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Research Article

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VALIDATED HPLC METHOD FOR SIMULTANEOUS QUANTITATION OF LEVOCETIRIZINE HYDROCHLORIDE AND NIMESULIDE IN BULK DRUG AND FORMULATION

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ABSTRACT

HPLC method has been described for simultaneous determination of Levocetirizine hydrochloride and Nimesulide in formulation. This method is based on HPLC separation of the two drugs on the HiQ Sil C_{18} HS (250 mm × 4.6 mm, 5.0 µ), Germany with isocratic conditions and simple mobile phase containing methanol: water (70: 30) at flow rate of 1 mL/min using UV detection at 239 nm. This method has been applied to formulation without interference of excipients of formulation. The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 1-6 µg/mL for Levocetirizine hydrochloride and 4-24 µg/mL for Nimesulide respectively. The mean values of the correlation coefficient, slope and intercept were 0.9990 ± 1.27, 34675 ± 1.18 and 8943.5 ± 1.82 for Levocetirizine hydrochloride and 0.9992 ± 0.78, 40863 ± 1.21 and 5209.2 ± 1.09 for Nimesulide respectively. The method was validated for precision, robustness and recovery. The limit of detection (LOD) and limit of quantitation (LOQ) was 0.25 µg/mL and 0.5 µg/mL for Levocetirizine hydrochloride and 2 µg/mL and 3 µg/mL for Nimesulide, respectively. Statistical analysis showed that the method is repeatable and selective for the estimation of Levocetirizine hydrochloride and Nimesulide.

Keywords: Levocetirizine hydrochloride, Nimesulide; HPLC, Validation.

INTRODUCTION

Levocetirizine (as levocetirizine hydrochloride) is [2-[4-[(R)-(4-Chlorophenyl) phenylmethyl]-1- piperazinyl] ethoxy] acetic acid hydrochloride (Figure **1**) is a third generation non-sedative antihistamine, developed from the second generation antihistamine cetirizine. Chemically, Levocetirizine is the active enantiomer of cetirizine. It is the L-enantiomer of the cetirizine racemate. Levocetirizine works by blocking histamine receptors. It does not prevent the actual release of histamine from mast cells, but prevents it 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. It is said to be more effective with fewer side effects than the second-generation drugs.[1]

Nimesulide, N-(4-Nitro-2-phenoxyphenyl) methane sulfonamide (Figure **2**) is a non-steroidal anti-inflammatory analgesic drug has a multifactorial mechanism of action that affects the activity of MMPs (metalloprotease) and other biochemical markers of joint destruction, reduces the release of ROS (reactive oxygen species) and other toxic substances from neutrophils, and reduces the production of pro inflammatory cytokines. Nimesulide has a rapid onset of the analgesic action. Its approved indications are the treatment of acute pain, the symptomatic treatment of osteoarthritis and primary dysmenorrheal in adolescents and adults above 12 years old. These unique characteristics make nimesulide an appealing therapeutic choice in the treatment of acute pain.[2]

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Figure 1. Structure of Levocetirizine hydrochloride

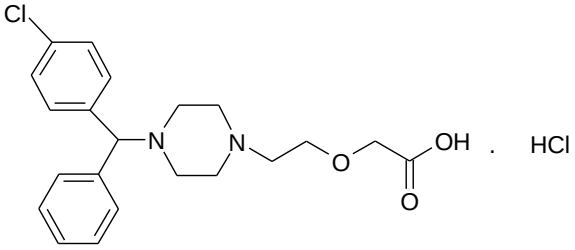
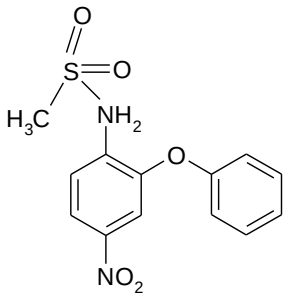


Figure 2. Structure of Nimesulide



Literature review reveals that methods have been reported for analysis of Levocetirizine hydrochloride and Nimesulide, HPLC method for the determination of Nimesulide in pharmaceutical preparations[3], RP-HPLC method for determination of Nimesulide in combination with drotaverine hydrochloride[4] and few bioanalytical methods are also reported.[5,6] HPTLC method for nimesulide[7], stability indicating HPTLC method for

simultaneous estimation of drotaverine and nimesulide in pharmaceutical dosage form[8] and some spectrophotometric methods for both Levocetirizine hydrochloride and Nimesulide are also reported.[9-12]

To date, there have been no published reports about the simultaneous quantitation of Levocetirizine hydrochloride and Nimesulide by chromatographic method in bulk drug and in tablet dosage form. This present study reports for the first time simultaneous quantitation of Levocetirizine hydrochloride and Nimesulide by HPLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH guidelines.

MATERIALS AND METHODS

Materials

Working standards of pharmaceutical grade Levocetrizine (Batch no. 1L16554) and Nimesulide (Batch no. 102/4321) were obtained as generous gifts from Cipla Limited, Patalganga (Maharashtra, India). They were used without further purification and certified to contain 99.86 % and 99.69 % on dry weight basis for Levocetrizine and Nimesulide, respectively. Fixed dose combination tablet (OPENOS, Batch no.1618502) containing 5 mg Levocetrizine and 100 mg Nimesulide was purchased from local market, Pune, India.

Instrumentation

The HPLC system consisted of Intelligent HPLC pump model (Jasco PU 2080 Plus) with sampler programmed at 20 µL capacity per injection was used. The detector consisted of a UV/ VIS (Jasco UV 2075 Plus). Another system consisted of Intelligent HPLC pump (Jasco PU 1580) with detector (Jasco UV-1575) and auto sampler. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was, HiQ Sil C_{18} HS (4.6 mm I.D. × 250mm L), Germany. Mobile phase consisted of a mixture of methanol: water (70: 30) at flow rate of 1 mL/min using UV detection at 239 nm. The mobile phase was filtered through a 0.2 micron membrane filter and degassed. The injection volume was 20 µL and analysis was performed at ambient temperature.

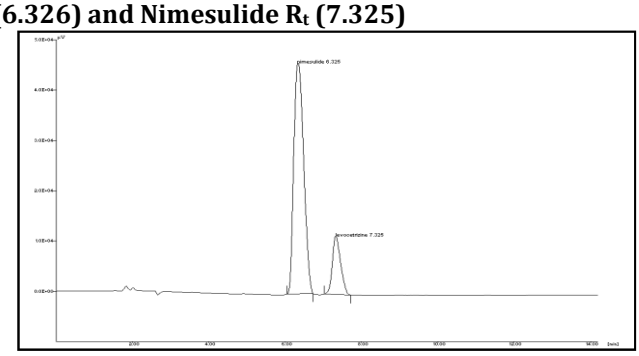
Preparation of Standard Stock Solutions

Standard stock solutions of concentration 1000 µg/mL of Levocetirizine hydrochloride and 1000 µg/mL of Nimesulide were prepared separately using methanol. The stock solution was stored at 2-8 °C protected from light. From the standard stock solution, the working standard solutions were prepared using methanol to get 5 µg/mL of Levocetirizine hydrochloride and 100 µg/mL of Nimesulide. The stock solutions were stored at 2-8 °C, protected from light.

Optimization of HPLC Method

All drugs were subjected to chromatographic analysis using mobile phases of differing pH, flow rate using the under mentioned chromatographic conditions. The changes in the retention time of all drugs were noted as a function of changing mobile phase, pH, flow rate, strength and selectivity. Initially methanol: water in the ratio of (80: 20) was tried but both the peaks merged. Later methanol: water in the ratio of (70: 30) was tried and it was found that both the peaks were well separated with acceptable resolution. Hence methanol: water in the ratio of (70: 30) at flow rate of 1 mL/min was finalized which gave acceptable retention time, plates and good resolution for Levocetirizine hydrochloride and Nimesulide (Figure **3**).

Figure 3. Chromatogrm of standard Levocetirizine R_t (6.326) and Nimesulide R_t (7.325)



Mobile phase: methanol: water (70: 30)
Concentration of drugs: 5 µg/mL and 100 µg/mL respectively
Injection volume: 20 µL

Validation of the method

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

Linearity and range: The mixed standard stock solution (100 µg/mL of Levocetirizine hydrochloride and 100 µg/mL of Nimesulide) was further diluted to get Levocetirizine hydrochloride and Nimesulide concentration in the range of 1-6 µg/mL and 4-24 µg/mL respectively. Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase in triplicate into the LC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision: The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations 2, 4, 6 µg/mL for Levocetirizine hydrochloride and 4, 12, 20 µg/mL for Nimesulide 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 quantitation: 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. To determine the LOD and LOQ, serial dilutions of mixed standard solution of Levocetirizine hydrochloride and Nimesulide was made from the standard stock solution. The samples were injected in LC system and measured signal from the samples was compared with those of blank samples.

Robustness of the method: To evaluate robustness of a HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate, percentage of methanol in the mobile phase and solvents from different lot were taken. Robustness of the method was done at three different concentration levels 2, 4, 6 µg/mL and 4, 12, 20 µg/mL for Levocetirizine hydrochloride and Nimesulide, respectively.

Specificity: The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. The peak purity of Levocetirizine hydrochloride and Nimesulide 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). Effect of excipients of formulation was studied for whether it interfered with the assay.

Accuracy: Accuracy of the method was carried out by applying the method to drug sample (Levocetirizine

hydrochloride and Nimesulide combination tablet) to which know amount of Levocetirizine hydrochloride and Nimesulide 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 Levocetirizine hydrochloride and Nimesulide in conventional tablet (Brand name: Openos, Label claim: 5 mg Levocetirizine hydrochloride 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 Levocetirizine hydrochloride and 100 mg Nimesulide was transferred into a 50 mL volumetric flask containing 30 mL methanol, sonicated for 30 min and diluted upto 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 Levocetirizine hydrochloride and Nimesulide, respectively). Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45-micron filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solution of 5 and 100 µg/mL for Levocetirizine hydrochloride and Nimesulide respectively. A 20 µL volume of sample solution was injected into HPLC, six times, under the conditions described above. The peak areas were measured at 239 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for Levocetirizine hydrochloride and Nimesulide in the current study involving methanol: water (70: 30) are given below.

Linearity: Levocetirizine hydrochloride and Nimesulide showed good correlation coefficient (r^2 = 0.9990 for Levocetirizine hydrochloride and 0.9992 for Nimesulide) in given concentration range (1-6 µg/mL for Levocetirizine hydrochloride and 4-24 µg/mL for Nimesulide). The mean values of the slope and intercept were 34675 ± 1.18 and 8943.5 ± 1.82 for Levocetirizine hydrochloride and 40863 ± 1.21 and 5209.2 ± 1.09 for Nimesulide respectively.

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[13-15].

Table 1. Precision studies

Concentration (µg/mL)	Measured concentration ± SD, RSD (%)	
	Repeatability(n= 6)	Intermediate precision (n= 6)
Levocetirizine		
1	0.98 ± 0.92, 0.61	0.99 ± 0.75, 0.11
3	2.97 ± 0.10, 0.82	3.01 ± 0.58, 0.70
5	5.00 ± 1.82, 1.01	4.98 ± 0.36, 0.82
Nimesulide		
4	3.93 ± 0.83, 0.82	4.012 ± 0.48, 0.21
12	12.07 ± 1.10, 0.09	12.203 ± 0.91, 0.19
20	19.98 ± 0.92, 0.87	20.004 ± 0.85, 0.20

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 0.25 µg/mL and 0.5 µg/mL for Levocetirizine hydrochloride and 2 µg/mL and 3 µg/mL for Nimesulide, respectively.

Robustness of the method : Each factor selected (except columns from different manufacturers) was changed at three levels (−0.1, 0 and 0.1). One factor at the time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of three chromatographic parameters (factors). Insignificant differences in peak areas and less variability in retention time were observed (Table **2**).

Table 2. Robustness testing[a] (n = 6)

Factor[a]	Level	Retention time	Retention factor	Asymmetry
Levocetirizine				
A: Flow rate (mL/min)				
0.9	-1	7.35	1.94	1.12
1.0	0	7.32	1.92	1.10
1.1	+1	7.29	1.91	1.09
Mean ± SD (n = 3)		7.32 ± 0.03	1.92 ± 0.02	1.10 ± 0.01
B: % of methanol in the mobile phase (v/v)				
69	-1	7.34	1.93	1.11
70	0	7.32	1.92	1.10
71	+1	7.30	1.92	1.10
Mean ± SD (n = 3)		7.32 ± 0.04	1.92 ± 0.01	1.10 ± 0.01
C: Solvents of different lots				
First lot		7.32	1.92	1.10
Second lot		7.35	1.94	1.13
Mean ± SD (n = 3)		7.33 ± 0.03	0.25 ± 0.01	1.11 ± 0.01
Nimesulide				
A: Flow rate (mL/min)				
0.9	-1	6.38	1.55	1.13
1.0	0	6.32	1.52	1.11
1.1	+1	6.29	1.51	1.07
Mean ± SD (n = 3)		6.33 ± 0.05	1.52 ± 0.03	1.10 ± 0.05
B: % of methanol in the mobile phase (v/v)				
69	-1	6.36	1.54	1.14
70	0	6.32	1.52	1.11
71	+1	6.30	1.52	1.09
Mean ± SD (n = 3)		6.32 ± 0.03	1.52 ± 0.01	1.11 ± 0.05
C: Solvents of different lots				
First lot		6.32	1.52	1.11
Second lot		6.35	1.54	1.10
Mean ± SD (n = 3)		6.33 ± 0.02	1.53 ± 0.02	1.10 ± 0.01

[a]Three factors were slightly changed at three levels (-0.1, 0, 0.1)

Specificity studies: The peak purity of Levocetirizine hydrochloride and Nimesulide was assessed by comparing their respective spectra at the peak start, apex and end positions i.e., r (S, M) = 0.9991 and r (M, E) = 0.9996. A good correlation (r = 0.9994) was also obtained between the standard and sample spectra of Levocetirizine hydrochloride and Nimesulide respectively. Also, excipients from formulation were not interfering with the assay.

Recovery: As shown from the data in Table **3** good recoveries of the Levocetirizine hydrochloride and Nimesulide in the range from 99 to 101.11 % were obtained at various added concentrations.

Table 3. Recovery studies (n = 6)

Label claim (mg/tablet)	Amount added (mg)	Total amount (mg)	Amount Recovered (mg) ± % RSD	% Recovery
Levocetirizine				
5	4 (80%)	9	9.11 ± 1.15	101.11
5	5 (100%)	10	9.90 ± 1.36	99.00
5	6 (120%)	11	11.21 ± 1.20	100.90
Nimesulide				
100	80 (80%)	180	179.8 ± 1.62	99.88
100	100 (100%)	200	199.9 ± 1.41	99.95
100	120 (120%)	220	220.2 ± 1.20	100.09

Analysis of a formulation: Experimental results of the amount of Levocetirizine hydrochloride and Nimesulide in

tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present. The drug content was found to be 99.40 % for Levocetirizine hydrochloride and 99.96 % for Nimesulide. Two different lots of Levocetirizine hydrochloride and Nimesulide combination tablets were analyzed using the proposed procedures as shown in Table **4**.

Table 4. Analysis of commercial formulation

Levocetirizine (5 mg)	**Levocetirizine found (mg per tablet)**	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	4.99 ± 0.82	99.80
2nd Lot	4.95 ± 0.76	99.00
Nimesulide (100 mg)	**Misoprostol found (mg per tablet)**	
	Mean ± SD (n= 6)	Recovery (%)
1st Lot	99.94 ± 1.01	99.94
2nd Lot	99.98 ± 0.82	99.98

CONCLUSION

HPLC method was developed and validated as per ICH guidelines. UV detection allowed an accurate quantitation of chromophoric compounds.

The drug was analysed by HPLC method using HiQ Sil C_{18} HS (250 mm × 4.6 mm, 5.0 µ), Germany with isocratic conditions and simple mobile phase containing methanol: water (70: 30) at flow rate of 1 mL/min using UV detection at 239 nm. The procedure has been evaluated for the linearity, accuracy, precision and robustness in order to ascertain the suitability of the analytical method. The method was also applied to marketed samples. It has been proved that the method is selective and linear between concentration range 1-6 µg/mL for Levocetirizine hydrochloride and 4-24 µg/mL for Nimesulide. LOD and LOQ were found to be 0.25 µg/mL and 0.5 µg/mL for Levocetirizine hydrochloride and 2 µg/mL and 3 µg/mL Nimesulide, respectively.

Statistical analysis proves that the method is suitable for the analysis of Levocetirizine hydrochloride and Nimesulide as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Levocetirizine hydrochloride and Nimesulide and also for its estimation in plasma and other biological fluids.

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