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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF LEVOCETIRIZINE HYDROCHLORIDE AND MONTELUKAST SODIUM AS BULK DRUGS AND IN TABLET DOSAGE FORM

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ABSTRACT

A new simple, accurate, precise and selective stability- indicating High Performance Thin Layer Chromatographic (HPTLC) method has been developed and validated for simultaneous determination of Levocetirizine Hydrochloride and Montelukast Sodium in combined tablet dosage form. The mobile phase selected was Toluene: Ethyl Acetate: Methanol (2.5: 5: 2.5, v/v/v) with UV detection at 240 nm. The retention factors for Levocetirizine Hydrochloride and Montelukast Sodium were found to be 0.17 ± 0.06 and 0.76 ± 0.04, respectively. The method was validated with respect to linearity, accuracy, precision and robustness as recommended by ICH. The drugs were subjected to stress condition of hydrolysis, oxidation, photolysis and thermal degradation. Results were found to be linear in the concentration range of 100-500 ng band^{-1} for Levocetirizine Hydrochloride and 200-1000 ng band^{-1} for Montelukast Sodium. The method has been successfully applied for the analysis of drug in pharmaceutical formulation. The % assay (Mean ± S.D.) was found to be 99.14 ± 0.36 for Levocetirizine Hydrochloride and 99.28 ± 0.66 for Montelukast Sodium, respectively. The developed method can be used for accessing the stability of Levocetirizine Hydrochloride and Montelukast Sodium in bulk drug and pharmaceutical tablet dosage form.

KEYWORDS: Levocetrizine hydrochloride, Montelukast Sodium, HPTLC, Forced degradation, Validation.

INTRODUCTION

Levocetirizine Hydrochloride (LEVO), chemically, 2-[2-[4-[(R)-(4-chlorophenyl)-phenyl methyl] piperazinyl-1-yl] ethoxy] acetic acid is a selective H1-antihistamine indicated for the treatment of allergic rhinitis and chronic idiopathic urticaria.[1] Montelukast Sodium (MONT), 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 is potent cysteinyl leukotriene receptor antagonist used in the treatment of asthma.[2]

Extensive review of literature revealed that several high performance liquid chromatographic (HPLC)[3-7] have been reported for the estimation of LEVO either as single drug or in combination with other drugs in pharmaceutical dosage form. Analytical methods reported for MONT includes high performance liquid chromatographic either as single or in combination with other drugs.[8-13] Several HPLC [14-17] and Spectrophotometric[18, 19] methods for simultaneous determination of LEVO and MONT in pharmaceutical dosage form were also reported.

To best of our knowledge, no reports were found for stability-indicating High Performance Thin Layer Chromatographic (HPTLC) method for simultaneous determination of LEVO and MONT in combined tablet dosage form. This paper describes simple, precise, accurate and selective stability indicating HPTLC method development and validation for simultaneous quantification of these drugs in combined tablet dosage form.

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure sample of LEVO and MONT were kindly supplied by Cipla Pharmaceuticals Ltd. (Mumbai, India). The pharmaceutical dosage form used in this study was Levomont tablets labeled to contain 5 mg of LEVO and 10 mg of MONT was procured from the local market. Toluene, Ethyl Acetate and Methanol (AR grade) were purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Chromatographic separation of drug was performed on precoated silica gel aluminium plate 60 F_{254} (20 ×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 8 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 20 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Toluene: Ethyl Acetate: Methanol (2.5: 5: 2.5, v/v/v) as mobile phase with chamber saturation time of 15 min. After development, TLC plates were dried and densitometric measurement was performed on CAMAG thin layer chromatography scanner-3 at 240 nm operated by winCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of standard solution

Working standard solution of LEVO was prepared by dissolving 5 mg of drug in 10 mL of methanol to get concentration of 500 ng μL^{-1} from which 1 mL was further diluted to 10 mL to get final concentration of 50 ng μL^{-1}. Stock solution of MONT was prepared by dissolving 10 mg of drug in 10 mL of methanol to get concentration of 1000 ng μL^{-1} from which 1 mL was further diluted to 10 mL to get final concentration of 100 ng μL^{-1}.

Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that both drugs showed maximum absorbance at 240 nm. So, 240 nm was selected as the wavelength for detection.

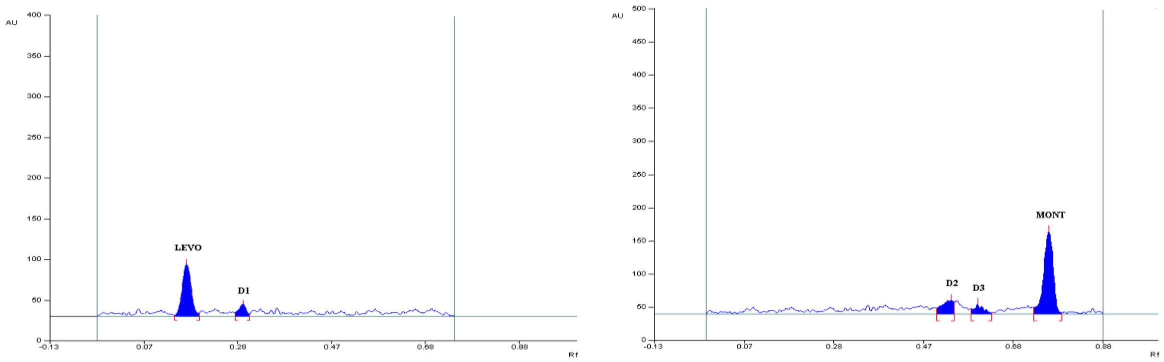
Tablet formulation analysis

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 5 mg of LEVO (10 mg of MONT) was weighed and transferred to a 10 mL volumetric flask containing 6 mL of methanol and the content was sonicated for 15 min. The solution was filtered and the volume was made up to the mark with methanol to obtain the final concentration of 500 ng $band^{-1}$ for LEVO and 1000 ng $band^{-1}$ for MONT. One mililitre volume of above solution was diluted with methanol to obtain final concentration of 50 ng $band^{-1}$ for LEVO and 100 ng $band^{-1}$ for MONT. Four µL volume of this solution was applied on TLC plate to obtain final sample concentration of 200 ng $band^{-1}$ for LEVO and 400 ng $band^{-1}$ for MONT. After chromatographic development peak areas of the bands were measured at 240 nm and the amount of each drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies of bulk drug: The bulk drugs were subjected to stress condition of hydrolysis, oxidation, photolysis and thermal degradation to check the stability.

Acid induced degradation

1 mL stock solution of LEVO (500 ng µL^{-1}) was mixed with 1 mL of 0.1 N HCl and 8 mL of methanol. Solution was refluxed for 1 h at 70°C. 8 µL volume of resulting solution was applied on TLC plate and developed under optimized chromatographic conditions. Same procedure was repeated for MONT (1000 ng µL^{-1}) to get final concentration 800 ng band^{-1} applied on the TLC plate. After acid hydrolysis, 12.27 % of degradation was observed for LEVO with additional degradation product at Rf 0.29 and 16.60 % of degradation was observed with two additional peaks for MONT. The representative densitogram obtained after acid degradation is shown in Fig. 1.

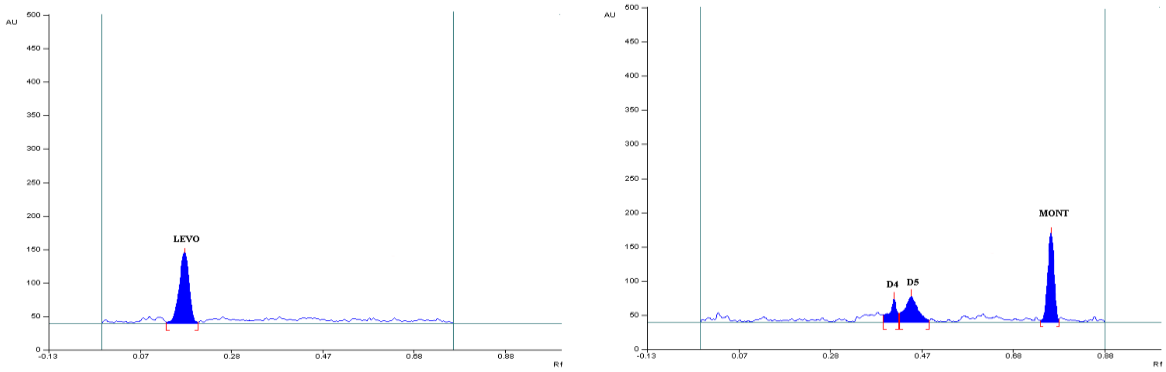


(a) **(b)**

Fig. 1: Representative densitogram of (a) LEVO with degradation product D1 (Rf= 0.29) (b) MONT with degradation products D2 (Rf= 0.52) and D3 (Rf= 0.61)

Base induced degradation

1 mL stock solution of LEVO (500 ng µL^{-1}) was mixed with 1 ml of 0.1 N NaOH and 8 mL of methanol. The solution was kept at room temperature for 24 h. 8 µL volume of resulting solution was applied on TLC plate and developed under optimized chromatographic conditions. Same procedure was repeated for MONT (1000 ng µL^{-1}) to get final concentration 800 ng band^{-1} applied on the TLC plate. Both the drugs were found susceptible to alkali degradation with % degradation of 9.44 and 26.12 for LEVO and MONT, respectively. The densitogram obtained after base degradation is shown in Fig. 2.

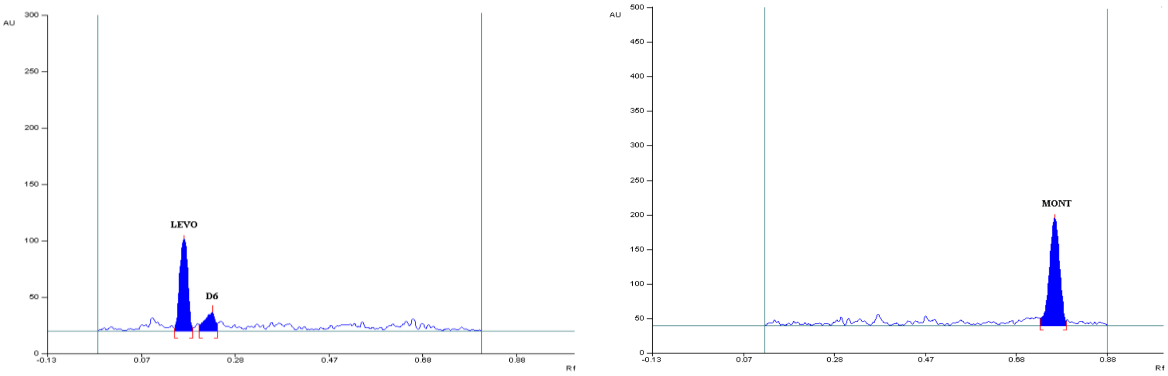


(a) **(b)**

Fig. 2: Representative densitogram of (a) LEVO (b) MONT with degradation products D4 (Rf= 0.40) and D5 (Rf= 0.45)

Neutral hydrolytic degradation

1 mL stock solution of LEVO (500 ng µL^{-1}) was mixed with 1 mL of water and 8 mL methanol. The solution was refluxed for 1 h at 70ºC. 8 µL volume of resulting solution was applied on TLC plate and developed under optimized chromatographic conditions. Same procedure was repeated for MONT to get final concentration 800 ng band^{-1} applied on the TLC plate. On neutral hydrolysis, 14.61 % degradation was observed with degradation product and 15.26% of MONT was degraded with reduction in peak area.



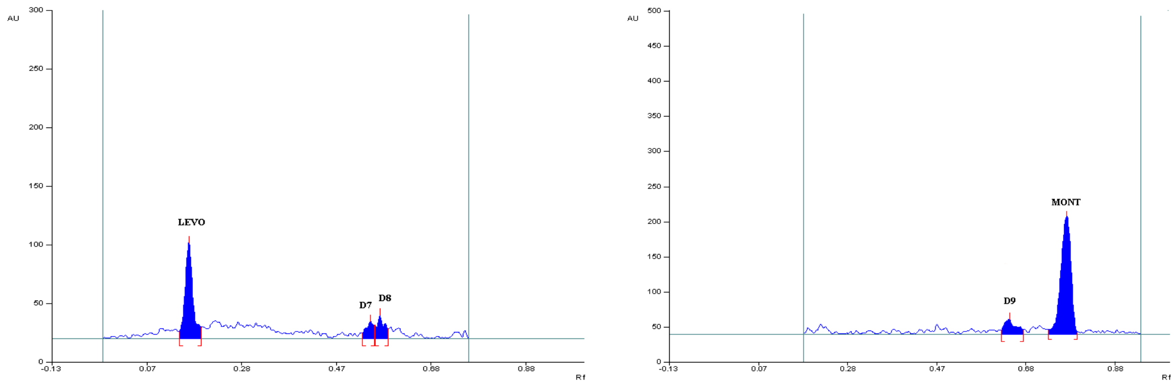
(a) **(b)**

Fig. 3: Representative densitogram of (a) LEVO with degradation product D6 (Rf= 0.21) (b) MONT

Oxidative degradation

1 mL stock solution of LEVO (500 ng µL^{-1}) was mixed with 1 mL of 30 % solution of H_2O_2 and 8 mL of methanol. The solution was kept at room temperature for 24 h and 8 µL of

resulting solution was applied on TLC plate and developed under optimized chromatographic conditions. Same procedure was repeated for MONT to get final concentration 800 ng band^{-1} applied on the TLC plate. Both drugs were found susceptible to oxidation with % degradation of 22.81 for LEVO and 14.38 for MONT. The densitogram obtained after oxidative degradation is shown in Fig. 4.

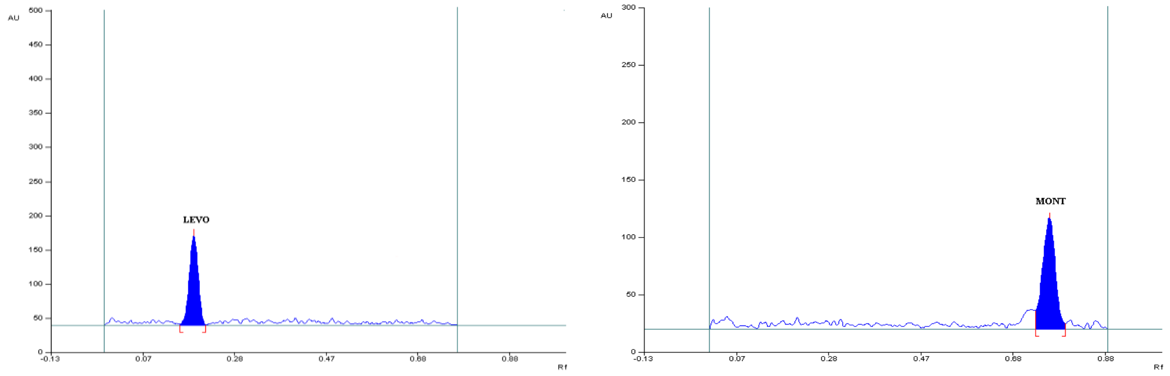


(a) **(b)**

Fig. 4: Representative densitogram of (a) LEVO with degradation product D7 (Rf= 0.54) and D8 (Rf= 0.58) (b) MONT with degradation product D9 (Rf= 0.65)

Photo-degradation

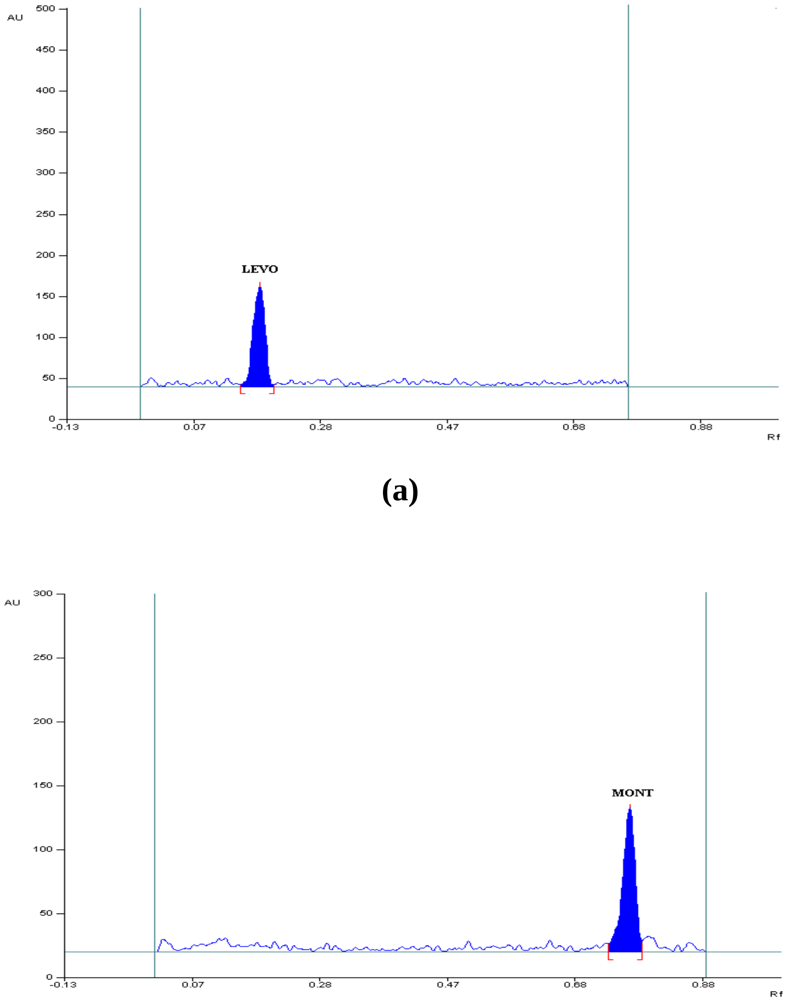
Photolytic studies were carried out by exposure of drugs to UV light up to 200 watt h square meter^{-1} for a period of 24 h. Samples were weighed, dissolved in methanol to get concentration of 50 ng µL^{-1} for LEVO and 100 ng µL^{-1} for MONT. 8 µL volume of the resulting solution was applied to HPTLC and analyzed. Both the drugs were found to be stable after exposure to UV light.



(a) **(b)**

Fig. 5: Representative densitogram of (a) LEVO (b) MONT after exposure to UV light

Dry heat degradation: Dry heat study was performed by keeping the drugs in oven at 80ºC for period of 24 h. Sample were withdrawn at appropriate time, weighed and dissolved in methanol to get solution of 50 ng µL^{-1} for LEVO and 100 ng µL^{-1} for MONT. 8 µL volume of the resulting solution was applied to HPTLC and analyzed. LEVO showed no degradation and 5.92% degradation was observed for MONT when subjected to dry heat in oven at 80ºC for 24 h. The desitogram of samples subjected to dry heat is shown in Fig. 6.



(a)

(b)

Fig. 6: Representative densitogram of (a) LEVO (b) MONT subjected to dry heat

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: The primary objective in developing this stability indicating HPTLC method is to achieve the resolution of MONT and LEVO and its degradation products. The chromatographic separation was achieved by linear ascending development in 20 cm × 10 cm twin trough glass chamber using Toluene: Ethyl Acetate: Methanol (2.5: 5: 2.5, v/v/v) as mobile phase and detection was carried out at 240 nm. The retention factors for LEVO and MONT were found to be 0.17 ± 0.06 and 0.76 ± 0.04, respectively. Representative densitogram of mixed standard solution of LEVO and MONT is shown in Fig. 7.

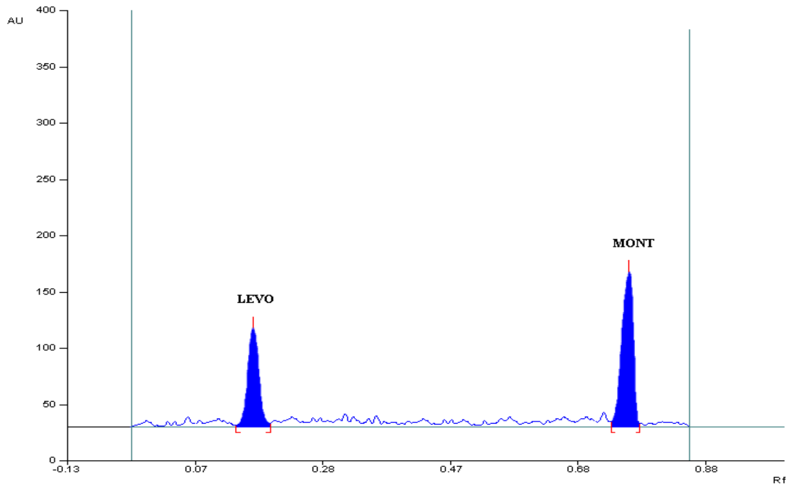


Fig. 7: Representative densitogram of mixed standard solution of LEVO (300 ng band^{-1}, Rf =0.17 ± 0.06) and MONT (600 ng band^{-1}, Rf =0.76 ± 0.04)

Result of forced degradation studies

Forced degradation study showed that both the drugs were susceptible to hydrolysis, oxidative stress conditions but stable under photolytic stress conditions. The degradation products formed during the study were well resolved from the drugs indicating specificity of the developed method. The forced degradation studies data are summarized in Table 1.

Table 1: Data of forced degradation studies of MONT and LEVO

Stress conditions/ duration	**MONT**		**LEVO**	
	% Assay of active substance	**Rf values of degraded products**	**% Assay of active substance**	**Rf values of degraded products**
Acid/ 0.1 N HCl/ Refluxed for 1 h at 70ºC	83.39	0.52, 0.61	87.72	0.29
Alkali/0.1 N NaOH/ Kept at RT for 24 h	73.87	0.40, 0.45	90.55	---
Oxidative /30 % H_2O_2 / Kept at RT for 24 h	85.61	0.65	77.19	0.54,0.58
Neutral/H_2O/ Refluxed for1 h at 70ºC.	84.73	---	85.38	0.21
Photolysis UV/200 watt h square meter^{-1} for 24 h	99.64	stable	99.66	stable
Dry heat/ 80ºC/ 24 h	94.08	---	99.50	stable

Method Validation

The method was validated as per ICH guidelines in terms of linearity, accuracy, intra-day and inter-day precision and robustness.[20, 21]

Preparation of Calibration Curve

Standard stock solutions of LEVO (50 ng μL^{-1}) and MONT (100 ng μL^{-1}) were applied by overspotting on TLC plate in range of 2, 4, 6, 8 and 10 µL. Straight-line calibration graphs were obtained in the concentration range of 100-500 ng $band^{-1}$ for LEVO and 200-1000 ng $band^{-1}$ for MONT with high correlation coefficient > 0.99.

Precision

Set of three different concentrations in three replicates of standard solutions of LEVO and MONT were prepared. All the solutions were analyzed on the same day in order to record any intra day variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.23-0.73 for LEVO and 0.33-0.78 for MONT. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.40-0.99 for LEVO and 0.42-1.05 for MONT. The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ for MONT were found to be 44.43 ng $band^{-1}$ and 134.66 ng $band^{-1}$, respectively. The LOD and LOQ for LEVO were found to be 29.12 ng $band^{-1}$ and 88.24 ng $band^{-1}$, respectively.

Recovery Studies

Accuracy of the method was performed by carrying out the recovery studies by adding standard drug to pre-analysed sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 200 ng $band^{-1}$ for LEVO and 400 ng $band^{-1}$ for MONT from tablet solution. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for estimation of drugs in tablet dosage form. The results obtained are shown in Table 2.

Table 2: Recovery Studies of LEVO and MONT

Drug	Amount taken (ng band^{-1})	Amount added (ng band^{-1})	Total amount found (ng band^{-1})	% Recovery	% RSD
LEVO	200	160	355.83	98.82	0.14
	200	200	398.78	99.83	0.26
	200	240	437.10	99.33	0.24
MONT	400	320	716.68	99.53	0.76
	400	400	797.35	99.66	0.12
	400	480	876.15	99.52	0.12

*Average of three determinations

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 995, indicating the no interference of any other peak of degradation product, impurity or matrix.

Robustness Studies

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase, wavelength was altered and their effect on the area of drug was noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% R.S.D. < 2). The results are given in Table 3.

Table 3: Robustness Data in Terms of Peak Area (% RSD)

Sr. No.	Parameter	(% RSD)*	
		MONT	LEVO
1	Detection wavelength (± 2 nm)	0.47	0.51
2	Mobile phase variation (± 2 % methanol)	0.78	0.75

*Average of three determinations

CONCLUSIONS

Stability indicating HPTLC-Densitometric method for simultaneous estimation of LEVO and MONT as bulk drugs and in tablet dosage form has been developed and validated as per ICH guidelines. The standard deviation, % RSD calculated for the method are low, indicating a high degree of precision of the method. The results of the recovery studies performed indicated that the method is accurate for estimation of drugs in tablet dosage form. The results of the stress studies indicated the specificity of the method. The method can be used to determine the purity of the drugs available from various sources by detecting the related impurities.

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