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**Der Pharmacia Lettre, 2010: 2 (1) 489-494**
*(http://scholarsresearchlibrary.com/archive.html)*

ISSN 0975-5071
USA CODEN: DPLEB4

# Development and Validation of TLC-Densitometry Method for Simultaneous Quantification of Montelukast Sodium and Levocetirizine Dihydrochloride Pharmaceutical Solid dosage form

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## Abstract

In this study, validated high performance thin liquid chromatographic (HPTLC) method for estimation of have been developed and validated for the simultaneous determination of Montelukast and Levocetirizine in combined pharmaceutical formulation. The chromatography estimation was performed using the following conditions: stationary phase was precoated silica gel 60 $F_{254}$ aluminum sheets (10 x 10 cm, E. Merck) and the mobile phase used was chloroform: methanol: toluene: glacial acetic acid (10:5:3:0.5 v/v/v/v). Chromatogram was developed in a camag twin trough chamber using a linear ascending technique. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 400 to 4500 ng with regression coefficient of 0.9998.The proposed method can be successfully used to determine the drug content of marketed tablet formulation.

**Keywords:** Montelukast, Levocetirizine, HPTLC, Densitometry estimation,

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## Introduction

Levocetrizine is a nonsedating antihistamine used in treatment of allergic diseases. Ion exchange resins are water-insoluble, cross-linked polymers containing salt forming groups in repeating position on the polymer chain. The unique advantage of ion exchange resins for complexation is due to the fixed positively or negatively charged functional groups attached to water insoluble polymer backbones. These groups have an affinity for oppositely charged counter ions, thus absorbing the ions into the polymer matrix. Since most of drugs possess ionic sites in their

molecule, the resins charge provides means to loosely bind such drugs. The binding is generally an equilibrium processes, resulting in continuous desorption or elution of drug from the resin as drug is absorbed into the body**.** [1, 2].Levocetirizine 2-[2-[4-[(*R*)-(4-chlorophenyl)-phenyl methyl] piperazinyl-1-yl] ethoxy] acetic acid, the *R*-enantiomer of racemic cetirizine, is a selective, potent, $H_1$-antihistamine compound indicated for the treatment of allergic, rhinitis and chronic idiopathic urticaria [3].

The recommended dosing of Levocetirizine is 5mg per day. It has a rapid onset, achieving maximum plasma concentration (*t* max) in 0.9 h, with peak serum levels (*C*max) of approximately 270 ng/mL [3]. In the plasma, 91% of the drug is bound to proteins and its volume of distribution (*V*d) is small (0.4 L/kg). The drug undergoes minimal metabolism, which increases the bioavailability and its half-life of elimination time is 8 hrs Levocetirizine is generally well tolerated in adults, adolescents and children with allergic conditions [4].Montelukast sodium 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 is a fast acting and potent cysteinyl leukotriene receptor antagonist which is being used in the treatment of asthma [5]. It can be administered orally once daily thereby increasing compliance over other common asthma treatments, has no known adverse effects or drug interactions, has demonstrated efficacy against allergen or exercise-induced bronchoconstriction (EIB) and is the only leukotriene modifier approved by the US Food and Drug Administration for use by children [6,7] from 2 to 12 years of age. A rapid onset of action is seen after the administration of Montelukast sodium, with improvement seen on the first day of treatment [8], and these positive effects may be additive to those of inhaled corticosteroids [9]. It should also be noted that for EIB which affects at least 70% of asthmatic patients, after 4 to 8 weeks of treatment, montelukast sodium has been demonstrated to provide superior protection compared to the long acting inhaled b -agonist, sal meterol, due to the progressive loss of protection of salmeterol against EIB [10].However, to our knowledge, there is no method for the simultaneous determination of these two drugs by high-performance thin-layer chromatography (HPTLC) in the literature. The aim of this work is to develop an accurate, specific, repeatable, and validated method for simultaneous determination of Montelukast and Levocetirizine in both tablet formulations.

## Materials and Methods

Montelukast sodium and Levocetirizine were generously given by Chemk chemicals (Solon, Himachal Pradesh, India). The tablet “L-MONTUS” with 10µg of Montelukast and 5µg of Levocetirizine dihydrochloride which was manufactured by “Swiss Garnier Life Sciences, Mehatpur, India” and marketed by “FOURRTS” was purchased from the market.

### *HPTLC method and chromatographic conditions:*

The chromatography estimation was performed using the following conditions: stationary phase was precoated silica gel 60 $F_{254}$ aluminum sheets (10 x 10 cm, E. Merck) and the mobile phase used was chloroform: methanol: toluene: glacial acetic acid (10:5:3:0.5 v/v/v/v). Chromatogram was developed in a camag twin trough chamber using a linear ascending technique. The chamber saturation time for mobile phase was optimized to 25 min. The length of chromatogram run was approximately 60 mm. Subsequent to the development; the TLC plates were dried in a current of air. The densitometric analysis was performed on a Camag TLC scanner III in the absorbance

mode at 302 nm with slit dimensions of 5.0 x 0.45 mm and scanning speed of 15mm/s were employed. Spotting parameters used were, 5 mm bandwidth, 15 mm space between two bands and spraying rate 20 s/µl.

***Calibration-curve***

Stock solutions of Montelukast sodium (10 mg/ml) and Levocetirizine (10 mg/ml) were prepared in glacial acetic acid. A series of standard curves were prepared over a concentration range of 200-3,200 ng for Montelukast sodium. For Levocetirizine the stock solution was spotted to give concentrations in the range of 400-1,300 ng. The data of spot area versus drug concentration was treated by linear least square regression analysis. Calibration curve was established by plotting peak area on ordinate and corresponding concentration on abscissa.

***Assay of tablets:***

Twenty tablets of Montelukast sodium and Levocetirizine were crushed and ground to fine powder. A powder equivalent to 20 mg of drug was transferred to a conical flask and extracted with glacial acetic acid (4 X 50 ml) by sonication. The extracts were filtered through Whatman No. 41 filter paper and the residue was washed with sufficient amount of methanol. The extract and its washings were pooled, transferred to a 10 ml volumetric flask and the final volume was made up to 10 ml with methanol to give a sample solution of 100 µg/ml. A fixed volume of 5 or 6 µl of working standard solutions (80 µg/ml) and 4 or 5 µl of sample solutions were spotted as sharp bands on the TLC plate and the plate was developed as mentioned above. The band of the drug was scanned at 302 nm.Precision of the method is expressed in terms of% RSD.

**Results and Discussion**

The method was validated in terms of linearity, accuracy, inter-day and intra-day precision, specificity, repeatability of measurement of peak area as well as repeatability of sample application. The limit of detection and limit of quantification were also determined. The TLC plates were pre-washed with methanol, and activated by keeping at 95 $^{o}$ for about 30 min. The stationary phase used was precoated silica gel 50 $F_{254}$. The mobile phase used was a mixture of chloroform: methanol: toluene: glacial acetic acid (10:5:3:0.5 v/v/v/v). The detection of spot was carried out at 269.0 nm,chamber saturation time 25 min, distance 30 mm, wavelength scanning at 269 nm, band width 9 mm, slit dimension keeping the slit dimension at 5 ×0.45 mm scanning speed 15 nm/sec, and the source of radiation of deuterium lamp. The plates were developed and scanned. The peak areas of each standard were obtained from the system, and a calibration graph was plotted with concentration vs. peak area. The method was validated for linearity, accuracy, limit of detection, limit of quantification, inter-day and intra - day assay precision, repeatability of measurement, and repeatability of sample application. Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (600 ng per spot of Montelukast sodium and 900 ng per spot Levocetirizine). The intra and interday variation for the determination of Montelukast sodium and Levocetirizine was carried out at three different concentration levels of 600, 1400, and 2600 ng per spot and 500, 1900, 3750 ng per spot, respectively. Robustness of the method was done at concentration of 1700 ng per spot for Montelukast sodium and 1100 ng per spot for Levocetirizine.In order to determine detection and quantification limit, drugs concentrations in the lower part of the linear range of the calibration curves were used. Stock solutions of 1,00µg/ml were prepared for both drugs and different

volume of stock solution 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 µl for Montelukast sodium and 1.40, 1.45, 1.50, 1.55, 1.60, 1.65 µl for Levocetirizine were spotted in triplicate. The amount of both drugs by spot versus average response (peak area) was graphed and the equations for this were determined. The standard deviations (SD) of responses were calculated. To study the accuracy of the proposed method recovery studies were carried out using standard addition method. The percent recovery was calculated by using the formula, % recovery= (T-A)/S×100, where T is total amount of drug estimated, A is the amount of drug contributed by tablet powder and S is the amount of pure drug added.

The specificity of the method was ascertained by analyzing standard drugs and samples. The spot for both drugs in sample was confirmed by comparing the $R_f$ values and spectra of the spot with that of standard. The peak purity of both drugs was accessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M), and peak end (E) positions of the spot. Recovery studies (table-2) the analyzed sample was over spotted with extra 80, 100 and 120 % of the standard drugs and it was analyzed by the proposed method. At each level of the amount, three determinations were performed. This was done to check the recovery of the drug at different level in the formulation. The resolution (Rs) between Montelukast sodium and Levocetirizine was 20.1. The efficiency of the method is studied by calculating number of theoretical plates and was found as 16,632. Peak area ratios of standard Montelukast sodium and Levocetirizine to that of internal standard were measured. A representative calibration graph of peak area ratio versus Montelukast sodium and Levocetirizine concentration (400 to 950 ng/spot) resulted in regression equation y=4.613 x+ 2.4721 (r = 0.9998).

The intra- and inter-day precision were carried out at three different concentration levels, i.e., 400, 1500, 3600 ng/spot; 700, 2500, 4600 ng/spot for the determinations of Montelukast sodium and Levocetirizine, respectively. The low values of percentage relative standard deviation (% RSD) for intra-and inter-day variation as shown in [Table-3] reveal that the proposed method is precise. Recovery studies of the drugs were carried out for the accuracy parameters.

**Table 1 Regression Analysis of Calibration Graph for Montelukast sodium and Levocetirizine**

| Parameter | Montelukast sodium | Levocetirizine |
|---|---|---|
| $R_F$ (SD) | 0.89 | 0.64 |
| Linearity and range (ng\spot) | 600 | 1200 |
| Linearity detection (ng\spot) | 55 | 87 |
| Limit of quantification (ng\spot) | 302 | 299 |
| Repeatability of application(%RSD) | 0.66 | 0.97 |
| Repeatability of measurement (%RSD) | 0.54 | 0.81 |
| Intraday (%RSD) | 0.43 | 0.36 |
| Inter day (%RSD) | 0.25 | 0.31 |
| LOD[a] | 1.536 | 2.864 |
| LOQ[b] | 2.536 | 3.453 |

$ SD = Standard Deviation

These studies were carried out at three levels i.e. multiple level recovery studies. Sample stock solution from tablet formulation of 100 µg/ml of was prepared. To the above prepared solutions, 80%, 100% and 120% of the standard drug solutions were added. Dilutions were made and recovery studies were performed. The assay value for the marketed formulation was found to be within the limits as listed in [Table 2]. The low RSD [11] value indicated the suitability of the method for routine analysis of Montelukast sodium and Levocetirizine in pharmaceutical dosage forms. The developed HPTLC technique is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible Montelukast sodium and Levocetirizine in bulk drug and tablet formulations. The $R_f$ values were found to be 0.89 and 0.64 for Montelukast sodium and Levocetirizine, respectively. Densitometric analysis of Montelukast sodium and Levocetirizine was performed at 302 nm. Adequate separation of the two drugs enabled the development of a selective and specific method of analysis.

**Table 2 -Recovery Studies**

| **Montelukast sodium** | | | | **Levocetirizine HCl** | | | |
|---|---|---|---|---|---|---|---|
| Label claimed | %Amount added | Found in(µg/ml) | %recovery | Label claimed | %Amount added | Found in(µg/ml) | %recovery |
| 10 | 80 | 9.99 | 99.99 | 5 | 80 | 4.98 | 99.98 |
| | 100 | 10.03 | 100.03 | | 100 | 5.01 | 100.01 |
| | 120 | 10.0 | 100.0 | | 120 | 5.09 | 100.09 |

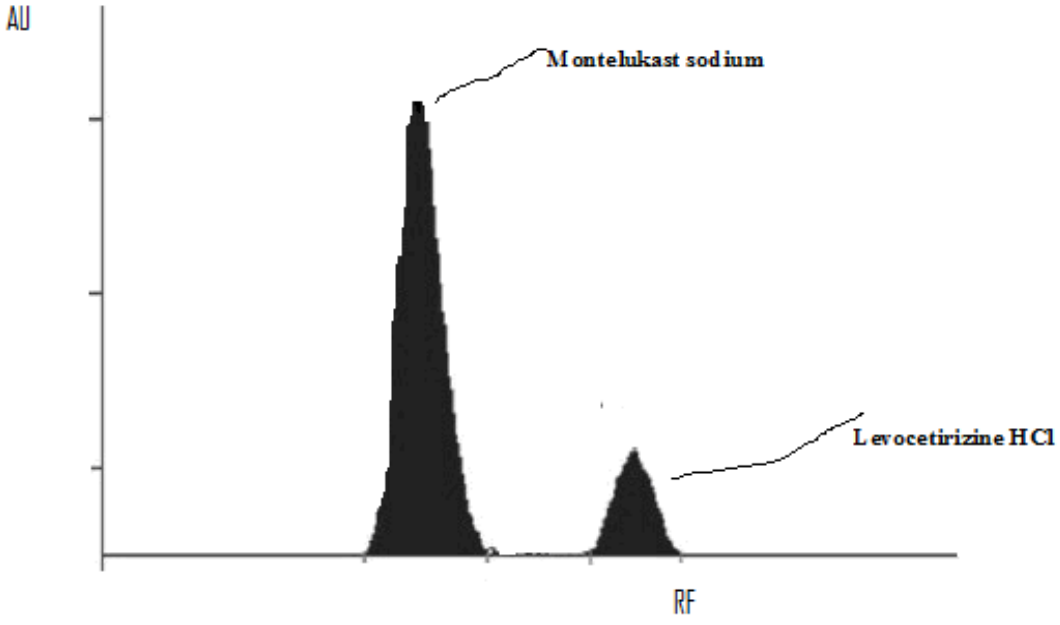


Figure- HPTLC Chromatogram **Montelukast sodium and Levocetirizine HCl**

## Conclusion

The statistical analysis proves that the method is reproducible and selective for the simultaneous estimation of Montelukast sodium and Levocetirizine as a bulk drug solution and in pharmaceutical formulations. The proposed HPTLC method was found to be rapid, specific, precise and accurate. The proposed method has advantage of simplicity and convenience for the separation and quantitation of Montelukast sodium and Levocetirizine in the combination and can be used for the assay of their dosage form.

## Acknowledgements

Authors are thankful to Prof.A.K.Singhei Department of Pharmaceutical Sciences Dr.Hari Singh Gour University Sagar (MP) India for encouragement and given valuable suggestion. The authors are thankful to Lupin Labs for the gifts sample of Pure Torsemide and Spironolactone.

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