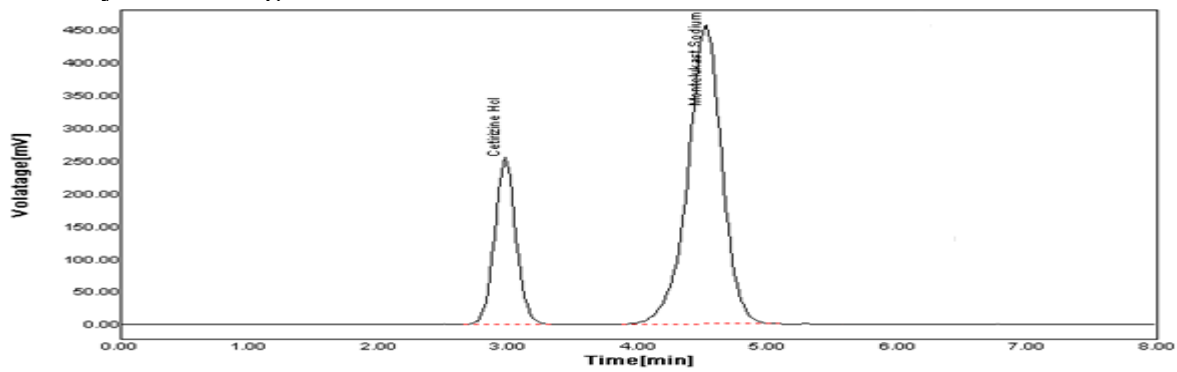


Fig: 21

Sample Name: Cit-Mont-80,160ppm-pH.7.2-2  
 Linearity Chromatogram 7:

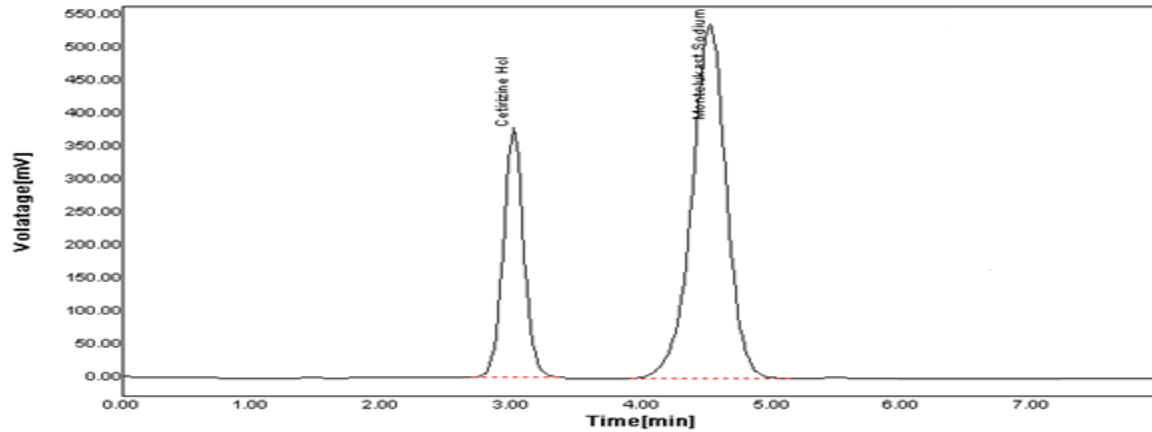


Fig: 22

Sample Name: Cit-Mont-100,200ppm-pH.7.2-2  
 Linearity Chromatogram 8:

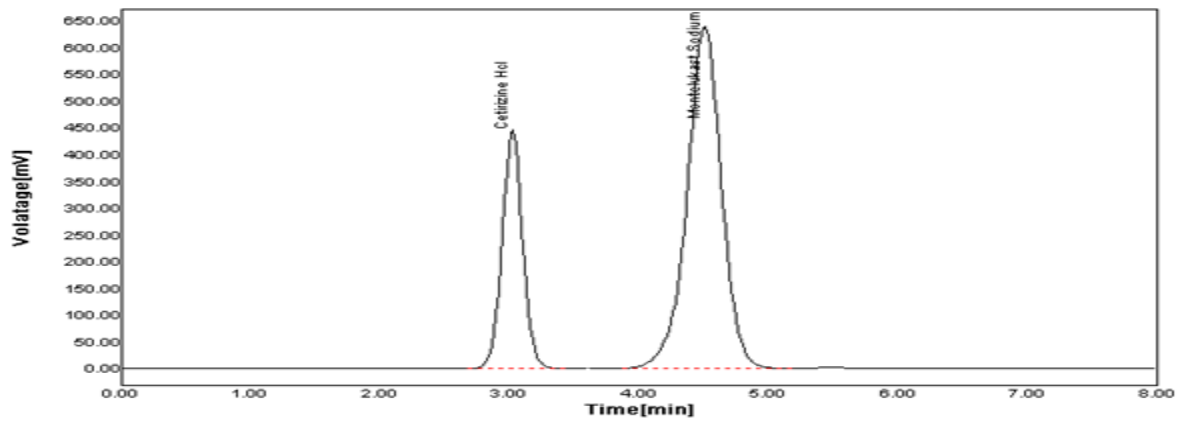


Fig: 23

Table No. 9 : regression characteristics of the linearity plot of Cetirizine HCl and Montelukast Sodium

Parameter	Values(Cetirizine HCl)	Values(Montelukast Sodium)
Linearity range (µg/ml)	1-100	2-200
Slope	37937.89	49328.87
Intercept	-14257.4	-7605.77
Correlation coefficient	0.999	0.999
Regression equation	$Y = 37937.89 X - 14257.4$	$Y = 49328.87 X - 7605.77$



Acceptance criteria: 'r' should be not less than 0.999.

#### 4. SPECIFICITY:

Standard chromatogram

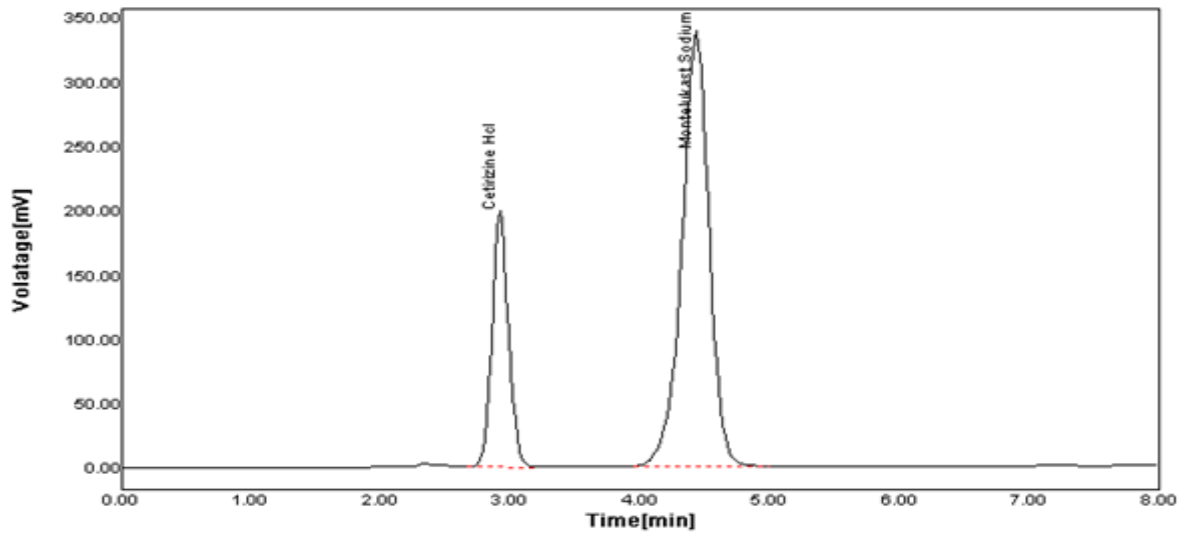


Fig: 24

Sample chromatogram:

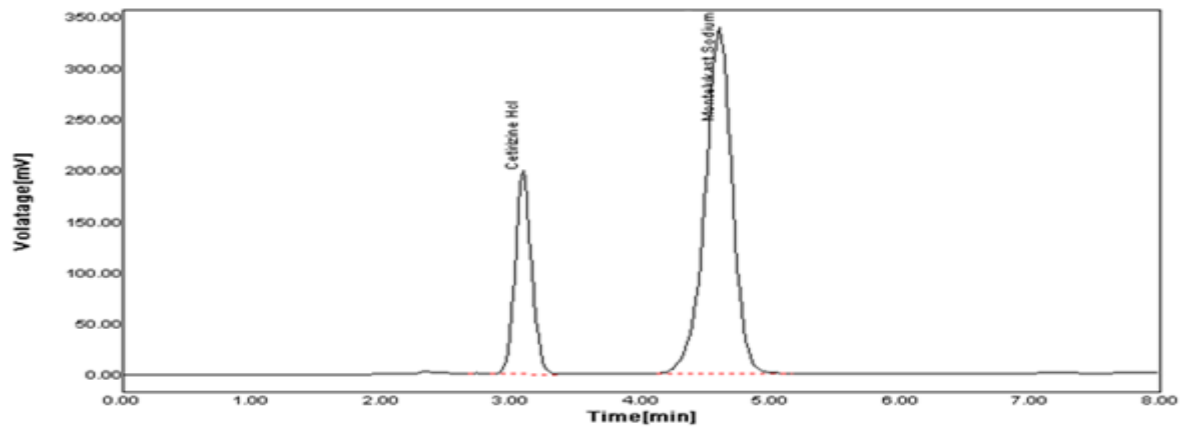


Fig: 25

Blank chromatogram:

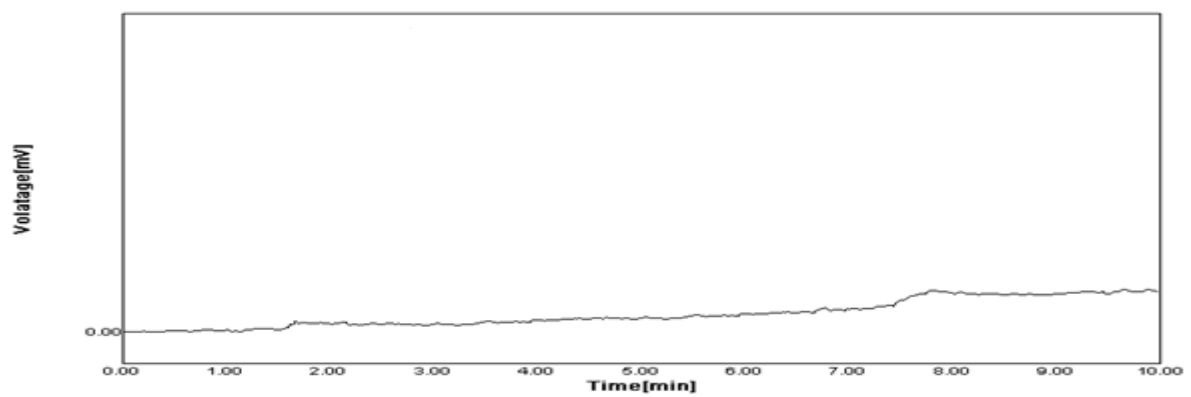


Fig: 26

Placebo chromatogram

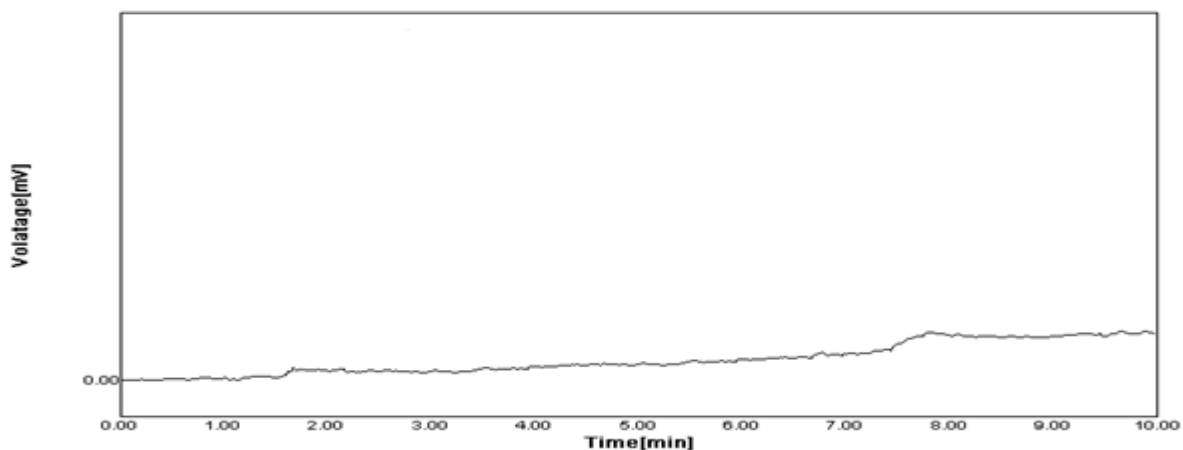


Fig: 27

### 5. LOD AND LOQ:

LOD were found to be 0.3 µg/ml for the Levocetirizine HCl and 0.4 µg/ml for Montelukast sodium LOQ were found to be 0.9 µg/ml for Levocetirizine HCl and 1.23 µg/ml for Montelukast sodium.

Table No.5.10: Summary of results of LOQ for Cetirizine HCl and Montelukast Sodium

Injec	Area of Ceti. (0.3ppm)	Area of Monte. (0.6ppm)
Injec-1	6704	18894
Injec-2	6797	18497
Injec-3	6671	18671
Injec-4	6798	18598
Injec-5	6783	18783
Injec-6	6687	18687
Mean	6740	18688.33
SD	58.86935	138.803
% RSD	0.873432	0.742725

Sample Name: Cit-Mont-0.3,0.6ppm-pH.7.2-2

### LOD Chromatogram

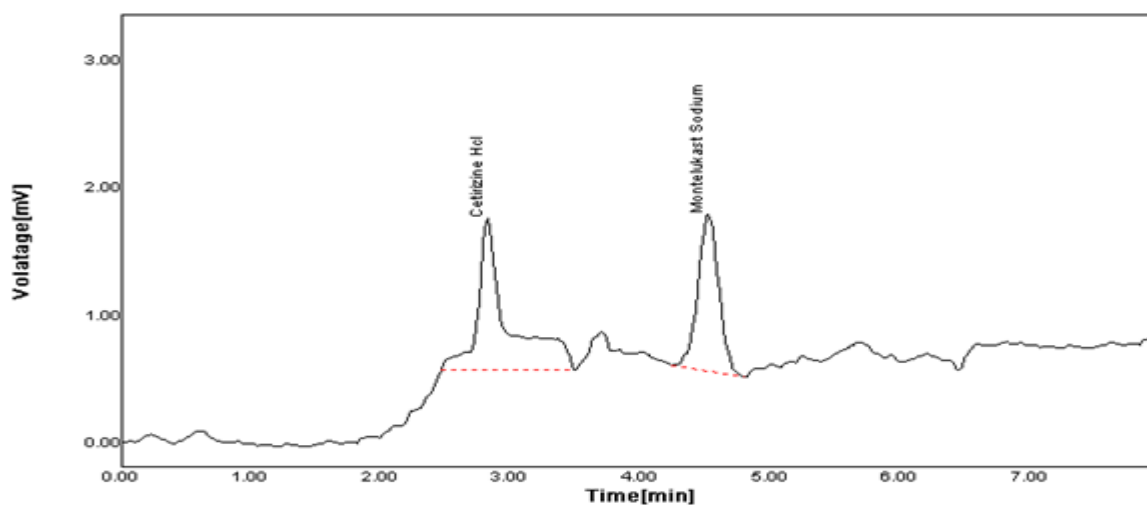


Fig: 27

## 6. ROBUSTNESS:

<b>Cetirizine HCl</b>					
<b>Parameters</b>	<b>Adjusted TO</b>	<b>Average Area<sup>a</sup></b>	<b>Retention time</b>	<b>Std deviation</b>	<b>% relative standard deviation</b>
<b>Flow Rate As per method 1.5ml/min</b>	<b>1.4 ml/min</b>	2135126.7	3.26	9505.82	0.45
	<b>As it is</b>	1737541.5	2.92	2562.12	0.15
	<b>1.6ml/min</b>	1362340.33	2.48	4341.03	0.32
<b>Mobilephase composition(Buffer: Methanol; 20:80)</b>	<b>Buffer: Methanol; 15:85)</b>	1985716.50	2.56	3784.67	0.19
	<b>As it is</b>	1737541.5	2.92	2562.12	0.15
	<b>Buffer: Methanol; 25:75)</b>	1483250.00	3.15	3340.39	0.23
<b>Montelukast Sodium</b>					
<b>Flow Rate As per method 1.5ml/min</b>	<b>Adjusted TO</b>	5370501.7	4.83	12826.87	0.24
	<b>1.4 ml/min</b>	4906217.3	4.42	2902.38	0.06
	<b>As it is</b>	4488153	3.96	7390.12	0.16
<b>Mobilephase composition(Buffer: Methanol; 20:80)</b>	<b>1.6ml/min</b>	5247538.83	4.13	15528.59	0.30
	<b>Buffer: Methanol; 15:85)</b>	4906217.3	4.42	2902.38	0.06
	<b>As it is</b>	4577340.2	4.78	8126.60	0.18

Robustness parameter results for Cetirizine HCl and Montelukast Sodium

Avg. Area = Six Repeatable injections.

## 7. SYSTEM SUITABILITY TESTING:

Parameters like TP, TF and resolution was determined by injecting Montelukast sodium and Levocetirizine HCl mixed standard preparation in replicate.

<b>Conc. of Ceti &amp; Mont</b>	<b>Injec</b>	<b>Area (Cetirizine)</b>	<b>Retention Time</b>	<b>Area (Monteluka st)</b>	<b>Retention Time</b>
<b>25 &amp; 50ppm</b>	<b>Injec-1</b>	1739825	2.91	4909825	4.433
	<b>Injec-2</b>	1741947	2.93	4901947	4.432
	<b>Injec-3</b>	1738245	2.91	4908245	4.423
	<b>Injec-4</b>	1739542	2.92	4919542	4.433
	<b>Injec-5</b>	1733846	2.92	4903846	4.434
	<b>Injec-6</b>	1737926	2.91	4907926	4.432
<b>Statistical Analysis</b>	<b>Mean</b>	1738555.167	2.916667	4908555.167	4.431167
	<b>SD</b>	2711.426002	0.008165	6142.624111	0.00407
	<b>% Relative standard deviation</b>	0.155958583	0.279942	0.125141185	0.091854
	<b>TF</b>	1.0556		1.0189	
	<b>PC</b>	3096.6		5321.6	

Suitability of the system for Cetirizine HCl and Montelukast Sodium

Acceptance Criteria: TF > 2, PC < 2000, Resolution 2.

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