International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 6(3): May-June 2016

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences

Review Article……!!!

Received: 25-04-2016; Revised: 10-05-2016; Accepted: 11-05-2016

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF NIMESULIDE AND CHLORZOXAZONE IN PHARMACEUTICAL TABLET DOSAGE FORM

Dhwani Shah[1]*, Ankita Bhavsar[1], Ramkishan Ajmeer[2]

1. Sat Kaival College of Pharmacy, Sarsa (Anand), Gujarat, India
2. CDSCO, FDA Bhavan, New Delhi

Keywords:

Nimesulide, Chlorzoxazone, Method development, Validation

For Correspondence:

Dhwani Shah

Sat Kaival College of Pharmacy, Sarsa (Anand), Gujarat, India

E-mail:

shahdhwani57@gmail.com

ABSTRACT

A rapid high performance liquid chromatographic method has been developed and validated for the simultaneous estimation of Nimesulide and Chlorzoxazone in combined dosage form. Estimation of these combined formulation was performed using Waters Symmetry C_8 column (150 x 4.6 mm, 5µm) using the mobile phase of composition Methanol : Acetonitrile : 1% Ammonium acetate buffer (25:25:50 % v/v/v). The flow rate was kept 1.0 ml/min and the separation was observed at 290 nm. The selected chromatographic conditions were found to effectively separate Nimesulide (R_t : 6.251 min) and Chlorzoxazone (R_t :11.642 min) having a resolution of 11.19. The method was validated in terms of linearity, accuracy, precision, specificity, limit of detection and limit of quantitation. Linearity for Nimesulide and Chlorzoxazone were found in the range of 100-300 µg/ml (r^2=0.999) and 250-750 µg/ml, (r^2=0.998) respectively. The percentage recoveries for Nimesulide and Chlorzoxazone ranged from 102.19-102.68 % and 100.78-103.02 % respectively. The percentage RSD for precision and accuracy of method was found to be less than 2 %. The lower limit of detection and limit of quantitation was within the limits. The method was found to be robust and can be successfully used to determine the drug content of marketed formulations. The method was validated according to the guidelines of International Conference on Harmonisation.

INTRODUCTION

Nimesulide chemically known as [N-(4-nitro-2-phenoxyphenyl)] methanesulfonamide (figure: 1) is non steroidal anti-inflammatory drug with good analgesic and anti-rheumatic properties. It is approved for used in treatment of musculoskeletal disorder, thrombophlebitis and dental pain, inflammation[1]. NIM is official in British pharmacopeia (BP 2010)[2]. Some HPLC and spectrophotometric method have been reported in literature for it's estimation.

Chlorzoxazone (CHZ) is a chemically 5-chloro-3Hbenzooxazol-2-one. (figure: 2). It acts by inhibiting multi synaptic reflexes involved in producing and maintain skeletal muscle spasm of varied aetiology. It acts on the spinal cord by depressing reflexes. CHZ is a synthetic compound, inhibits antigen-induced broncho spasms and hence, is used to treat asthma and allergic rhinitis. CHZ inhibits degranulation of mast cells, subsequently preventing the release of histamine and slow-reacting substance of anaphylaxis (SRS-A), mediators of type I allergic reactions. CHZ may also reduce the release of inflammatory leukotrienes. CHZ is used to relieve pain and stiffness caused by muscle strains and sprains[3]. RP-HPLC method has been reported in literature for estimation of Chlorzoxazone alone and in combination of other drug. CHZ is official in United States Pharmacopeia (USP 2010).[4] Currently there is no validation method has been reported for combination of NIM and CHZ till date. The combination of these two drugs is not official in any pharmacopoeia.

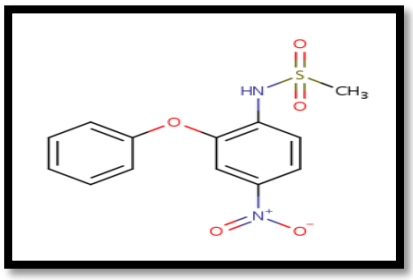

FIG NO-1: STRUCTURE OF NIM

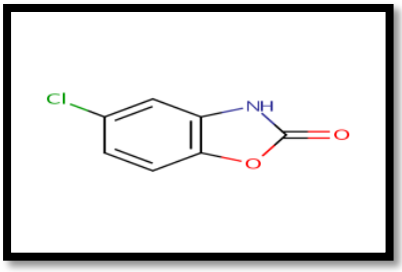

FIG NO-2: STRUCTURE OF CHZ

MATERIALS AND METHODS

Instrumentation

A Waters 600 HPLC system with Csw software with UV/Vis detector and a universal injector 7725i (Phenomex) with 20.0µL loop was used.

Selection of analytical wave length

The standard solution of NIM (10 µg/ml) and CHZ (10 µg/ml) in methanol was scanned in the UV region of 200-400 nm using methanol as a blank. The overlain spectra was recorded and 290 nm analytical wavelength was selected for estimation of NIM and CHZ. Chromatogram shown in figure 3.

Chromatographic condition

Chromatographic separations were achieved using a C_8 column (150 x 4.6 mm, 5µm). The mobile phase consisting of methanol: (Acetonitrile)ACN:1% ammonium acetate (25:25:50% v/v/v) was passed through 0.45µ membrane filter and degassed by ultra- sonication. The flow rate was maintained at 1.0 ml/min and the measurements were made at 290 nm. The column and HPLC system was kept at ambient temperature.

Selection of mobile phase

For the selection of mobile phase, various solvents individually and in combinations were tried and a mobile phase containing methanol: ACN: 1% ammonium acetate (25:25:50% v/v/v) was selected, as both the drugs Nimesulide and Chlorzoxazone were resolved with reasonable retention times with sharp peaks.

Preparation of mobile phase

A mixture of 25 ml of methanol and 25 ml of ACN in this solution add 50 ml volume of 1% ammonium acetate (1gm in 100 ml water) and sonicated for 10 minutes.

Preparation of standard solution of NIM and CHZ

Accurately weighed NIM 50 mg and 125 mg CHZ in 50 ml volumetric flask and make up with methanol. From this 2 ml was taken and diluted up to 10 ml with methanol. This solution was containing 200 µg/ml of NIM and 500 µg/ml of CHZ. Solution was injected in HPLC Chromatogram shown in figure: 4.

Preparation of sample solution

Twenty tablets were weighed and powdered equivalently to 50 mg of NIM and 125 mg of CHZ were transferred in 50 ml volumetric flask. 25 ml of methanol was added, sonicated for 20 minute and diluted with methanol up to mark. The solution was filtered . First few ml of filtrate was discarded. 2 ml of filtrate was diluted to 10 ml with methanol to make 200 µg/ml NIM and 500 µg/ml of CHZ. Chromatogram is shown in figure: 5.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITION

Parameters	Condition
Mobile phase	Methanol : ACN : 1%ammonium acetate (25:25:50%v/v/v)
Stationary Phase	ECO-C_8 (15mm*4.6mm*5µ) (particle size) suniest-eco-C_8
Flow rate	1 ml/min
Run time	20 min
Volume of injection	20 µL
Detection wave length	290 nm

METHOD VALIDATION

System suitability parameters

The column efficiency, resolution, peak asymmetry, retention time, area, were calculated from the chromatogram of standard solution containg 200 µg/ml NIM and 500 µg/ml CHZ. The value obtained demonstrated the suitability of the system for analysis of this drug combination. Summary is shown in table 2.

Linearity, limit of detection (LOD) and limit of quantification (LOQ)

Standard calibration samples were prepared by making serial dilutions (1,1.5,2,2.5,3 ml) from the stock solution of NIM and CHZ. Calibration curve of concentration versus peak area ratio was plotted at concentration range of 100-300 µg/ml for NIM and 250-750 µg/ml for CHZ. Calibration curves shown in figure 6 and 7. LOD and LOQ As per ICH guideline, limit of detection and quantitation of the developed method were calculated from the standard deviation of the response(σ) and slope of the calibration curve(S) of each drug using the formula, Limit of detection=3.3*σ/S; Limit of quantitation=10*σ/S. Result are shown in Table.8

Precision

Repeatability, intermediate precision were evaluated by injecting the sample solutions in to the HPLC system on the same day (repeatability) and on different instrument (intermediate precision) at 200µg/ml of NIM and 500µg/ml of CHZ. The results were reported in term of % RSD. Acceptance criteria =<2% Result are shown in Table 3 and 4.

Accuracy(% recovery)

The Standard was spiked with Formulation at these concentration levels of 50, 100, 150% and the mixture were analyzed by the proposed method. The experiment was conducted in triplicate. Result are shown in Table 5.

Robustness

It is the capacity of the analytical method to remain unaffected by small but deliberate variation in method parameters. The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate, wave length etc.

Effect of variation of flow rate- The flow rate is varied between 0.8-1.2 ml/min and the chromatogram was recorded. The result are summarized in Table 6.

Specificity

The specificity of the method was determined by analyzing standard drug and sample**.**

Chromatogram of blank, placebo, standard and sample are shown in 8,9,10. respectively.

Quantitative estimation of marketed pharmaceutical formulation.

Twenty tablets were weighed and powdered equivalently to 50 mg of NIM and 125 mg of CHZ were transferred in to a 100 ml volumetric flask and dissolved in methanol, it was shaken for 30 min and the volume was made up to the mark with methanol, the content was ultra sonicated for 20 min it contains 1000 µg/ml NIM and 2500 µg/ml of CHZ. The solution was filtered through 0.45µm glass paper. Again from this solution 5 ml taken and transferred in to 50 ml volumetric flask and make up with methanol up to the mark. It contain NIM 200 µg/ml and CHZ 500 µg/ml. The assay result obtained for NIM and CHZ was comparable with the corresponding labelled amount result are given in Table 7.

RESULTS

Selection of analytical wave length

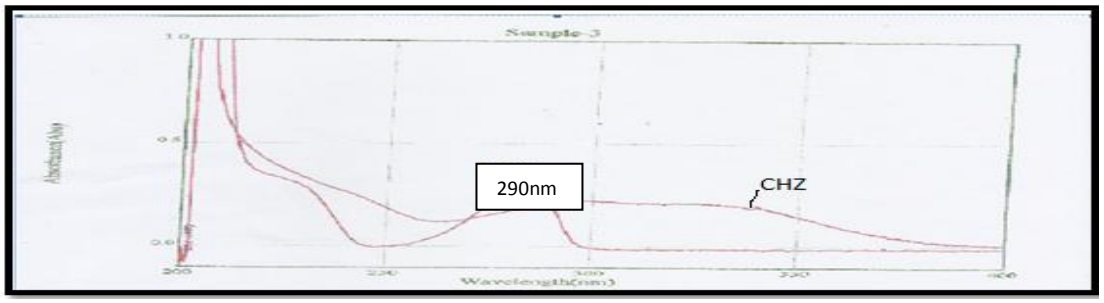

FIG NO-3: OVERLAIN SPECTRA OF NIM AND CHZ

Method development

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for NIM and CHZ was obtained with a mobile phase methanol: ACN: 1% ammonium acetate (25:25:50%v/v/v) at a flow rate of 1 ml/min to get better resolution and repeatability. Quantification was carried out at 290 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained Figure 4.

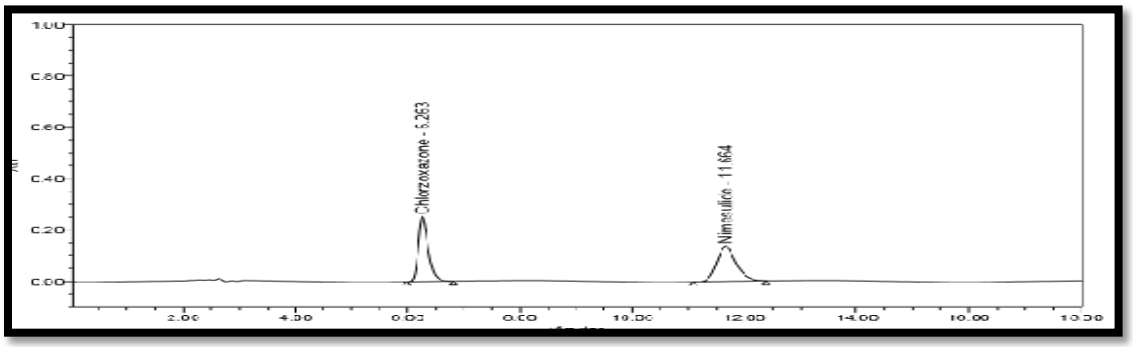

FIG NO-4: CHROMATOGRAM OF STANDARD SOLUTION (NIM AND CHZ)

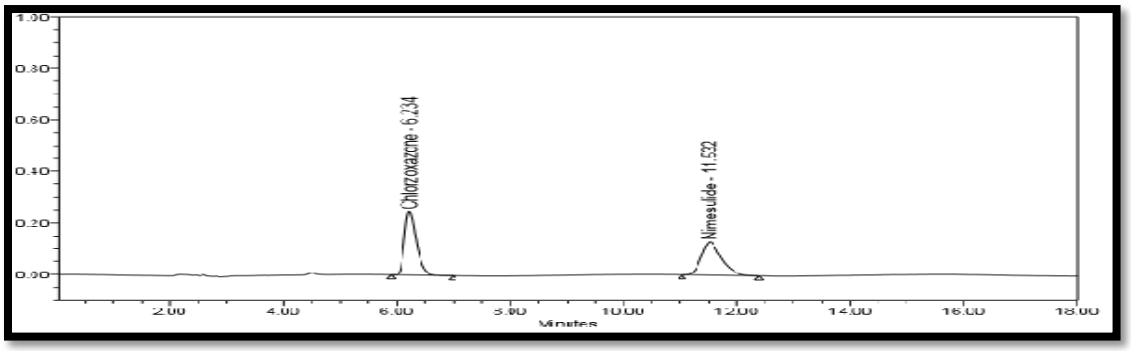

FIG NO-5: CHROMATOGRAM OF MARKETED SOLUTION

TABLE: 2 SYSTEM SUITABILITY DATA OF NIM AND CHZ

Parameters	NIM±RSD	CHZ±RSD	Acceptance criteria
Theoretical plates	5247±1.41	5464±0.17	>2000
Tailing factor	1.29±1.37	1.20±0.14	<2
Resolution	11.19±0.40		>2
Retention time	6.251±1.10	11.642±1.69	<20
Area	3303181.66±1.58	3267149.66±1.54	-
Flow rate	1ml/min	1ml/min	-

Linearity and Range

Linear correlation obtained between peak area Vs concentration range of 100-300 µg/ml for NIM and 250-750 µg/ml for CHZ. Calibration curve of these two drugs at 290 nm are shown in figure.6 and 7.

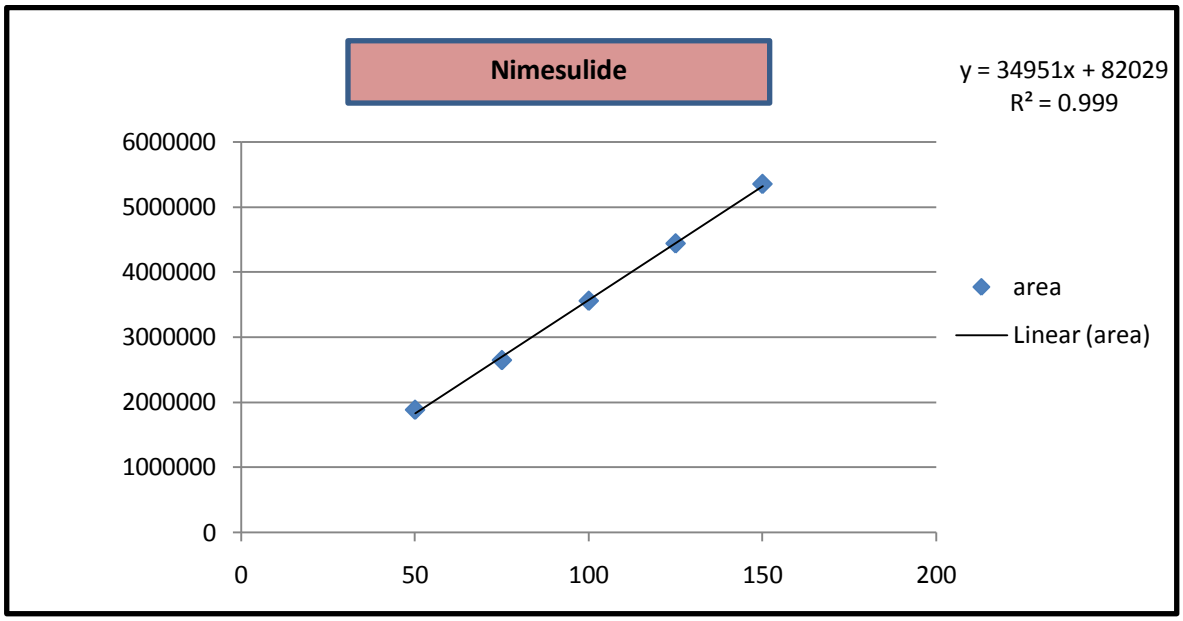

FIG NO-6: CALIBRATION CURVE OF NIM

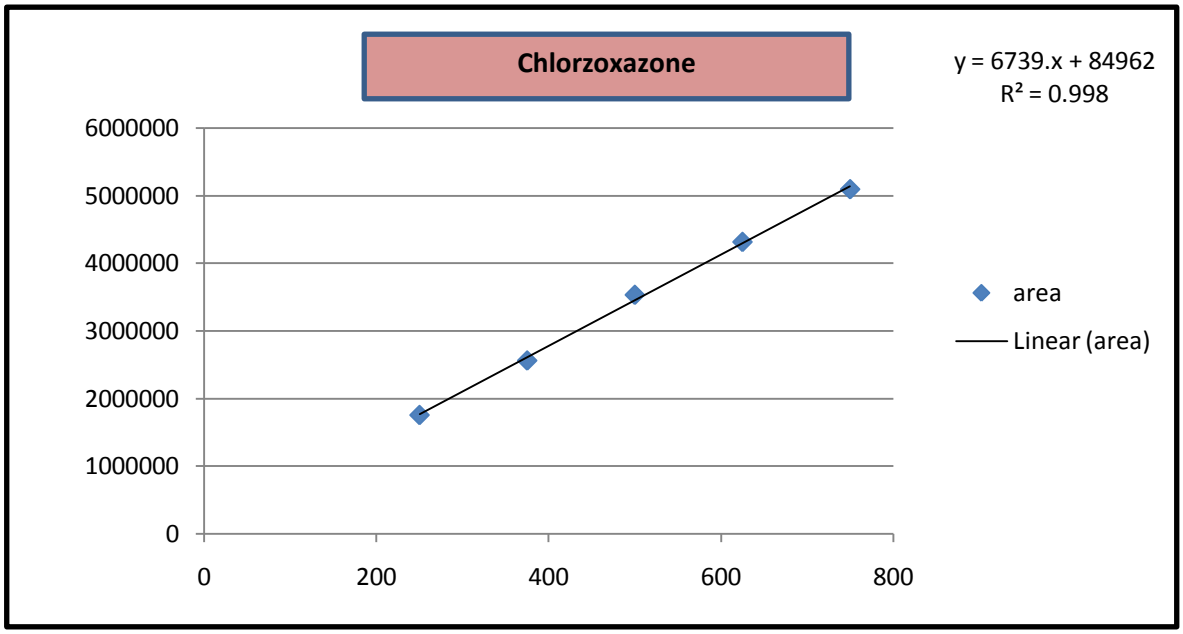

FIG NO-7: CALIBRATION CURVE OF CHZ

Precision

Repeatability, intermediate precision were evaluated by injecting the sample solutions in to the HPLC system on the same day (repeatability) and on different instrument (intermediate precision) at 200 µg/ml of NIM and 500 µg/ml of CHZ. The results were reported in term of % RSD. The result are given in table 3 and 4.

TABLE: 3 DETERMINATION OF REPEATABILITY

Sr no	Peak area(n=6)		Retention time(min)		Tailing factor	
	NIM	CHZ	NIM	CHZ	NIM	CHZ
1	3237637	3435553	11.532	6.234	1.35	1.43
2	3439336	3551497	11.509	6.245	1.32	1.38
3	3372778	3533257	11.515	6.247	1.27	1.37
4	3353587	3553629	11.528	6.246	1.29	1.44
5	3345390	3590264	11.496	6.230	1.32	1.50
6	3390147	3611965	11.466	6.223	1.40	1.50
Mean	**3346479**	**3546028**				
SD	56675.79	61253.61				
%RSD	1.69%	1.72%				

Limit:%RSD for area NMT 2.0%

n=number of replicate injection

TABLE: 4 INTERMEDIATE PRECISION

Sr no	Peak area(n=6)		Retention time(min)		Tailing factor	
	NIM	CHZ	NIM	CHZ	NIM	CHZ
1	3376740	3312582	12.055	6.836	1.33	1.53
2	3309679	3364135	12.036	6.833	1.34	1.54
3	3270652	3445617	12.017	6.836	1.34	1.53
4	3316936	3342306	11.981	6.817	1.30	1.50
5	3200591	3399743	11.920	6.804	1.29	1.49
6	3316574	3378074	11.887	6.805	1.30	1.49
Mean	**3298529**	**3373743**				
SD	58778.74	46220.98				
%RSD	1.78%	1.37%				

Limit:%RSD for area NMT 2.0%

n=number of replicate sample

Accuracy(% Recovery)

The recovery experiment was performed by the standard addition method. The % recoveries obtained were 102.36% and 102.07% for NIM and CHZ respectively. The low value of standard deviation indicates that the proposed method is accurate. Result are shown in table: 5.

TABLE: 5 ACCURACY DATA OF NIM AND CHZ.

Drug	% level	Amount of sample taken(mg)	Mean Area (n=3)	Amount of standard recover(mg)	% recovery	%RSD
	50	10	1769599	10.48	102.23	1.37
	100	20	3399871	20.81	102.19	0.43
NIM	150	30	5032115	30.80	102.68	0.83
	50	25	1721904	26.06	102.02	0.99
	100	50	3344298	50.62	100.78	0.32
CHZ	150	75	5082078	76.93	102.43	0.15

n= number of replicate injection.

The percentage recovery for each level should be between 98-102% as per ICH guideline. The percentage recovery of NIM and CHZ are found to be 102.36% and 102.07% respectively which is within the limit.

Robustness

The content of the drug was not adversely affected by small deliberate changes in flow rate. As evident from the low value of relative standard deviation indicating that the method was robust. Result is shown in table 6.

TABLE: 6 DATA OF ROBUSTNESS

variation		NIM (n=3)		CHZ (n=3)	
		RT (min)	Peak area	RT (min)	Peak area
Flow rate	+0.2	9.891 min	3150064	5.584 min	3230460
(1 ml/min)	-0.2	12.427min	3636328.3	6.781 min	3792518
% RSD		0.896		0.405	

Limit:%RSD for area NMT 2.0%

n=number of replicate sample

Specificity

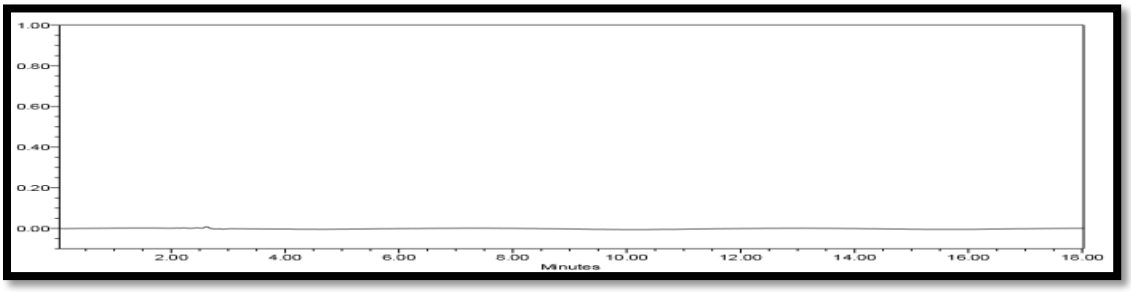

FIG NO- 8: BLANK CHROMATOGRAM OF NIM AND CHZ

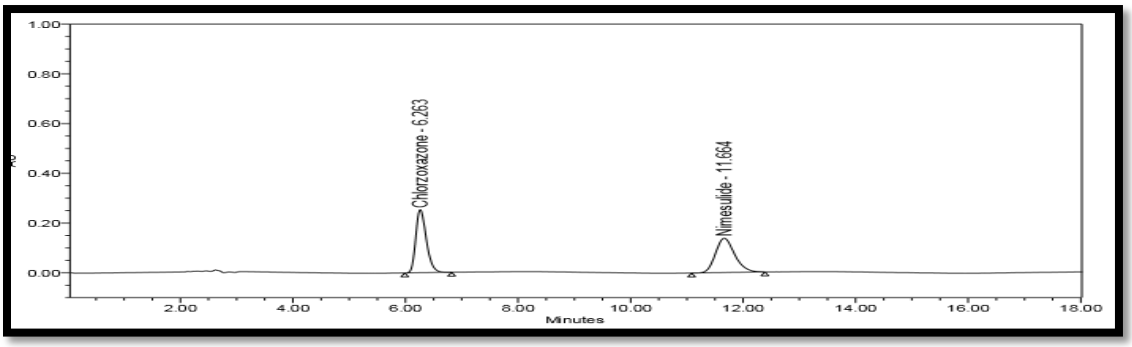

FIG NO-9: CHROMATOGRAM OF NIM AND CHZ MIX STD

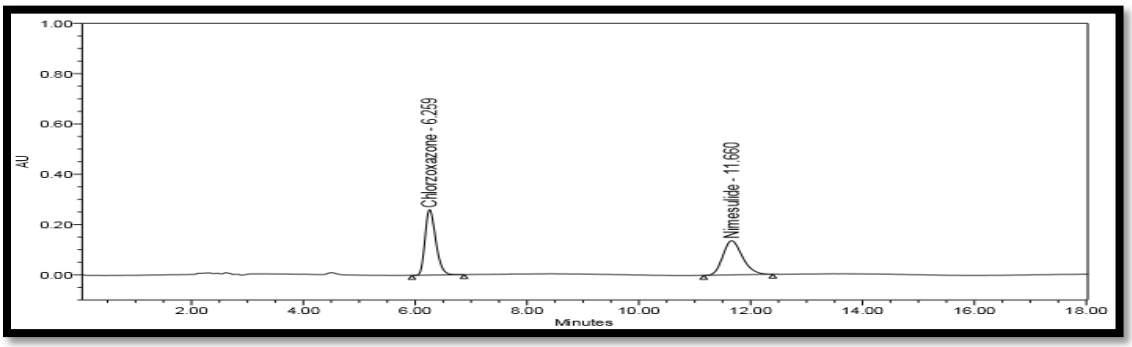

FIG NO-10: CHROMATOGRAM OF NIM AND CHZ FORMULATION

Quantitative Estimation of Marketed Pharmaceutical Formulation

The proposed validated method was successfully applied to determine NIM and CHZ in combined pharmaceutical formulation. The assay result obtained for NIM and CHZ was comparable with the corresponding labelled amount.

TABLE: 7 ANALYSIS OF MARKETED FORMULATION

Sr no	Actual concentration.		Concentration found		%assay	
	NIM (mg/ml)	CHZ (mg/ml)	NIM (mg/ml)	CHZ (mg/ml)	NIM (%)	CHZ (%)
1	50	125	50.009	132.49	100.1	105.99
2	50	125	51.16	132.33	102.32	105.86
3	50	125	49.83	131.27	99.67	105.02
		Mean			100.69	105.62
		SD			1.422	0.526
		%RSD(Limit: NMT 2%)			1.41	0.49

TABLE: 8 SUMMARY OF VALIDATION PARAMETER FOR PROPOSED METHOD

Parameters	Result	
	NIM	CHZ
Linear range(n=6) µg/ml	100-300 µg/ml	250-750 µg/ml
Slope	34951	6739
Correlation Co-efficient(r^2)	0.999	0.998
Regration equation	Y=34951x+82029	Y=6739x+84962
% recovery	102.19-102.68%	100.78-103.02%
Repeatability(%RSD)	1.69%	1.72%
Intermediate precision(%RSD)	1.78%	1.37%
Limit of Detection(LOD)	4.71 µg/ml	29.870 µg/ml
Limit of Quantification(LOQ)	14.273 µg/ml	90.517 µg/ml
Specificity	Specific	Specific
Robustness	Robust	Robust

DISCUSSION

The present method for the determination of NIM and CHZ is sensitive, rapid, specific, accurate and robust. The excellent separation is demonstrated in the chromatograms and no interfering peaks were observed. The calibration curve was linear. The accuracy of the method was in compliance with the proposed limits and the precision of the method was satisfactory. The system suitability of the method shows that the performance of the chromatographic system is not significantly influenced by variations of the operational parameters inside an accepted domain. This method shows the system suitability parameters are within the limits only. The HPLC method described here successfully applied for estimation of NIM and CHZ in marketed formulation.

REFERENCES

1. Bennett A and Villa G.,"Nimesulide: an NSAID that preferentially inhibits COX-2,and has various unique pharmacological activities," Expert Opin Pharmacother**.** 200; vol.1:277–286.
2. British pharnacopoiea;6th Edn; the stationary office,london medicines and health care product Regulatory Agency,2009,vil II.
3. Neil M J., The Merck Index An encyclopedia of chemicals, drugs and biological; 14th Edition; Merck Research Laboratories,USA,(2004),pp 48.
4. United state oharmacopoiea-34 and national formulary-29; asia;united state pharmacopoeia convention, Rockville MD, USA,2011 vol II.