Development and Validation of RP-HPLC Method for Simultaneous Estimation of Nimesulide, Cetrizine and Pseudo Ephedrine HCl In Tablet Dosage Form

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ABSTRACT

A simultaneous estimation of RP-HPLC method was developed and validated for the estimation of Nimesulide, Cetrizine and Pseudo ephedrine HCl in tablet dosage form using C18 column (250mm 4.6mm, 5mm) with mobile phase consisting of Methanol: Water (65:35v/v) with a flow rate of 1.0ml/min (UVdetection 220nm). Linearity was observed over the concentration range 0.1–5 µg/ml (R^2=0.996) with regression equation y = 0.501x + 0.043 for Nimesulide and for Cetrizine 0.1–5 µg/mL (R^2=0.994) with regression equation y = 0.4519x + 0.1656 and PseudoephidrineHcl 0.1–5 µg/mL (R^2=0.997) with regression equation y = 0.3554x + 0.0088. The method was validated as per ICH guidelines.

Keywords:HPLC,Nimesulide, Cetrizine and Pseudoephedrine HCl.

INTRODUCTION

Nimesulide is N-(4-nitro-2-phenoxyphenyl) methane sulphonamide. It is used in the treatment of musculoskeletal disorder, dyemenorrhoea, thrombophlebitis, dental pain and inflammation. It is a centrally and peripherally acting non-opioid analgesic and antipyretic.

Cetirizine hydrochloride is (±) - [2- [4- [(4-chlorophenyl)phenylmethyl] -1- piperazinyl] ethoxy]acetic acid dihydrochloride and is official in the United States Pharmacopoeia, British Pharmacopoeia and Indian Pharmacopoeia. Cetirizine hydrochloride is a White crystalline powder and is water soluble. The molecular mass is 461.81g/mol.Cetirizine hydrochloride an antihistamine, is a major metabolite of hydroxyzine, and a racemic selective H1 receptor inverse agonist used in the treatment of allergies, fever, angioedema and urticaria. Like many other antihistamine medications, cetirizine is commonly prescribed in combination with pseudoephedrine hydrochloride, a decongestant.

Pseudoephedrine hydrochloride is 2-methylamino-1-phenyl-1-propanol hydrochloride and is official in the United States Pharmacopoeia,British Pharmacopoeia, and Indian Pharmacopoeia. Pseudoephedrine hydrochloride is a white crystalline powder and the molecular mass is 201.69 g/mol. Pseudoephedrine is a decongestant that shrinks blood vessels in the nasal passages. It is used to relieve nasal congestion caused by colds, allergies and fever. Pseudoephedrine occurs naturally as an alkaloid in certain plant species, the majority of pseudoephedrine produced for commercial use is derived from yeast fermentation of dextrose in the presence of benzaldehyde. The salts like pseudoephedrine hydrochloride and pseudoephedrine sulfate are found in many of the counter preparations either as single-ingredient preparations or more commonly in combination with antihistamines active substances including cetirizine in capsule or coated tablet forms for the treatment of seasonal allergic rhinitis.

MATERIALS AND METHODS

HPLC method development

Chemicals and reagents

Cetrizine, Nimesulide and Pseudo ephedrine HCl were obtained from Lincoln Pharmaceuticals Ltd. Methanol, water were of HPLC grade reagents obtained from Finar chemicals (Ahmedabad), India.

Instruments and chromatographic conditions[6,7]

The Lab India UV-3000+ model was used to determine the absorption maxima (λ_{max}) of Cetrizine, nimesulide and Pseudo ephedrine hcl. The analysis was performed by using UFLC Shimadzu LC-

20AD Pump, Photo Diode Array Detector (Japan). The reverse phase chromatography was performed with an analytical Kromasil C18 column. Methanol: Water (65:35) was used as the mobile phase. The flow rate was set at 1 ml/min and the injection volume was 20 µL. The UFLC detector was set at a wavelength of 220nm. The column and UFLC instrument was maintained at room temperature.

Preparation of standard solutions

Accurately weighed quantity of 10mg Nimesulide, Cetrizine and Pseudo ephedrine HCl were transferred into 10 ml volumetric flasks separately, dissolved and diluted up to the mark with HPLC grade water to give a stock solution having a strength of 1 mg/ml.

Standard graph procedure

Calibration curves were prepared by taking appropriate aliquots of Nimesulide, Cetrizine and Pseudo ephedrine Hclstandard stock solutions in different 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain final concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5µg/ml of Nimesulide, Cetrizine and Pseudo ephedrine HCl. Standard solutions were injected into system and the chromatograms were obtained. The effluent was monitored at 220 nm. Calibration curve was constructed by plotting average peak area against concentration and regression equations were computed.

Assay

10 tablets of Nimesulide, Cetrizine and Pseudo ephedrine HCl were taken and powdered. The total weight of the powder was noted and the average wt of each tablet was determined. 10 mg equivalent of Nimesulide was weighed and dissolved in 10 ml of methanol and the resultant solution was filtered and further diluted to make a solution which contains 5 µg/ml of Nimesulide, 0.25 µg/ml of Cetrizine and 6 µg/ml of Pseudo ephedrine.

HPLC method validation[7-14]

Linearity

Calibration curves were plotted by taking Peak area of Nimesulide, Cetrizine and Pseudo ephedrine HCl on Y-axis and Concentration of corresponding values on X-axis. The minimally acceptable correlation coefficient (r^2) for the calibration curve was 0.996, 0.994 and 0.997 respectively.

Precision and Accuracy

In order to assess the intra- and inter-day precision and accuracy for the assay Nimesulide, Cetrizine and Pseudo ephedrine HCl samples at (0.1µg/ml), (1.0µg/ml) and (5.0µg/ml) concentrations were prepared as described above. The intra-day precision of the assay was

assessed by calculating the coefficient of variation (CV) for the analysis of samples in three replicates and twice in a day. And inter-day precision was determined by the analysis of samples on three consecutive days. Accuracy was calculated by comparing the measured values and the True values and was expressed in percent. The precision was accepted when the standard deviation for each concentration doesn't exceed ± 20 and accuracy was accepted when the average values are > 95% of true concentration except for the LOQ where the limit was > 92%.

Robustness

In the robustness study the influence of small deliberate variations of the analytical parameters on retention time of the drugs were examined. The following two factors were selected for change: flow rate of mobile phase (1.0, 0.9, and 1.1) and wave length (220nm, 225nm) for Nimesulide, Cetrizine and Pseudo ephedrine HCl. It was accepted because there were no marked changes in the chromatogram,which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION

UV-Visible Spectrum of Nimesulide, Cetrizine and Pseudo ephedrine HCl

UV-Visible spectrum of Nimesulide, Cetrizine and Pseudo ephedrine HCl were obtained in the wave length range of 200-400 nm by using Lab India UV-visible spectrophotometer (UV3000+). Isobestic point of above drugs were found at a wave length of 220nm in water hence wavelength of 220nm was used as λ max for the determination of Nimesulide, Cetrizine and Pseudo ephedrine HCl by using RP-HPLC. Results were shown in Figure 1.
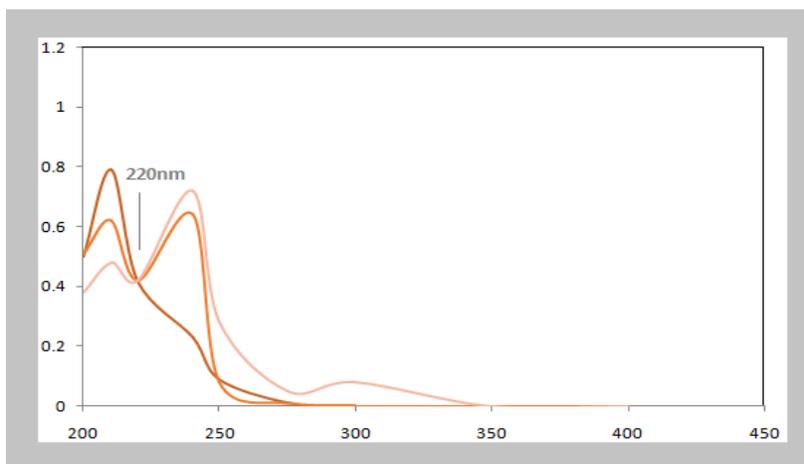

Figure-1: UV-Visible spectrum of Nimesulide, Cetrizine and Pseudo ephedrine HCl Within a range of 200-400nm

HPLC method validation

Nimesulide, Cetrizine and Pseudo ephedrine HCl was determined using a simple, sensitive and specific RP-HPLC method. An optimum separation was achieved using a composition of methanol

and water (65:35v/v) as a mobile phase. During the preliminary investigations different columns, different mobile phases were studied to select optimum conditions for the determination of the Nimesulide, Cetrizine and Pseudo ephedrine HCl. Separation of Nimesulide, Cetrizine and Pseudo ephedrine HCl were achieved with a flow rate of 1mL/min at 220 nm. Under these conditions the retention times of Nimesulide, Cetrizine and Pseudo ephedrine HCl were 5.5 min, 3.4 min and 8.1min respectively. Results were shown in Figure 2.
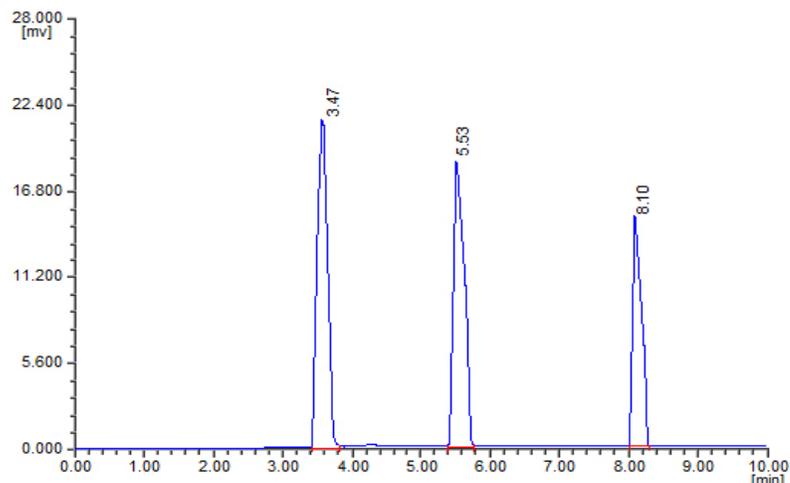

Figure-2: Sample Chromatogram of Cetrizine, Nimesulide and Pseudo ephedrine hcl

Linearity and range

The linearity of Nimesulide,Cetrizine and Pseudo ephedrine HCl were found to be in the range of 0.1-5µg/ml with correlation co-efficient of 0.996, 0.994 and 0.997 respectively. Calibration data were shown in Table- 1, 2 & 3 and calibration curves were shown in Figure 3,4&5.
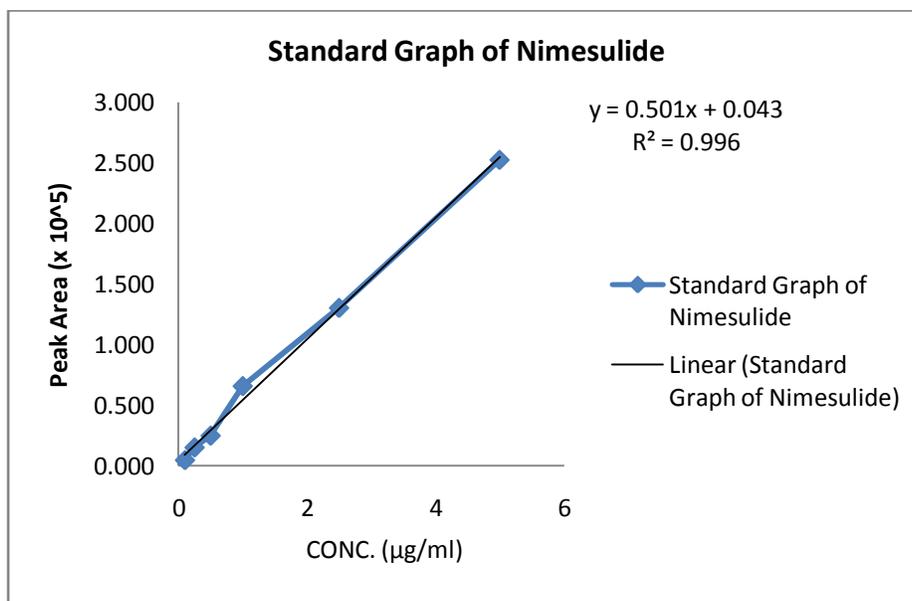

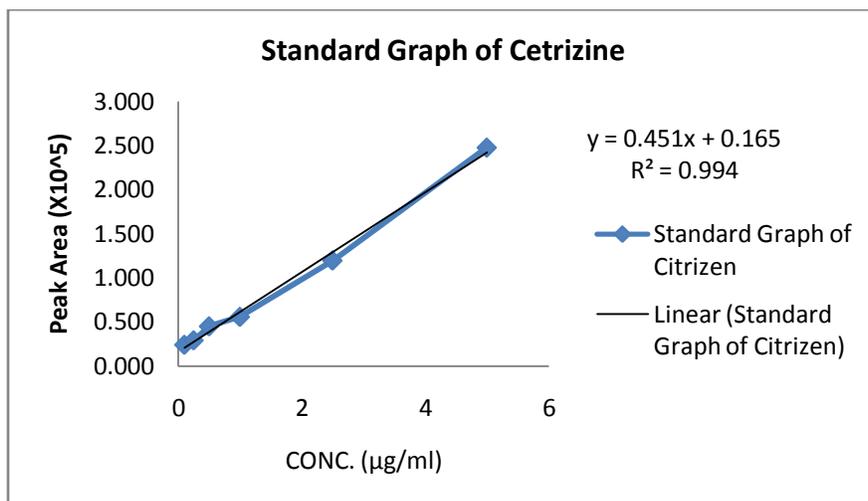**Figure-3: Standard graph of Nimesulide**
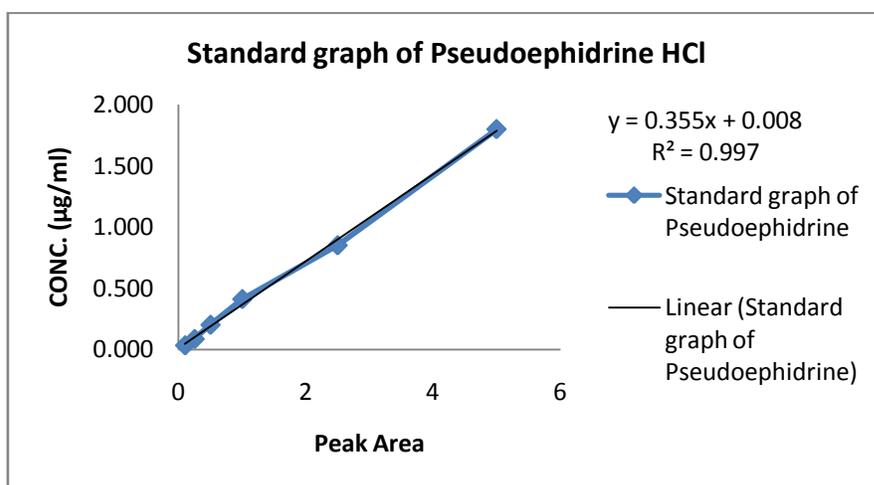
Figure-4: Standard graph of Cetrizine.

Figure-5: Standard graph of Pseudoephidrine HCl.

Table-1: Calibration data of Nimesulide.

S.No	Concentration(µg/ml)	Peak area
1	0.1	0.048
2	0.25	0.153
3	0.5	0.250
4	1	0.660
5	2.5	1.305
6	5	2.526

Table-2: Calibration data of Cetrizine.

S.No	Concentration(µg/ml)	Peak area
1	0.1	0.242
2	0.25	0.292
3	0.5	0.451
4	1	0.558
5	2.5	1.197
6	5	2.479

Table-3: Calibration data of Pseudo ephedrine HCl.

S.No	Concentration(µg/ml)	Peak area
1	0.1	0.032
2	0.25	0.084
3	0.5	0.200
4	1	0.410
5	2.5	0.850
6	5	1.800

Precision and Accuracy

Intra- day and Inter-day accuracy and precision were determined using three quality control samples of concentrations of 0.1, 1 and 5 µg/mL and Intraday and interday accuracy values of Nimesulide were 92.17, 97.87, 99.62 , 95.66, 97.36 and 99.19 respectively. Intraday and interday accuracy values of Cetrizine were 99.33, 97.3,99.1, 91.73, 98.03 and 98.66. respectively. Intraday and inter day accuracy values of Pseudo ephedrine were found to be 94.0, 98.5, 97.2, 95.3, 97.7, and 96.8 respectively Therefore, the intra- and inter- day accuracies (% deviation) were within < ±10% for the QC samples. The intra- and inter-day assay precision (CV) range of Nimesulide from 4.65 to 1.66 and 3.67 to 0.78 % respectively. Intra- and inter-day assay precision (CV) range of Cetrizine from 4.19 to 0.91 and 2.62 to 0.65. Intra- and inter-day assay precision (CV) range of Pseudo ephedrine hcl from 1.73 to 1.83 and 2.51 to 1.40. The accuracy and Precision data shows that the %RSD not more than ±2 which demonstrated that the method shows good accuracy and precision, the results were shown in Table-4, 5 &6.

Table-4: Intra and Inter day variations of Nimesulide.

Nim Conc	Intraday Accuracy and Precision					
	Trail 1	trail 2	Trail 3	Mean	SD	%RSD
0.1	93.4	87.4	95.7237	92.17	4.29	4.65
1.0	97.9	95.5	100.2237	97.87	2.36	2.41
5.0	101.5	98.38	98.98	99.62	1.65	1.66
	Interday Accuracy and Precision					
0.1	92	96	99	95.66	3.51	3.67
1.0	97.1	95.5	99.5	97.36	2.01	2.06
5.0	99.08	98.48	100.02	99.19	0.77	0.78

Table-5:Intra and Inter day variations of cetrizine.

Cet Conc	Intraday Accuracy and Precision					
	Trail 1	trail 2	Trail 3	Mean	SD	RSD
0.1	98	96	104	99.33	4.16	4.19
1.0	94.4	96.8	100.9	97.36	3.28	3.37
5.0	99.68	99.58	98.06	99.10	0.90	0.91

Interday Accuracy and Precision						
0.10	94	92	89.2	91.73	2.41	2.62
1.0	95.5	98.7	99.9	98.03	2.27	2.32
5.0	99.04	97.92	99.04	98.66	0.64	0.65

Table-6: Intra and Inter day variations of Pseudo ephedrine HCl.

Pseudo Conc. (µg/ml)	Intraday Accuracy and Precision				
	Trail 1	trail 2	Trail 3	Mean	SD
0.1	95	92	95	94.000	1.732
1.0	98.9	96.5	100.2	98.533	1.877
5.0	95.1	98.38	98.18	97.220	1.839
Interday Accuracy and Precision					
0.1	93	95	98	95.333	2.517
1.0	98.1	98.5	96.5	97.700	1.058
5.0	97.08	98.08	95.3	96.820	1.408

Robustness

In the robustness study the influence of small deliberate variations of the analytical parameters on retention time of the drugs were examined. The following two factors were selected for change: flow rate of mobile phase (1.0, 0.9, and 1.1) and Wavelength for Nimesulide, Cetrizine and Pseudo ephedrine HCl which demonstrated that the RP-HPLC method developed is robust the results were shown in Table- 7, 8 & 9.

Table-7: Robustness data of Nimesulide.

Parameter	Variations	Peak area	%RSD	Avg%RSD	Rt
Flow rate	1.0ml/min	116348	0.527245	0.44	3.47
	0.9ml/min	117200	0.378548		3.83
	1.1ml/min	115872	0.43755		3.31
wavelength	220nm	116214.7	0.513584	0.47	3.47
	225nm	115872.7	0.43755		3.44

Table-8: Robustness data of Cetrizine.

Parameter	Variations	Peak area	%RSD	Avg%RSD	Rt
Flow rate	1.0ml/min	622295	0.10	0.13	5.53
	0.9ml/min	622523	0.18		5.91
	1.1ml/min	621821	0.11		5.29
Wavelength	220nm	622195	0.13	0.11	5.53
	225nm	621788	0.09		5.51

Table-9: Robustness data of Pseudo ephedrine HCl.

Parameter	Variations	Peak area	%RSD	Avg%RSD	Rt
Flow rate	1.0ml/min	136348	0.449907	0.382139	8.10
	0.9ml/min	137200	0.323366		8.63
	1.1ml/min	135872	0.373144		7.81
Wavelength	220nm	136297	0.509535	0.423582	8.10
	225nm	136528	0.337609		8.17

Limit of detection and Limit of quantification

Limit of detection (LOD) for Nimesulide, Cetrizine and Pseudo ephedrine HCl were found to be 0.44μg/ml,0.52μg/ml and 0.33μg/ml respectively. Limit of quantification (LOQ) for Nimesulide, Cetrizine and Pseudo ephedrine HCl were found to be 1.51μg/ml, 1.59μg/ml and 1.0 μg/ml respectively. The above LOD, LOQ values were very low which indicates that the method is sensitive.

CONCLUSION

A simple and isocratic reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for quantitative determination of Nimesulide, Cetrizine and Pseudo ephedrine Hcl in formulation. It was analyzed by using Kromasil – C18 (250 mm x 4.6 mm, 5μ) at ambient temperature, with isocratic elution of Methanol: Water as mobile phase (65:35). The flow rate was set 1 ml/min and the analysis was performed at a wavelength of 220 nm using UV detector. The retention time (Rt) for Nimesulide, Cetrizine and Pseudo ephedrine HCl were 5.5, 3.4 and 8.1 min respectively. The calibration curves were linear over a concentration range from 0.1-5μg/ml. Limit of detection (LOD) for Nimesulide, Cetrizine and Pseudo ephedrine HCl was 0.44μg/ml,0.52μg/ml.0.33μg/ml and Limit of quantitation (LOQ) Nimesulide, Cetrizine and Pseudo ephedrine HCl was 1.51μg/ml, 1.59μg/ml and 1.0μg/ml respectively. The proposed HPLC method is precise, sensitive, accurate, specific and efficient and can be used in routine analysis in quality control laboratories

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