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Determination of montelukast sodium and levocetrizine hydrochloride by using HPTLC method

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ABSTRACT

A simple, precise, accurate and rapid high-performance thin-layer chromatographic method has been developed and validated for the estimation of Montelukast sodium and Levocetrizine Hydrochloride simultaneously in combined dosage forms. The stationary phase used was precoated silica gel 60F 254. The mobile phase used was a mixture of Chloroform: Benzene: Methanol: Toluene (5:7.2:1:0.2 v/v/v/v). The detection of spots was carried out at 286 nm. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 500-1500 ng spot^{-1} for Montelukast sodium 1000-5000 ng spot^{-1} for Levocetrizine Hydrochloride. The limit of detection and the limit of quantification for Montelukast sodium were found to be 170 ng/spot and 570 ng/spot respectively, for Levocetrizine Hydrochloride and Levocetrizine Hydrochloride, 20 ng/spot and 70 ng/spot respectively. The proposed method can be successfully used to determine the drug content of marketed formulation.

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. 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, H1-antihistamine compound indicated for the treatment of allergic, rhinitis and chronic idiopathic urticarial [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. 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]. A rapid onset of action is seen after the administration of Montelukast sodium, with improvement seen on the first day of treatment[6], and these positive effects may be additive to those of inhaled corticosteroids[7]. 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, salmeterol, due to the progressive loss of protection of salmeterol against EIB [8].However, to our knowledge, there is few methods 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, simple, specific, repeatable, and validated method for simultaneous determination of Montelukast and Levocetirizine in both tablet formulations. This paper now describes an HPTLC method for the determination of Montelukast sodium and Levocetrizine Hydrochloride in tablets. The method is rapid, accurate and precise.

Materials and methods

MONT and LVC were gift sample from Ranbaxy Laboratories Ltd., Gurgaon. The commercial fixed dose combination product (L MONTUS containin10 mg Montelukast Sodium and 5 mg Levocetirizine Hydrochloride) was procured from the local market. Silica gel 60F 254 TLC plates (E. Merck, Mumbai) were used as a stationary phase. A Camag HPTLC system comprising of Camag Linnomate V automatic sample applicator, Hamilton syringe, Camag TLC Scanner 3, Camag WinCATS software, Camag twin-trough chamber and ultrasonicator was used during the study.

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Preparations of standard solution

Working standards of Montelukast sodium and Levocetrizine Hydrochloride were weighed accurately and diluted with methanol to obtain a final concentration of 1 mg/ml for Montelukast sodium and 100µg/ml for Levocetrizine Hydrochloride. 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 mg/ml. A fixed volume of 5 or 6 ml of working standard solutions (80 mg/ml) and 4 or 5 ml 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 286nm.Precision of the method is expressed in terms of% RSD.

Chromatographic conditions

The chromatographic estimations were performed using stationary phase, precoated silica gel 60F 254 aluminium sheets (20 × 10 cm, prewashed with methanol and dried in an oven at 50° for 5 min); mobile phase, Chloroform: Benzene: Methanol: Toluene (5:7.2:1:0.2 v/v/v/v); chamber and plate saturation time of 30 min. Migration distance allowed was 72 mm; wavelength scanning was done at 286 nm.

Calibration-curve

Stock solutions of Montelukast sodium (10 mg/ml) and Levocetirizine (10 mg/ml) were prepared in methanol. A series of standard curves were prepared over a concentration range of 500-1500 ng/ml for Montelukast sodium. For Levocetirizine the stock solution was spotted to give concentrations in the range of 1000-5000 ng/ml. 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.

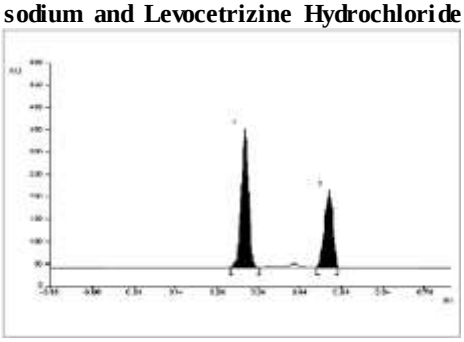
Validation Procedure

Aliquots of 0.8, 0.9, 1, 2, 3 and 4 µl of standard solution of Montelukast sodium and 1, 3, 6, 8 and 10 µl of standard solution of Levocetrizine Hydrochloride were applied on the TLC plate. The TLC plate was dried, developed and analyzed photometrically as described earlier. The calibration curves were prepared by plotting peak area versus concentration (µg/spot) corresponding to each spot. The method was validated by establishing linearity, accuracy, inter-day and intra-day precision, specificity, repeatability of measurement of peak, as well as repeatability of sample application. The limit of detection and limit of quantification were also determined. The related impurities were determined by spotting higher concentration of the drugs so as to detect and quantify them.

Sample Preparation

For the analysis of the marketed formulations, 2 µl (for Montelukast sodium) and 5 µl (for Levocetrizine Hydrochloride) of filtered solutions of the marketed formulations were spotted onto the same plate, followed by development scanning. The analysis was repeated six times. The spots were resolved into two peaks in the chromatogram of drug samples extracted from the marketed formulations. The content of the drug was calculated from the peak areas recorded. A solvent system that would give dense and compact spots with appropriate and significantly different Rf values was desired for quantification of Montelukast sodium and Levocetrizine Hydrochloride in pharmaceutical formulations. The mobile phase consisting of Chloroform: Benzene: Methanol: Toluene (5:7.2:1:0.2 v/v/v/v) gave Rf values of 0.3 (±0.04) and 0.53 (±0.04) for Montelukast sodium and Levocetrizine Hydrochloride respectively [Figure - 1].

Figure-1 Representative chromatogram peak of Montelukast sodium and Levocetrizine Hydrochloride



Linearity range for Montelukast sodium and Levocetrizine Hydrochloride was found to be in the range of 0.8-4.0 µg/spot and 0.1-1.0 µg/spot, with a correlation coefficient of 0.9992 and 0.9995, respectively. The LOD and LOQ for Montelukast sodium were found to be 170 ng/spot and 570 ng/spot for Levocetrizine Hydrochloride, 20 ng/spot and 70 ng/spot respectively.

Precision [9, 10]

The intra-day and inter-day precision (RSD) values were determined for standard Montelukast sodium (400-3000ng/spot) and Levocetrizine Hydrochloride (600-4200ng/spot) six times on the same day and over a period of 1 week. The intra-day and inter-day coefficients of variation are given in [Table - 1].

Results and discussion

Repeatability of sample application was assessed by spotting 2 µl of Montelukast sodium and 5 µl of Levocetrizine Hydrochloride solution six times on a TLC plate, followed by development of plate and recording the peak area for 6 spots. The % RSD for peak area values of Montelukast sodium and Levocetrizine Hydrochloride was found to be 1.09 and 1.17 respectively. Repeatability of measurement of peak area was determined by spotting 2 µl of Montelukast sodium and 5 µl of Levocetrizine Hydrochloride solution on a TLC plate and developing the plate. The separated spot was scanned five times without changing the position of the plate and % RSD for measurement of peak area of Montelukast sodium and Levocetrizine Hydrochloride was found to be 0.143 and 0.072 respectively. To confirm the specificity of the proposed method, the solution of the formulation was spotted on the TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the peaks of Montelukast sodium and Levocetrizine Hydrochloride

Recovery Study

Recovery studies of drugs were carried out for accuracy parameters. These studies were carried out at three levels, i.e., multiple level recovery studies. Sample stock solution from tablet formulation of 1 mg/ml and 100 µg/ml of Montelukast sodium and Levocetrizine Hydrochloride respectively was prepared. To the above prepared solution, 50%, 100%, 150% of the standard Montelukast sodium solution and 20%, 40% and 60% of the standard Levocetrizine Hydrochloride solution were added. Dilutions were made and recovery studies were performed. Percentage recovery was found to be within limits,

as listed in [Table - 2]. For the detection of the related impurities, Montelukast sodium and Levocetrizine Hydrochloride (0.1 g each) were dissolved separately in 10 ml of methanol, and these solutions were termed as sample solutions (10 mg/ml). One millilitre of each sample solution was diluted to 10 ml with methanol, and these solutions were termed as standard solutions (1000 µg/ml). Aliquots of both the standard solutions (2 µl) and sample solutions (20 µl) were spotted on the plate and chromatography performed as described earlier. The sample solution of Montelukast sodium showed three unknown additional spots at Rf of 0.06, 0.41 and 0.47. The sample solution of Levocetrizine Hydrochloride showed three unknown additional spots at Rf of 0.37, 0.70 and 0.76. However, the areas of these spots were found to be less than 0.04% as compared to the areas of standard solution spots.

Assay

The assay value for the marketed formulation was found to be within the limits, as listed in [Table - 3]. The low RSD value indicated the suitability of the method for routine analysis of Montelukast sodium and Levocetrizine Hydrochloride in pharmaceutical dosage forms.

Conclusion

The developed HPTLC technique is simple, precise, specific and accurate, and the statistical analysis proved that method is reproducible and selective for the analysis of Montelukast sodium and Levocetrizine Hydrochloride in bulk drug and tablet formulations.

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Table – 1 A summary of validation parameters of Montelukast sodium and Levocetrizine Hydrochloride

Parameters	Results	
	Montelukast sodium	Levocetrizine Hydrochloride
Linearity	500-1500 ng/ml	1000-5000 ng/ml
Correlation coefficient	0.9992	0.9995
Precision(%CV)	400-3000(ng/spot)	600-4200 (ng/spot)
Intra day (n=6)	1.39-1.50	1.45-1.89
Inter day (n=6)	1.09	1.17
Repeatability of sample application (n=6)	0.14	0.07
Repeatability of Peak area (n=6)	570	70
Limit of Detection (ng/spot)	Specific	Specific
Limit of Quantification(ng/spot)		
Specificity		

Table – 2 Recovery study of Montelukast sodium and Levocetrizine Hydrochloride

Label Claim mg/tablet	Amount added	Total amount added (mg)	Amount recovered*(mg) ± SD	% Recovery ± SD	% RSD
Montelukast sodium	50	15	15.26 ± 0.30	102.4 ± 1.36	1.36
	100	20	19.40 ± 0.44	98.00 ± 1.63	1.63
	150	25	25.68 ± 0.29	102.7 ± 1.16	1.16
Levocetrizine Hydrochloride	20	12	11.98 ± 0.12	99.87 ± 1.02	1.02
	40	14	14.37 ± 0.22	102.65± 1.59	1.59
	60	16	16.20 ± 0.16	101.23± 0.72	0.72

Recovery study of Montelukast sodium and Levocetrizine Hydrochloride * indicates that each value is a mean ± Standard deviation of three determinations.

Table – 3 Assay

Label Claim (mg/tablet)	Amount Found *	% of Drug found*	% RSD
Montelukast sodium	10.04	100.40	1.96
Levocetrizine Hydrochloride	5.02	98.40	0.767

*Each value is mean of six determinations