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Development of Validated HPLC and HPTLC Methods for Simultaneous Determination of Levocetirizine Dihydrochloride and Montelukast Sodium in Bulk Drug and Pharmaceutical Dosage Form

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Abstract

Two chromatographic methods have been described for the simultaneous determination of levocetirizine dihydrochloride and Montelukast sodium in tablets. The first method was a high performance thin layer chromatographic (HPTLC) separation followed by densitometric measurements on normal phase silica gel 60 F_{254}. The second method was a high performance liquid chromatographic (HPLC) separation on a BDS Hypersil C_{18} column using disodium hydrogen phosphate buffer (0.02 M): Methanol (25: 75, v/v) pH adjusted to 7 with ortho-phosphoric acid as the mobile phase. The proposed methods were validated as per ICH guidelines and successfully applied for the determination of investigated drugs in tablets.

Keywords: Levocetirizine dihydrochloride; Montelukast sodium; HPLC; HPTLC; Method validation

Introduction

Levocetirizine (Figure 1a), chemically 2-[2-[4-[(*R*)-(4-chlorophenyl)-phenyl-methyl]piperazin-1-yl]ethoxy]acetic acid, is a third-generation non-sedative antihistamine and used in the form of levocetirizine dihydrochloride for the treatment of allergic rhinitis and chronic idiopathic urticaria. It is an active R-enantiomer of cetirizine, orally active, potent, selective and long acting H1-histamine receptor antagonist with no anticholinergic activity [1-2]. Montelukast (Figure 1b), chemically 2-[1-[[(1*R*)-1-[3-[2-(7-chloroquinolin-2-yl)ethenyl] phenyl]-3-[2-(2-hydroxypropan-2yl)phenyl]propyl]sulfanylmethyl] cyclopropyl] acetic acid is a selective and orally active leukotriene receptor antagonist that inhibits the cysteinyl leukotriene ($CysLT_1$) receptor in the lungs and bronchial tubes. It is used in the form of montelukast sodium for the treatment of asthma and to relieve symptoms of seasonal allergies [3-6]. It has been demonstrated by recent studies that the treatment of allergic rhinitis with concomitant administration of an antileukotriene (Montelukast sodium) and an antihistamine (levocetirizine), shows significantly better symptom relief compared with the modest improvement of rhinitis symptomatically with each of the treatments alone [7-8]. Literature review reveals that some analytical methods have been reported for levocetirizine dihydrochloride [9-12] and montelukast sodium [13-15] individually as stability indicating and in biological fluids or in combination with other drugs in pharmaceutical dosage forms. Recently HPLC and HPTLC methods has been reported for simultaneous estimation of levocetirizine dihydrochloride and Montelukast sodium in pharmaceutical dosage forms which are either tedious or expensive methods [16,17]. The HPLC method uses acetonitrile which is expensive than methanol as the mobile phase whereas HPTLC method uses cholorform as a component of mobile phase which undesirable in the TLC separation. In the present study we have proposed new validated simple HPTLC and reliable LC methods for the simultaneous determination of levocetirizine dihydrochloride and Montelukast sodium in their combined tablet formulation.

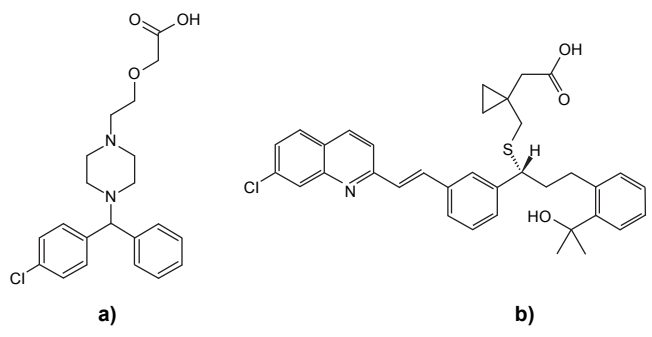

Figure 1: (a) structure of levocetirizine dihydrochloride **(b)** structure of Montelukast sodium.

Material and Methods

Chemicals

Working standards of pharmaceutical grade levocetirizine dihydrochloride (99.78 %, w/w) and Montelukast sodium (99.30 %, w/w) were obtained as gift samples from Unichem Laboratories Ltd. Bardez Goa, India and Lupin Ltd. Mulshi, Pune, India respectively. Fixed dose combination Tablets (Montair-LC) containing 5 mg of levocetirizine dihydrochloridedihydrochloride and 10 mg of Montelukast sodium sodium were purchased from a local pharmacy, Pune, India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India. High purity

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deionized water was obtained from Millipore, Milli-Q (Bedford, MA, USA) water purification system.

Instrumentation and chromatographic conditions

For HPTLC, the samples were spotted in the form of bands of 6 mm width with a Camag 100 microlitre sample syringe (Hamilton, Bonaduz, Switzerland) on silica gel precoated aluminum plate 60 F_{254}, [(20 × 10 cm) with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Techno, Mumbai] using a Camag Linomat IV applicator (Switzerland). The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. A constant application rate of 0.1 µLs^{-1} was used and the space between two bands was 6 mm. The slit dimension was kept at 5mm × 0.45 mm and the scanning speed was 10 mm s^{-1}. The mobile phase was consisted of toluene: ethyl acetate: methanol: ammonia (2.5: 7: 2.5: 1, v/v/v/v) and 15 mL of the mobile phase was used for chromatography. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25°C ± 2). The length of chromatogram run was 8 cm. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode and operated by CATS software (V 3.15, Camag). The source of radiation used was a deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression. The HPLC system (Jasco corporation, Tokyo, Japan) consisted of a Pump (model Jasco PU- 2080 Plus) along with manual injector sampler programmed at 20 µl capacity per injection was used. The detector consisted of UV/ VIS (model Jasco UV 2075). LC separations were performed on a BDS Hypersil C_{18} analytical column Dim. (mm) 250 × 4.6, Particle Sz. (µ) 5 (Thermo Scientific, Waltham, USA). Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The mobile phase was consisted of a mixture of Sodium dihydrogen phosphate buffer (0.02 M): Methanol (25: 75, v/v) pH adjusted to 7 with ortho-phosphoric acid. The mobile phase was degassed and filtered by passing through a 0.45 µm pore size membrane filter (Millipore, Milford, MA, USA) prior to use. The flow rate was 1 mL min^{-1}. All determinations were performed at ambient temperature with a detection wavelength of 231 nm.

Preparation of standard stock and working solutions

Mixed standard stock solution containing 1.00 mg mL^{-1} of levocetirizine dihydrochloride and 2.00 mg mL^{-1} of Montelukast sodium was prepared in methanol. For HPTLC, the working standard solution of levocetirizine dihydrochloride and Montelukast sodium was prepared at concentration of 500 ng µL^{-1} and 1000 ng µL^{-1} respectively, by diluting the standard stock solution in methanol. For HPLC, working standard solutions were prepared by diluting the above standard stock solution in mobile phase to reach a concentration range of 1 – 10 µg mL^{-1} for levocetirizine dihydrochloride and 2 – 20 µg mL^{-1} for Montelukast sodium. The stock solution was stored at 2–8°C protected from light.

Selection of analytical wavelength

Stock solutions of drugs were prepared in methanol separately. UV spectrum of 10 µg mL^{-1} of each individual drug was taken. Levocetirizine dihydrochloride and montelukast sodium showed maximum absorbance at 230nm and 344 nm, respectively. Isobestic point was found at 231 nm and was selected as the detection wavelength (Figure 2a). Further, in situ HPTLC spectral overlain of levocetirizine dihydrochloride and montelukast sodium was taken. Isobestic point at 231 nm was confirmed and was selected as scanning wavelength (Figure 2b).

Optimization of HPTLC and HPLC method

The HPTLC and HPLC procedures were optimized with a view to develop a simultaneous assay method for levocetirizine dihydrochloride and montelukast sodium. For HPTLC, the mixed standard stock solution containing 1.00 mg mL^{-1} of levocetirizine dihydrochloride and 2.00 mg mL^{-1} of montelukast sodium was spotted onto HPTLC plates and run in different solvent systems. Several mobile phases were tried on trial and error basis and finally a mobile phase consisted of toluene:ethyl acetate:methanol:ammonia (2.5: 7: 2.5: 1, v/v/v/v) was selected. In order to reduce the neckless effect the TLC chamber was saturated for 30 min using saturation pads. The mobile phase was run up to a distance of 8 cm; which takes approximately 25 min. for complete development of the TLC plate.

For HPLC, The mixed standard stock solution was diluted in mobile phase to a concentration containing 10 µg mL^{-1} of levocetirizine dihydrochloride and 20 µg mL^{-1} of Montelukast sodium. Then, the stock solution is injected into the BDS Hypersil C_{18} analytical column. Different ratios of Disodium hydrogen phosphate buffer (0.02 M) and Methanol at different pH were tried. The optimum mobile phase was found to be Disodium hydrogen phosphate buffer (0.02 M): Methanol (25: 75 v/v), pH adjusted to 7 with ortho-phosphoric acid. The

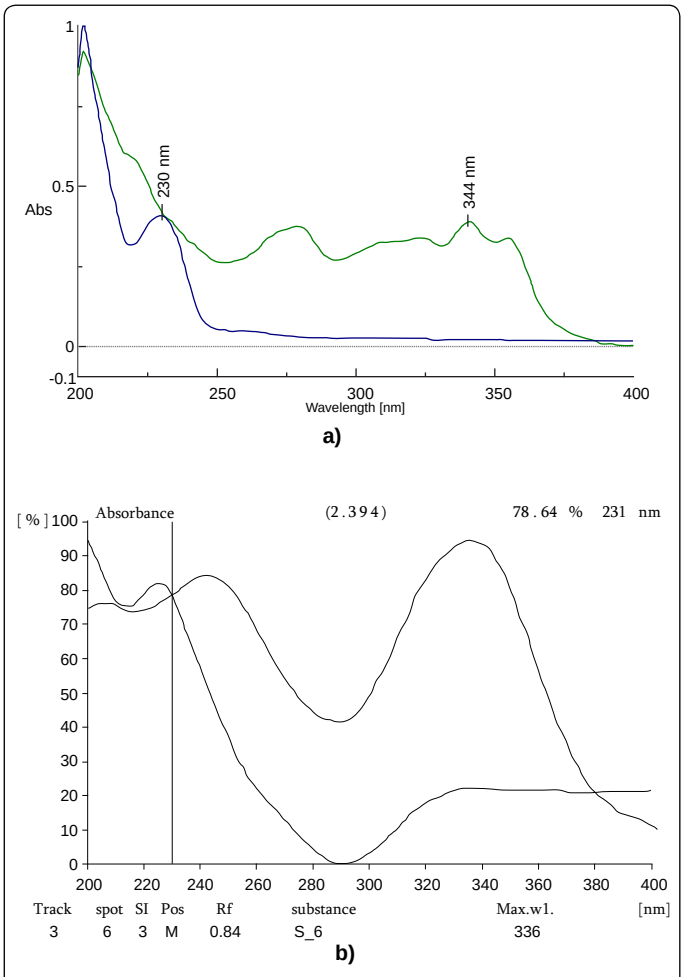

Figure 2: (a) UV spectrum overlay of levocetirizine dihydrochlorideand Montelukast sodium **(b)** In situ HPTLC spectral overlain of levocetirizine dihydrochlorideand Montelukast sodium.

separation was carried out at ambient temperature with a flow rate of 1.0 mL min^{-1}. The retention times for levocetirizine dihydrochloride and montelukast sodium were found to be 3.558 ± 0.03 and 7.450 ± 0.04 min, respectively. Acceptable retention time (t_R), plates, asymmetry and good resolution for levocetirizine dihydrochloride and Montelukast sodium were obtained.

Validation of HPTLC and HPLC method

The optimized HPTLC & HPLC method was validated with respect to the following Parameters. The validation was performed as per the ICH guidelines [18,19].

Linearity: For HPTLC, 1 to 5 µL volumes of the working standard stock solution were spotted in triplicate on HPTLC plate to obtain a final concentration range of 500-2500 ng $spot^{-1}$ for levocetirizine dihydrochloride and 1000-5000 ng $spot^{-1}$ for montelukast sodium. The plate was then developed using the previously described mobile phase. For HPLC, 20-µL of working standard solution was injected into the HPLC system six times for each concentration and chromatographed under the above mentioned conditions. Linear calibration curves were generated using least-squares linear-regression analysis by plotting the peak area against concentration of the drug. The limit of detection (LOD) and limit of quantification (LOQ) were determined by diluting known concentrations of standard stock solution until the average responses were approximately three (For LOD) or ten times (for LOQ) the responses of the blank.

Precision: The precision of the method was analyzed by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations of 500, 1500, 2500 ng $spot^{-1}$ and 1000, 3000, 5000 ng $spot^{-1}$ for levocetirizine dihydrochloride and Montelukast sodium, respectively by HPTLC and 1, 4, 10 µg mL^{-1} and 2, 8, 20 µg mL^{-1} for levocetirizine dihydrochloride and Montelukast sodium, respectively by HPLC. Method repeatability was achieved from RSD% values obtained by repeating the assay six times on the same day for intra-day precision. The intermediate (interday) precision of the method was checked by performing same procedure on different days under the same experimental conditions.

Robustness: The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. For HPTLC method, following the introduction of small changes in the mobile phase composition (±0.1 mL for ammonia), the effect on the results was examined. Mobile phases having different proportions of components, e.g. toluene: ethyl acetate: methanol: ammonia (2.6: 7: 2.5: 1, v/v/v/v), (2.4: 7: 2.5: 1, v/v/v/v), (2.5: 7: 2.6: 1, v/v/v/v), (2.5: 7: 2.4: 1, v/v/v/v) etc., were tried and chromatograms were run. The amount of mobile phase was varied over the range of ±5%. The time from spotting to chromatography and from chromatography to scanning was varied by 10 min and analysed. The robustness of the method was determined at three different concentration levels of 500, 1500, 2500 ng $spot^{-1}$ for levocetirizine dihydrochloride and 1000, 3000, 5000 ng $spot^{-1}$ for montelukast sodium. For HPLC, robustness of the method was studied by deliberately varying parameters like flow rate (±0.1 mL min^{-1}) and mobile phase composition (±1 mL).

Specificity: The ability of an analytical method to unequivocally assess the analyte in the presence of other components (impurities, degradents and excipients) can be demonstrated by evaluating specificity. The specificity of the HPTLC method was determined by analyzing standard drug and test samples. The spot for levocetirizine dihydrochloride and montelukast sodium in the samples was confirmed by comparing the R_F and spectrum of the spot to that of a standard. The peak purity of levocetirizine dihydrochloride and montelukast sodium was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E). For HPLC, The specificity of the method was determined by injecting excipient solution having the same concentration as that of the tablet solution.

Accuracy: Accuracy of the two proposed methods was carried out by applying the methods to drug sample (Levocetirizine dihydrochloride and Montelukast sodium combination tablets) to which known amount of levocetirizine dihydrochloride and montelukast sodium standard powder corresponding to 50, 100 and 150% of label claim had been added (standard addition method). The absolute recovery was calculated by comparing the peak areas obtained from standard solution of levocetirizine dihydrochloride and montelukast sodium with the peak areas of samples of different concentration.

Analysis of marketed formulation (Assay): Ten tablets of Montair - LC (labeled to contain 5 mg levocetirizine dihydrochloride and 10 mg montelukast sodium, Cipla Ltd.) were weighed and powdered. An accurate weight of the powder equivalent to 5 mg of levocetirizine dihydrochloride and 10 mg of Montelukast sodium was transferred into a 25 mL volumetric flask containing 15 mL methanol, sonicated for 30 min and diluted up to 25 mL with methanol. This solution was filtered through a 0.45 µm membrane filter. For HPTLC, concentration achieved after the above dilution was 200 ng $µL^{-1}$ of levocetirizine dihydrochloride and 400 ng $µL^{-1}$ of montelukast sodium. 4 µL volume was spotted for six times to achieve a final concentration of 800 ng $spot^{-1}$ and 1600 ng $spot^{-1}$ for levocetirizine dihydrochloride and montelukast sodium, respectively. For HPLC, Suitable dilutions were made using mobile phase to prepare final tablet solution containing 10 µg mL^{-1} for levocetirizine dihydrochloride and 20 µg mL^{-1} for montelukast sodium. Tablet solutions thus prepared were filtered then analyzed as mentioned under the construction of calibration graphs in the above section. The analysis was repeated for six times.

Results

Optimization of chromatographic conditions

The experimental conditions for HPTLC such as wavelength of detection and mobile phase composition were optimized to provide accurate, precise and reproducible results. A scanning wavelength of 231 nm obtained as a common wavelength concluded from UV spectrum overlay and in situ HPTLC spectral overlay was used. Initially, toluene, acetone and methanol were tried in different ratio. Formic acid was then added to improve the peak shape. But

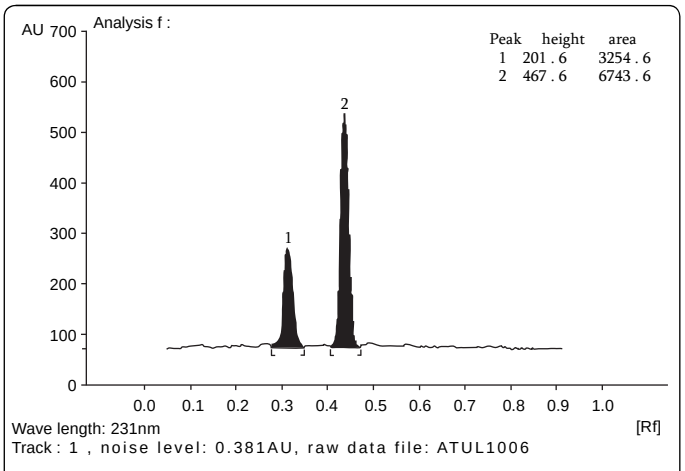

Figure 3: (Densitogram of a formulation containing 800 ng spot-1 of levocetirizine dihydrochloride(RF 0.31) and 1600 ng spot-1 of Montelukast sodium (RF 0.44) showing no interference of excipients in analysis.

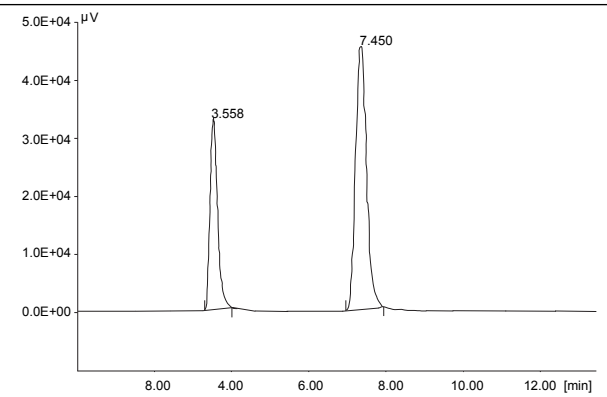

Figure 4: Chromatogram of a formulation containing 10 µg mL-1 of levocetirizine dihydrochloride(tR 3.558) and 20 µg mL-1 of Montelukast sodium (tR 7.450) showing no interference of excipients in analysis.

resolution achieved was not satisfactory. Finally, toluene, ethyl acetate, methanol and ammonia were tried in different ratio. The optimum mobile phase was found to be consisted of toluene: ethyl acetate: methanol: ammonia (2.5: 7: 2.5: 1, v/v/v/v). The drugs were satisfactorily resolved with R_F values at 0.31 ± 0.01 and 0.44 ± 0.01 for levocetirizine dihydrochloride and montelukast sodium, respectively. A good resolution and sharp peaks were obtained with minimum tailing with the proposed mobile phase (Figure 3).

To optimize the HPLC assay conditions, different ratios of Sodium dihydrogen phosphate buffer (0.02 M) and methanol at different pH were tried. The optimum mobile phase was found to be consisted of sodium dihydrogen phosphate buffer (0.02 M) and methanol (25: 75, v/v), pH adjusted to 7 with ortho-phosphoric acid. The separation was carried out at ambient temperature with a flow rate of 1.0 mL min^{-1}. The retention times for levocetirizine dihydrochloride and montelukast sodium were found to be 3.558 ± 0.03 and 7.450 ±

(a) HPTCL

Parameters	Levocetirizine	Montelukast
Linearity range	500-2500 ng $spot^{-1}$	1000-5000 ng $spot^{-1}$
r^2 ± S.D.	0.9981 ± 0.0007	0.9982 ± 0.002
Slope ± S.D.	2.768 ± 0.005	1.756 ± 0.001
Intercept ± S.D	961 ± 1.92	3602 ± 3.40
Confidence limit of slope[a]	2.547-2.990	1.618-1.893
Confidence limit of intercept[a]	594.2-1328	3146-4057
Sy .X	109.9	136.5

(b) By HPLC

Parameters	Levocetirizine	Montelukast
Linearity range	1 – 10 µg mL^{-1}	2 – 20 µg mL^{-1}
r^2 ± S.D.	0.9987 ± 0.00	0.998 ± 0.01
Slope ± S.D.	40810 ± 19.44	56040 ± 21.61
Intercept ± S.D	-4930 ± 8.04	15460 ±6.36
Intercept ± S.D	960.4 ± 1.92	3601 ± 3.40
Confidence limit of slope[a]	39120 - 42510	1.618 -1.893
Confidence limit of intercept[a]	-14250 - 4389	3146 - 4057
Sy .X	5126	136.5

*p<0.0001 - Slope significantly different from zero
[a]95% confidence limit.
Sy.X- Standard deviation of residuals from line.

Table 1: Linear regression data for calibration curves.

(a) By HPTCL

Drugs	Conc. (ng $spot^{-1}$)	Repeatability (n= 6)		Intermediate precision (n= 6)	
		Found conc. ± SD	RSD (%)	Found conc. ± SD	RSD (%)
Levocetirizine	500	504.07 ± 2.12	0.42	499.95 ± 0.99	0.20
	1500	1497.51± 27.10	1.81	1505.03± 27.84	1.85
	2500	2509.37± 37.39	1.49	2501.53± 33.27	1.33
Montelukast sodium	1000	998.50 ± 9.19	0.92	1009.04 ± 1.92	0.19
	3000	3035.91 ± 6.07	0.20	2989.31± 12.26	0.41
	5000	4955.98± 60.96	1.23	4911.08± 44.20	0.90

(b) By HPCL

Drugs	Conc. (µg mL^{-1})	Repeatability (n= 6)		Intermediate precision (n= 6)	
		Found conc. ± SD	RSD (%)	Found conc. ± SD	RSD (%)
Levocetirizine	1	0.98 ± 0.002	0.20	1.008 ± 0.000	0.06
	4	4.05 ± 0.014	0.34	4.142 ± 0.012	0.28
	10	9.97 ± 0.007	0.07	10.280 ± 0.005	0.04
Montelukast sodium	2	1.98 ± 0.002	0.10	1.960 ± 0.003	0.15
	8	8.82 ± 0.050	0.06	8.180 ± 0.006	0.07
	20	20.860 ± 0.020	0.10	19.910 ± 0.008	0.04

Table 2: Precision studies

(a) By HPTLC

Parameters	SD of peak area*		% RSD	
	LEVO.	MONT.	LEVO.	MONT.
Mobile phase composition (± 0.1 ml of ammonia)	2.78	3.12	0.75	1.12
Amount of mobile phase (±5 %)	1.54	2.36	0.17	0.32
Time from spotting to chromatography (10 min)	2.53	2.61	0.42	0.54
Time from chromatography to scanning (10 min)	1.74	2.42	0.14	0.33

(b) By HPTLC

Factor	Level	Retention time		Asymmetry	
		LEVO.	MONT.	LEVO.	MONT.
A: Flow Rate (mL min^{-1})					
0.9	-1	3.643	7.49	1.24	1.06
1.0	0	3.558	7.45	1.23	1.03
1.1	+1	3.462	7.41	1.19	1.04
Mean ± S.D (n =3)		3.56± 0.095	7.45 ± 0.040	1.21± 0.032	1.04 ± 0.036
B: % of methanol in the mobile phase (v/v)					
24	-1	3.629	7.59	1.23	1.06
25	0	3.558	7.45	1.13	1.03
26	+1	3.487	7.31	1.17	1.04
Mean ± S.D (n =3)		3.558± 0.071	7.55 ± 0.14	1.17± 0.05	1.04± 0.02

*SD – (Standard deviation n=3)

Table 3. Robustness studies.

(a) By HPTLC

Drugs	Label claim mg tab^{-1}	Amount added in mg (%)	Total amount (mg)	Actual conc. taken (ng spot^{-1})	For HPTLC (n= 6)		
					calculated conc. ± SD	RSD (%)	Recovery (%)
Levocetirizine	5	2.5	7.5	600	605.61± 3.15	0.52	101.87
		5	10	800	799.0± 10.47	1.31	99.75
		7.5	12.5	1000	998.6± 9.19	0.92	99.72
Montelukast sodium	10	5	15	1200	1212.0± 7.15	0.59	102.00
		10	20	1600	1604.0± 6.73	0.42	100.55
		15	25	2000	2001.9± 23.22	1.16	100.19

(b) By HPLC

Drugs	Label claim mg tab^{-1}	Amount added in mg (%)	Total amount (mg)	Actual conc. Taken (µg mL^{-1})	For HPLC (n= 6)		
					calculated conc. ± SD	RSD (%)	Recovery (%)
Levo cetirizine	5	2.5 (50%)	7.5	3	3.028 ± 0.03	0.98	100.94
		5 (100%)	10	4	4.048 ± 0.03	0.79	101.20
		7.5 (150%)	12.5	5	5.006± 0.02	0.39	100.12
Montelukast sodium	10	5 (50%)	15	6	6.16 ± 0.03	0.48	102.66
		10 (100%)	20	8	8.092 ± 0.06	0.74	101.16
		15 (150%)	25	10	10.04 ± 0.02	0.19	100.40

Table 4: Accuracy studies.

0.04 min, respectively (Figure 4). Acceptable retention time (t_R), theoretical plates, asymmetry and good resolution for levocetirizine dihydrochlorideand montelukast sodium were obtained.

Method validation

Linearity: Linear relationships were observed by plotting drug concentrations against peak areas for each compound, for both chromatographic methods. For HPTLC, levocetirizine dihydrochloride and montelukast sodium showed linear response in the concentration range of 500-2500 ng spot^{-1} and 1000-5000 ng spot^{-1}, respectively. The corresponding linear regression equation was $y = 2.768 x + 961$ and $y = 1.756 x + 3602$ with square of correlation coefficient (R^2) of 0.9981 and 0.9982 for levocetirizine dihydrochloride and montelukast sodium respectively. Similarly for HPLC, levocetirizine dihydrochloride and montelukast sodium showed linear response in the range of 1 – 10 µg mL^{-1} and 2 – 20 µg mL^{-1}, respectively. The corresponding linear regression equation was $y = 40810 x - 4930$ and $y = 56040 x - 15460$ with square of correlation coefficient (R^2) of 0.9987 and 0.9980 for levocetirizine dihydrochloride and montelukast sodium, respectively. An excellent correlation existed between the peak areas and concentration of levocetirizine dihydrochloride and Montelukast sodium (Table 1). The signal: noise ratios of 3:1 and 10:1 were considered as LOD and LOQ respectively. In HPTLC method, the limit of detection (LOD) and the limit of quantitation (LOQ) were found to be 90 ng spot^{-1} and 200 ng spot^{-1} for Levocericine dihydrochloride and 50 ng spot^{-1} and 110 ng spot^{-1} for montelukast sodium respectively. In HPLC method, the limit of detection (LOD) and the limit of quantitation (LOQ) were found to be 0.5 µg mL^{-1} and 0.8 µg mL^{-1} for Levocetricine dihydrochloride and 0.2 µg mL^{-1} and 0.6 µg mL^{-1} for montelukast sodium respectively.

Precision: The results of the repeatability and intermediate precision experiments are shown in Table 2. The developed methods were found to be precise as the RSD values for repeatability and intermediate precision studies were <2%, respectively as recommended by ICH guidelines.

(a) By HPTLC

Drugs	Label claim mg tablet^{-1}	For HPTLC (n= 6)	
		Drug content (%) ± SD	RSD (%)
Levocetirizine	5	99.72 ± 0.97	0.98
Montelukast sodium	10	100.19 ± 1.06	1.06

(b) By HPLC

Drugs	Label claim mg tablet^{-1}	For HPLC (n= 6)	
		Drug content (%) ± SD	RSD (%)
Levocetirizine	5	99.76± 0.55	0.56
Montelukast sodium	10	100.15 ± 0.71	0.71

Table 5: Analysis of a marketed formulation (Assay).

Robustness: The standard deviation of the peak areas was calculated for each parameter and the RSD was found to be less than 2 % for HPTLC. For HPLC, robustness of the method was studied by deliberately varying parameters like flow rate (±0.1 mL min^{-1}) and mobile phase composition (±1 mL).The low values of the RSD %, as shown in Table 3. indicated the robustness of the two proposed methods.

Specificity: The specificity of both methods was noticed by the complete separation of levocetirizine dihydrochloride and montelukast sodium peaks in the presence of tablet excipients. The peak purity of levocetirizine dihydrochlorideand Montelukast sodium was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9979 and r (M, E) = 0.9986. A good correlation (r^2 = 0.9981) was also obtained between the standard and sample spectra of levocetirizine dihydrochloride and Montelukast sodium, respectively. For HPLC, no interference was observed due to any unknown excipients of tablet dosage forms at the retention times of levocetirizine dihydrochloride and montelukast sodium. The peaks obtained were sharp and had clear baseline separation for both the methods.

Accuracy: As shown from the data in Table 4, satisfactory recoveries % with small relative standard deviations (RSD%) were obtained at various added concentrations for both the methods. The results indicate that the methods are highly accurate for simultaneous determination of the two drugs.

Analysis of a marketed formulation (Assay): Using the proposed chromatographic methods, assays of levocetirizine dihydrochloride and montelukast sodium in their tablets were carried out. Satisfactory results were obtained for both drugs in a good agreement with the label claims thereby suggesting suitability of the method (Figure 3 and Figure 4). The recovery % ± RSD % of six replicate determinations were 99.72 ± 0.98 (levocetirizine), 100.19 ± 1.06 (montelukast sodium) for HPTLC and 99.76 ± 0.56 (levocetirizine), 100.15 ± 0.71 (montelukast sodium) for HPLC (Table 5).

Conclusion

The proposed HPTLC and HPLC methods provide simple, accurate and reproducible methods of quantitative analysis for simultaneous determination of levocetirizine dihydrochloride and montelukast sodium in bulk and in pharmaceutical formulation. Both methods were validated as per ICH guidelines. The methods are specific and there is no interference from any of the sample components. It was concluded that the developed method offered several advantages such as rapid, cost effective, simple mobile phase and sample preparation steps, improved sensitivity and comparative short run time made it specific, reliable and easily reproducible in any quality control set-up providing all the parameters are followed accurately for its intended use.

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