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Validated HPTLC Method for Simultaneous Estimation of Levocetirizine Hydrochloride and Nimesulide in Formulation

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ABSTRACT

This paper describes a new, simple, precise, and accurate HPTLC method for simultaneous estimation of levocetirizine hydrochloride and nimesulide as the bulk drug and in tablet dosage forms. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F_{254} as the stationary phase and the solvent system consisted of toluene: ethyl acetate: methanol: ammonia 9: 1: 1: 0.5(v/v/v/v). Densitometric evaluation of the separated zones was performed at 238 nm. The two drugs were satisfactorily resolved with R_F values 0.21 ± 0.02 and 0.26 ± 0.02 for levocetirizine and nimes ulide, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (100–350 ng/spot for levocetirizine and 400-1400 ng/spot for nimesulide), precision (intra-day RSD 1.03–1.41 % and inter-day RSD 1.10–1.85 % for levocetirizine, and intra-day RSD 0.07–0.25 % and inter-day RSD 0.28–0.75 % for nimesulide), accuracy (100.06 ± 1.16 % for levocetirizine and 99.48 ± 0.50 % for nimesulide), and specificity, in accordance with ICH guidelines.

Keywords: Thin layer Chromatography, Densitometry, Validation and Quantification, Levocetirizine Hydrochloride & Nimesulide.

INTRODUCTION

Levocetirizine Hydrochloride is chemically (LEVO), 2-[2-[4-[(R)-(4-Chlorophenyl phenyl methyl]-piperazine-1-yl] ethoxy]-acetic acid dihydrochloride **(Figure 1).** LEVO is an active enantiomer of cetirizine, which is third-generation non-sedative antihistamine. LEVO works by blocking histamine receptors. It does not prevent the actual release of histamine from mast cells, but prevents its binding to its receptors. This in turn prevents the release of other allergy

chemicals and increased blood supply to the area, and provides relief from the typical symptoms of hayfever [1].

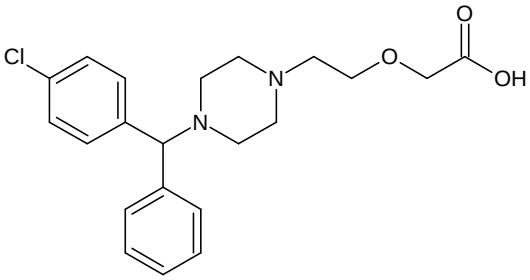

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Figure1 The structure of Levocetirizine Hydrochloride

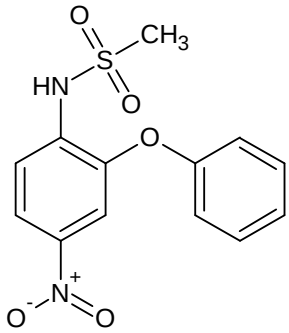

Figure 2 Structure of Nimesulide

Nimesulide (NIME), N-(4-Nitro-2-phenoxyphenyl)methanesulfonamide **(Figure 2)** is a relatively COX-2 selective, non-steroidal anti-inflammatory drugs (NSAID) with analgesic and antipyretic properties. Its approved indications are the treatment of acute pain, the symptomatic treatment of osteoarthritis and primary dysmenorrhoe [2].

Literature review reveals that methods have been reported for analysis of NIME and LEVO in bulk and pharmaceutical formulation by UV spectrophotometric method [3, 4] and analysis of Montelukast Sodium and Levocetirizine Dihydrochloride by HPTLC [5] and for estimation of NIME by HPTLC either alone or in combination with other drugs [6-9].

Today TLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC - thus reducing the analysis time and cost per analysis.

To date, there have been no published reports about the simultaneous estimation of LEVO and NIME by TLC in bulk drug and in pharmaceutical dosage forms. This present study reports for the first time simultaneous estimation of LEVO and NIME by TLC in bulk drug and in pharmaceutical dosage forms.

MATERIALS AND METHODS

Working standards of pharmaceutical grade LEVO (batch no. LE/002/1128) and NIME (batch no. NI/6543/241) were obtained as generous gifts from AGIO PHARMA, Pune (Maharashtra, India). It was used without further purification and certified to contain 99.02 % and 99.30 % (w/w) on dry weight basis LEVO and NIME, respectively. Fixed dose combination tablets (OPENOS) containing 5 mg LEVO and 100 mg NIME were procured from Panacia Biotech Pvt. Ltd. India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60_{F-254} plates, [20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany)] using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110 ^{0}C for 5 min prior to chromatography. A constant application rate of 0.1 µL/s was used and the space between two bands was 6 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 mm/s. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase consisted of toluene: ethyl acetate: methanol: ammonia (9: 1: 1: 0.5) (v/v/v/v) and 11.5 mL of mobile phase was used per chromatography run. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25 ^{o}C ± 2) at relative humidity of 60 % ± 5. Each chromatogram was developed over a distance of 8 cm. following the development the TLC plates were dried in a stream of air with the help of an air dryer in a wooden chamber with adequate ventilation. The flow of air in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 238 nm and operated by CATS software (V 3.15, Camag). The source of radiation used was 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 performed by linear regression of peak areas determined by UV absorption as a function of sample amounts.

Preparation of Standard Stock Solutions

Standard stock solutions of concentration 50 µg/mL of LEVO and 1000 µg/mL of NIME were prepared separately using methanol. From the standard stock solution, the mixed standard solution was prepared using the methanol to contain 5 µg/mL of LEVO and 100 µg/mL of NIME. The stock solution was stored at 2-8 °C protected from light.

Optimization of the HPTLC method

The TLC procedure was optimized with a view to develop a simultaneous assay method for LEVO and NIME respectively. The mixed standard stock solution (5 µg/mL of LEVO and 100 µg/mL of NIME) and 10 µL of band were spotted on to TLC plates and run in different solvent systems. Initially, toluene, ethyl acetate, methanol and ammonia were tried in different ratios. Toluene was used to impart the necessary non-polarity to mobile phase to obtain a suitable R_F

value. Initially, toluene, ethyl acetate, methanol, and ammonia in the ratio of 8: 1: 1: 1 v/v/v/v was selected but R_F was found less than 0.15 and tailing with peaks was observed. Then volume of ethyl acetate was increased by 1 mL and toluene was decreased by 0.5 mL to increase R_F and for improving peak shape. Finally, the mobile phase consisting of toluene: ethyl acetate: methanol: ammonia in the ratio of 9: 1: 1: 0.5 v/v/v/v was found optimum **(Figure 3).** In order to reduce the neckless effect TLC chamber was saturated for 20 min using saturation pads. The mobile phase was run upto a distance of 8 cm; which takes approximately 20 min for complete development of the TLC plate.

Validation of the method

Validation of the optimized TLC method was carried out with respect to the following parameters.

Linearity and range

From the mixed standard stock solution 50 µg/mL of LEVO and 200 µg/mL of NIME, 2 to 7 µL solution spotted on TLC plate to obtain final concentration 100-350 ng/spot for LEVO and 400-1400 ng/spot for NIME. Each concentration was applied six times to the TLC plate. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (100, 200, 300 ng/spot for LEVO and 400, 800, 1200 ng/spot for NIME) of the drug six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and limit of quantification

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background by spotting a blank and calculating the signal-to-noise ratio for LEVO and NIME by spotting a series of solutions until the S/N ratio 3 for LOD and 10 for LOQ. To determine the LOD and LOQ, serial dilutions of mixed standard solution of LEVO and NIME were made from the standard stock solution in the range of 10–100 ng/spot. The samples were applied to TLC plate and the chromatograms were run and measured signal from the samples was compared with those of blank samples.

Robustness of the method

Following the introduction of small changes in the mobile phase composition, the effects on the results was examined. Mobile phases having different compositions, e.g. toluene: ethyl acetate: methanol: ammonia (9: 1: 1: 0.4 v/v/v/v), (9.5: 1: 1: 0.5 v/v/v/v), (9: 0.9: 1: 0.5 v/v/v/v), (9: 1: 0.9: 0.5 v/v/v/v), were tried and chromatograms were run. The amount of mobile phase was varied over the range of ± 5 %. The plates were prewashed with methanol and activated at 60 °C for 2, 5, and 7 min respectively prior to chromatography. The time from spotting to chromatography and from chromatography to scanning was varied from ± 10 min. The

robustness of the method was determined at three different concentration levels 100, 200 and 300 ng/spot and 400, 800 and 1200 ng/spot for LEVO and NIME respectively.

Specificity
The specificity of the method was determined by analyzing standard drug and test samples. The spot for LEVO and NIME in the samples was confirmed by comparing the R_F and spectrum of the spot with that of a standard. The peak purity of LEVO and NIME 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).

Accuracy
Accuracy of the method was carried out by applying the method to drug sample (LEVO and NIME combination tablet) to which know amount of LEVO and NIME standard powder corresponding to 80, 100 and 120% of label claim had been added (Standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

Analysis of a marketed formulation
To determine the content of LEVO and NIME in conventional tablet (Brand name: OPENOS, Label claim: 5 mg levocetirizine hydrocloride and 100 mg nimesulide per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 5 mg of LEVO and 100 mg NIME was transferred into a 50 mL volumetric flask containing 30-35 mL of methanol sonicated for 30 min and diluted to 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (100 and 2000 µg/mL for LEVO and NIME respectively). Then 5 mL of the above filtered solution was diluted to produce a concentration of 50 and 1000 µg/mL for LEVO and NIME respectively and 1 µL of this solution (50 and 1000 ng/spot for LEVO and NIME respectively) was applied to a TLC plate which was developed in optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for LEVO and NIME in the current study involving toluene: ethyl acetate: methanol: ammonia (9: 1: 1: 0.5, v/v/v/v) as the mobile phase for TLC are given below.

Linearity
The drug response was linear (r^2 = 0.9974 for LEVO and 0.9965 for NIME) over the concentration range between 100-350 ng/spot for LEVO and 400-1400 ng/spot for NIME. The mean (± RSD) values of the slope, intercept and correlation coefficient for LEVO and NIME were 1.1177 (± 0.465), 35.181 (± 1.24) and 0.9974 (± 1.4), and 2.5357 (± 0.234), 251.86 (± 1.57), and 0.9965 respectively.

Table 1Precision studies

Concentration (ng/spot)	Repeatability (n=6)			Intermediate precision (n=6)		
	Measured conc. ±SD	(%) RSD	Recovery (%)	Measured conc. ±SD	(%)RSD	Recovery (%)
Levocetirizine Hydrochloride						
100	98.25±1.7	1.12	98.25	98.35 ± 1.9	1.10	99.15
200	196.88 ± 2.6	1.03	98.45	195.86 ± 2.2	1.15	100.02
300	299.71 ± 5.2	1.41	99.90	300.25 ± 6.1	1.85	100.85
Nimesulide						
400	403.58± 3.26	0.25	100.89	410.48±3.46	0.35	100.98
800	813.63± 4.23	0.18	101.70	820.73±4.50	0.28	100.95
1200	1212.34± 2.42	0.07	101.02	1220.35± 3.42	0.75	98.45

Table 2 Robustness testing (n = 6)

Parameter	SD of peak area for Levocetirizine Hydrochloride	% RSD	SD of peak area for Nimesulide	% RSD
Mobile phase composition (± 0.1 ml)	4.96	0.28	5.14	0.79
Amount of mobile phase (± 5%)	6.56	0.89	2.76	0.82
Time from spotting to chromatography (± 10 min.)	6.78	0.74	4.98	0.67
Time from chromatography to scanning (± 10 min.)	5.80	0.75	2.26	0.55

Table 3 Recovery studies (n = 6)

Drug	Label claim (mg per tablet)	Amount added (%) (Total amount present)	Amount recovered (mg) ± % RSD	Recovery (%)
Levocetirizine HCl	5mg	80 (9mg)	8.93 ± 1.71	99.22
		100 (10mg)	10.10 ± 0.11	101.01
		120 (11 mg)	10.99 ± 0.96	99.96
Nimesulide	100 mg	80 (180mg)	176.92 ± 0.18	98.29
		100 (200mg)	198.42 ± 1.73	99.21
		120 (220mg)	222.09 ± 1.86	100.95

Table 4 Analysis of commercial formulation

Levocetirizine hydrochloride (5 mg)	LEVO found (mg per tablet)	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	4.85 ± 1.12	99.77
2nd Lot	4.78 ± 1.04	99.56
Nimesulide (100 mg)	NIME found (mg per tablet)	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	99.75 ± 1.09	99.75
2nd Lot	99.18 ± 1.11	99.18

Precision

The results of the repeatability and intermediate precision experiments are shown in **Table 1**. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines.

LOD and LOQ

Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 60 ng/spot and 80 ng/spot for LEVO and 100 ng/spot and 150 ng/spot for NIME, respectively.

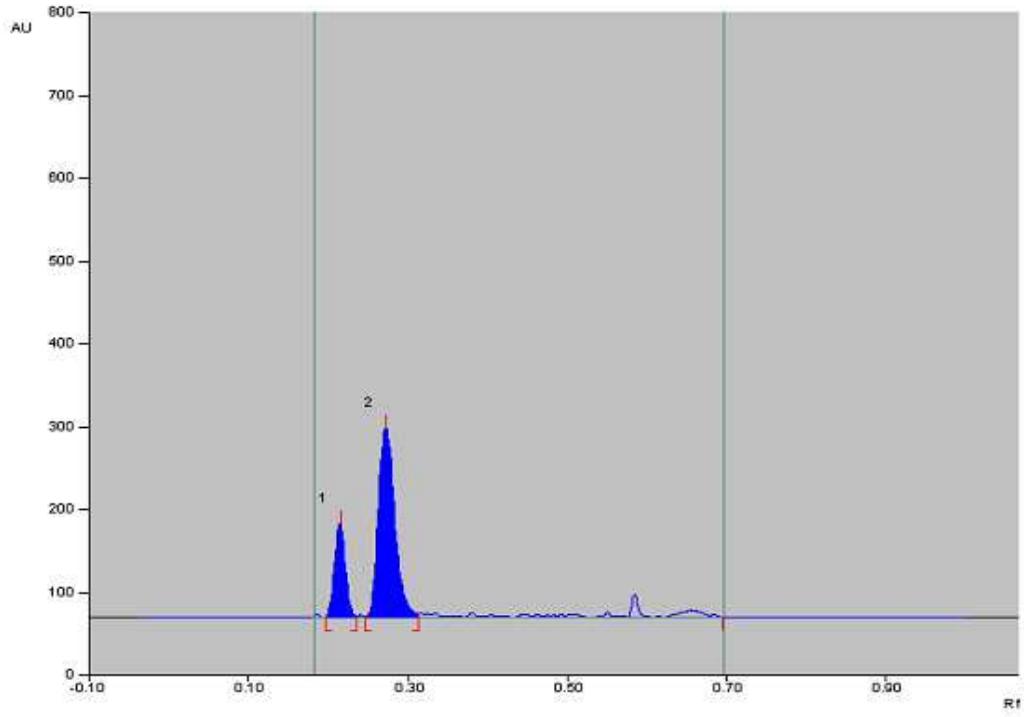

Figure 3 Densitogram of Levocetirizine hydrochloride R_F (0.21) and Nimesulide R_F (0.26)

Robustness of the method

The standard deviations of peak areas were calculated for each parameter and the % RSD was found to be less than 2 %. The low values of the % RSD, as shown in **Table 2** indicated robustness of the method.

Specificity

The peak purity of LEVO and NIME was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9974 and r (M, E) = 0.9969. A good correlation (r = 0.9979) was also obtained between the standard and sample spectra of LEVO and NIME respectively.

Recovery Studies

As shown from the data in **Table 3** good recoveries of the LEVO and NIME in the range from 99.21 to 101.01 % were obtained at various added concentrations. The average recovery of three levels (nine determinations) for LEVO and NIME were 100.06 % and 99.48 % respectively.

Analysis of a formulation

Experimental results of the amount of LEVO and NIME in tablets, expressed as a percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets. The drug content was found to be 99.66 % (± 0.83) and 99.46 % (± 0.78). Two different lots of LEVO and NIME combination tablets were analyzed using the proposed procedures and the results are summarized in **Table 4**.

CONCLUSION

Introducing TLC into pharmaceutical analysis represents a major step in terms of quality assurance. The developed TLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of LEVO and NIME as bulk drug and in pharmaceutical formulation without any interference from the excipients. It was concluded that the developed method offered several advantages such as rapid, cost effective, simple mobile phase and sample preparation steps and improved sensitivity made it specific, reliable and easily reproducible in any quality control set-up providing all the parameters are followed accurately for its intended use. It may be extended to study the degradation kinetics of LEVO and NIME and also for its estimation in plasma and other biological fluids. The proposed TLC method is less expensive, simpler, rapid, and more flexible than HPLC.

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