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# Simultaneous RP-HPLC Estimation of Levocetirizine Hydrochloride and Montelukast Sodium in Tablet Dosage Form

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**Abstract:** In the present study a simple, accurate and precise reverse phase liquid chromatographic method has been developed for simultaneous estimation of Levocetirizine Hydrochloride and Montelukast Sodium from tablet dosage form. The method was developed using Waters HPLC system on a L7 column (Hypersil Gold: 250mm x 4.6 mm, 5µm) using a mixture of 0.05 (M) Potassium Dihydrogen Phosphate Buffer of pH 7.5 and Methanol in the ratio 20:80 v/v as mobile phase in an isocratic elution mode at a flow rate of 1.2 ml/min, at 35ºC with a load of 10µl. The detection was carried out at 225 nm. The retention time of Levocetirizine and Montelukast were found to be around 3.2 min and 4.2 min respectively. The method was validated with respect to linearity, robustness, precision and accuracy and was successfully applied for the simultaneous quantitative determination of Levocetirizine Hydrochloride and Montelukast Sodium from the tablet dosage form.

**Keywords:** Levocetirizine Hydrochloride, Montelukast Sodium, RP-HPLC.

## INTRODUCTION AND EXPERIMENTAL

Levocetirizine is the *R*-enantiomer of Cetirizine, a non sedating antihistamine and used for the treatment for the symptomatic relief of allergic conditions including rhinitis and chronic urticaria [1]. Chemically it is 2-[2-[4-[(*R*)-(4-chlorophenyl)-phenyl-methyl] piperazin-1-yl]ethoxy]acetic acid dihydrochloride. Montelukast is a potent cysteinyl leukotriene receptor antagonist which is being used very effectively in the treatment of chronic asthma and chemically it is 2- [1-[(R)-[3-[2(E)-(7-chloroquinolin-2-yl) vinyl] phenyl] - 3-[2- (1-hydroxy-1-methylethyl) phenyl] propyl - sulfanylmethyl] cyclopropyl] acetic acid sodium salt. It has been found to be very effective for asthma in 6-14 years children [2].

Literature survey reveals separate HPLC method of analysis for Levocetirizine Hydrochloride in bulk as well as from tablet is available [3]. Furthermore HPLC assay methods for Montelukast in human plasma were established [4-5]. Several methods [6-9] also have been reported for simultaneous determination of Levocetirizine Hydrochloride and Montelukast Sodium from various formulations which include TLC, Ratio Derivative Spectroscopy, HPTLC as well as HPLC. The objective of this work is to develop an accurate, specific, repeatable and validated HPLC method for simultaneous determination of

Levocetirizine dihydrochloride and Montelukast Sodium from tablet dosage form. The proposed method was validated as per the International Conference on Harmonization (ICH) guidelines [10].

## MATERIALS AND METHODS

Methanol used was of HPLC grade of Merck and Milli Q water was used for the preparation of the mobile phase. All other reagents like $KH_2PO_4$, KOH, $H_3PO_4$ used were of AR/GR grade. All the glass wares used were of standard quality.

### Drugs used

Levocetirizine dihydrochloride (Assay: 100.91%, $H_2O$: 0.33%) and Montelukast sodium (Assay: 98.6%, Loss on Drying: 0.039%) reference standards of The Central Drugs Laboratory, Kolkata (Govt. of India) were used. Tablet formulation containing Levocetirizine dihydrochloride 5 mg and Montelukast sodium equivalent to Montelukast 10 mg was used as the sample during the method development process.

### Instrumentation

An Isocratic Waters HPLC with a 515 pump, 2487 dual λ UV-Visible detector and L7 column (Hypersil Gold: 250mm x 4.6mm, 5µm) were used for the analysis. The HPLC system was well equipped with Empower 2 software for data processing.

### Chromatographic Condition

The mixture of 0.05 (M) Potassium Dihydrogen Phosphate Buffer of pH 7.5 (adjusted with 10% $H_3PO4$/ 10% KOH) and Methanol in the ratio 20:80 v/v was used as mobile phase. The buffer was filtered through 0.22 micron membrane prior to mix with Methanol. The mobile phase was ultrasonicated for 5 minutes to degas the mixture and then used. The separation was achieved on a L7 column (Hypersil Gold: 250 mm x 4.6mm, 5µm). The flow rate of 1.2 ml/min was set for the isocratic elution and detection was carried out at 225 nm. All determinations were performed at a constant column temperature of 35ºC with a load of 10µl. The mobile phase was used as the diluent for the preparation of sample and standard solutions. The summary of chromatographic condition is given in TableI.

### Solutions Preparation

A mixed standard solution was prepared by dissolving 5.5 mg Levocetirizine 2HCl and 10 mg Montelukast sodium in 50 ml mobile phase in order to obtain a working concentration of Levocetirizine 2HCl around 0.1 mg/ml and Montelukast around 0.2 mg/ml.

## RESULTS AND DISCUSSION

### Method Development

Different composition of mobile phases containing a mixture (v/v) of $KH_2PO_4$ Buffer and Methanol were tried but the mixture of 0.05 (M) $KH_2PO_4$ Buffer of pH 7.5 and Methanol in the ratio 20:80 v/v was selected as optimal for obtaining well defined and well resolved peaks of Levocetirizine and Montelukast at a flow rate of 1.2 ml/min at 35ºC on a L7 column. 225 nm was selected as the optimum wavelength for detection and quantitation, at which best detector response for both Levocetirizine and Montelukast were obtained. The mean retention time and standard deviation for Levocetirizine and Montelukast were found to be 3.2± 0.002 min and 4.2 ± 0.01 min.

### Tablet Assay

Twenty tablets were weighed and powdered. An accurately weighed quantity of tablet powder equivalent to about 5mg Levocetirizine 2HCl and 10 mg Montelukast was transferred to 50 ml volumetric flask and 30 ml of mobile phase was added to it. The solution was then sonicated for 10 minutes. Finally the volume was made up to the 50 ml mark with the diluent. Solution was mixed and filtered through 0.22 µm membrane filter and then analyzed under optimized chromatographic condition. A representative chromatogram of mixed standard solution of Levocetirizine 2HCl (~0.1 mg/ml) and Montelukast (~0.2 mg/ml) is given in Figure 1.The average result of six estimations is given in Table II.

**Table I: Summary of chromatographic condition:**

| Parameters | Condition |
|---|---|
| Column | Hypersil Gold L7 (4.6 x 250 mm, 5µm) |
| Mobile Phase | 0.05 M $KH_2PO_4$ Buffer pH 7.5: Methanol 20: 80 v/v |
| Flow Rate | 1.2 ml/min |
| Temperature | 35°C |
| Detection Wavelength | 225 nm |
| Injection Volume | 10 µl |
| Diluent | Mobile phase |

**Figure 1 Representative chromatogram**

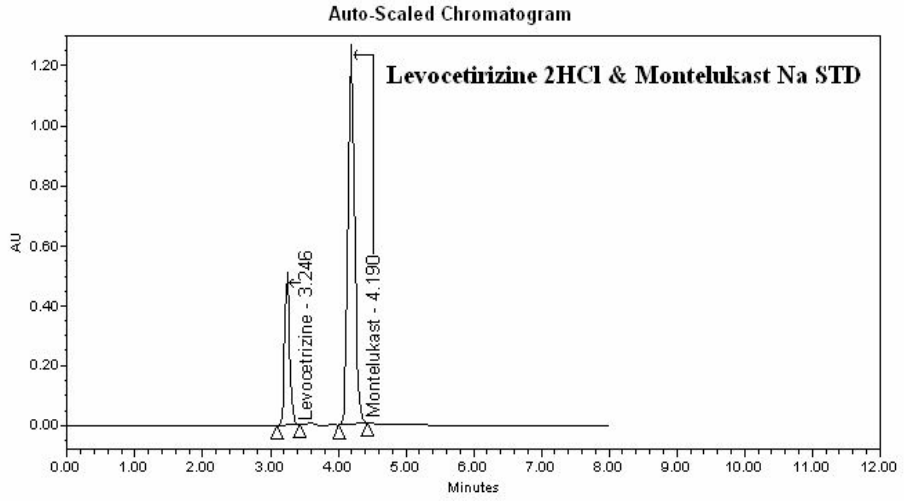
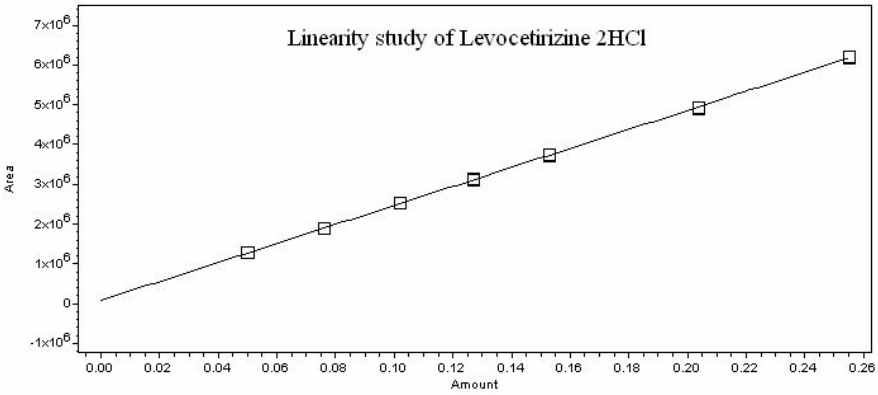
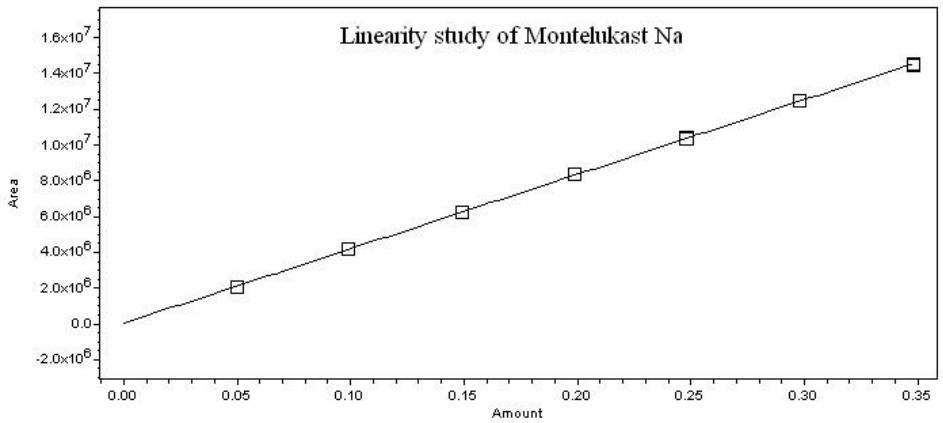



**Figure 2: Linearity of Levocetirizine 2HCl**




Peak Name: Levocetrizine 2HCl; RT: 3.236; Fit Type: Linear (1st Order); Cal Curve Id: 3061; R: 0.999941; R^2: 0.999883; Weighting: None; Equation: Y = 2.39e+007 X + 8.12e+004; Normalized Intercept/Slope: 0.022273; RSD(E): 0.521723

**Figure 3 Linearity of Montelukast Sodium**




Peak Name: Montelukast; RT: 4.116; Fit Type: Linear (1st Order); Cal Curve Id: 3019; R: 0.999973; R^2: 0.999947; Weighting: None; Equation: Y = 4.16e+007 X + 4.36e+004; Normalized Intercept/Slope: 0.005266; RSD(E): 0.380759

**Method Validation**

**Linearity**

The linearity of the method is the ability to elicit test results that are directly proportional to the concentration of the analyte in samples. The linearity study was made from a series of standard solutions of Levocetirizine and Montelukast. For Levocetirizine suitable volumes of stock solution was diluted to obtain a series of solutions having concentration of 0.05, 0.076, 0.102, 0.127, 0.153, 0.204 and 0.255 mg/ml of Levocetirizine 2HCl. Again for Montelukast a standard stock solution was diluted suitably to obtain a series of solutions having concentration of 0.05, 0.099, 0.149, 0.199, 0.248, 0.298 and 0.348 mg/ml of Montelukast. Each solution was injected in replicate and chromatograms were recorded. The average peak areas were plotted against concentration to obtain calibration curves for Levocetirizine 2HCl and Montelukast. The Calibration curves were linear in the range 10-260 mcg/ml for Levocetirizine 2HCl and 10-350 mcg/ml for Montelukast. The calibration curves for Levocetirizine 2HCl and Montelukast are shown in Figure 2 & Figure 3 and Linear Regression Analysis results are summarized in Table III.

**Precision**

Precision study was assessed by injection repeatability and sample repeatability tests. For injection repeatability mixed standard solution of Levocetirizine 2HCl and Montelukast Sodium was injected in replicate. For sample repeatability study, six sample solutions of tablet were prepared following the method described under Tablet Assay and they were assayed. The % RSD of the six separate assay results was calculated. Injection repeatability was confirmed from the low %RSD values of peak area for both the components. The %RSD for assay results of six determinations was less than 1% for both the components, which confirms the high degree of precision of the method.

**Robustness**

To evaluate the robustness, the developed method was subjected to small deliberate variations in the optimized method parameters like variation of flow rate ±0.05 ml/min (i.e. 1.15 ml/min, 1.2 ml/min and 1.25 ml/min) and detection wavelength i.e. 225±1 nm. The mixed standard solution containing 0.1046 mg/ml Levocetirizine 2HCl and 0.1994 mg/ml Montelukast was injected in replicate under varied chromatographic conditions and the standard deviation of the retention time of each analyte were calculated. The method was found to be robust as the slight deliberate variation in detection wavelength and flow rate did not lead to changes in retention times of peak of interest. While evaluating the robustness data it was observed that system suitability parameters (e.g. Tailing Factor, Plate counts, Resolutions etc) were found to be within the specified limits under those deliberately varied conditions, which ensures that the validity of the analytical procedure was maintained whenever used. The result of robustness study is summarized below in Table IV.

**Table II: Result of HPLC Assay**

| Component | Amount present (mg) | Amount Found (mg) | % Estimation |
|---|---|---|---|
| Levocetirizine 2HCl | 5 mg | 4.96 mg | 99.2% |
| Montelukast | 10 mg | 10.03 mg | 100.3% |

**Table III: Linear Regression Data**

| Parameter | Levocetirizine2HCl | Montelukast |
|---|---|---|
| Concentration Range (mcg/ml) | 10-260 | 10-350 |
| Slope | 23900000 | 41600000 |
| Intercept | 81200 | 43600 |
| $R^2$ | 0.9998 | 0.9999 |

**Table IV: Robustness Data in terms of Retention Time (mean ± S.D.)**

| Level | Wavelength [a*] | | Flow Rate [b*] | |
|---|---|---|---|---|
| | Levocetrizine2HCl | Montelukast | Levocetrizine2HCl | Montelukast |
| - | 3.27 ± 0.002 | 4.26 ± 0.01 | 3.39 ± 0.001 | 4.41 ± 0.001 |
| 0 | 3.26 ± 0.001 | 4.23 ± 0.003 | 3.26 ± 0.001 | 4.23 ± 0.003 |
| + | 3.26 ± 0.002 | 4.25 ± 0.01 | 3.13 ± 0.002 | 4.08 ± 0.002 |

a* Wavelength 225± 1 nm
b* Flow Rate 1.2±0.05 ml/min

**Table V: Accuracy Results**

| Level of Standard Addition | Levocetirizine 2HCl | | | | Montelukast | | | |
|---|---|---|---|---|---|---|---|---|
| | Sample | Standard | | | Sample | Standard | | |
| | Added (mg) | Added (mg) | Recovered (mg) | % Recovery | Added (mg) | Added (mg) | Recovered (mg) | %Recovery |
| 80% | 4.95 | 4.22 | 4.25 | 100.71 | 10.02 | 7.88 | 7.88 | 100.00 |
| 100% | 4.95 | 4.83 | 4.85 | 100.41 | 10.02 | 9.69 | 9.63 | 99.38 |
| 120% | 4.95 | 5.93 | 5.95 | 100.34 | 10.01 | 11.3 | 11.09 | 98.14 |

**Accuracy (Recovery Study)**

The accuracy was evaluated from the recovery study at three different levels. It was carried out by spiking standard of Levocetirizine 2HCl and Montelukast Sodium to the pre-analyzed sample at three different levels i.e. 80%, 100% and 120% of the amount of each component contributed from the tablet powder. Each solution was injected in triplicate. The amount of drug recovered is calculated in each case. The % recovery was also calculated by using the following formula = (Amount of drug recovered/ Amount of drug added) x 100. The recovery of the added standard was studied at three different levels and the result is given in Table V.

**LOD and LOQ Determination**

Limit of detection can be calculated by using following formula:

LOD =3.3 σ/S

Limit of quantitation can be calculated based on standard deviation of the response and the slope.

LOQ =10 σ/S

Where σ = Standard deviation of the Y intercept of regression line [11].

S = Slope of the calibration curve

The Limit of Detection (LOD) were found to be 2.26 mcg/ml for the Levocetirizine 2HCl and 2.41 mcg/ml for Montelukast while Limit of Quantitation (LOQ) were found to be 6.85 mcg/ml for Levocetirizine 2HCl and 7.3 mcg/ml for Montelukast.

**System Suitability Testing**

System suitability testing is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability was assessed by injecting Levocetirizine 2HCl and Montelukast sodium mixed standard preparation in replicate. Parameters such as theoretical plates, tailing factor, resolution were determined. The System suitability parameters for the method are listed below in the Table VI.

**Table VI: System Suitability Parameters**

| Parameter | Levocetirizine 2HCl | Montelukast |
|---|---|---|
| Calibration Range (mcg/ml) | 10-260 | 10-350 |
| Theoretical Plates | 7500 | 8000 |
| Tailing Factor | 1.13 | 1.14 |
| Resolution | - | 5.95 |
| LOD (mcg/ml) | 2.26 | 2.41 |
| LOQ (mcg/ml) | 6.85 | 7.3 |

## CONCLUSION

The reported RP-HPLC method was proved to be simple, rapid and reproducible. The validation data indicate good precision, accuracy and reliability of the method. The developed method offers several advantages in terms of simplicity in mobile phase, isocratic mode of elution, easy sample preparation steps and comparative short run time which makes the method specific and reliable for its intended use in simultaneous determination of Levocetirizine dihydrochloride and Montelukast sodium in tablet dosage forms.

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