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STABILITY-INDICATING HPLC METHOD FOR DETERMINATION OF NIMESULIDE IN BULK POWDER AND IN TABLET DOSAGES FORM

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ABSTRACT

A novel stability-indicating high pressure liquid chromatographic (HPLC) method was developed and validated for quantitative determination of Nimesulide (NIM) in bulk drug and in tablets. Isocratic, HPLC method, using a C_{18} reversed phase column with mobile phase 50% acetonitrile, 50% aqueous contained 0.05% orthophosphoric acid as mobile phase adjust PH at 2.8 and temperature at 40°C. The proposed method was investigated to separate the drug from its stress degradation products. The flow rate was 1.5 ml/min, column oven temperature was ambient and detection of column effluent was performed at 220 nm. NIM was subjected to the stress conditions of hydrolysis (acid and base), oxidation and heat degradation. Stress degraded samples were analyzed by the developed procedure. The analyte was well separated from its degradants. The described method showed excellent linearity over a range of 1-200 µg/ml. The determination coefficient for NIM was 0.9998. Limit of detection for NIM was 0.98 µg/ml and limit of quantification was 2.97 µg/ml respectively. Degradation of NIM was observed in acid, base and in 35% H_2O_2 conditions only. The drug was found to be stable in the other stress conditions attempted. The percentage recovery of NIM ranged from (99.72% to 100.1176%) in tablets. The developed method was validated with respect to the linearity, accuracy (recovery), precision, specificity and robustness. The forced degradation studies proved stability indicating power of the method.

KEYWORDS

Nimesulide, HPLC, Bulk Powder and Tablets.

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INTRODUCTION

Nimesulide, N-(4-nitro-2-phenoxyphenyl) methane sulfonamide, is a derivative of p-nitrophenyl methane sulfonamide. It belongs to selective COX-2inhibitors, with a potent anti-inflammatory and analgesic activity, when administered orally, rectally, or topically. It is widely used for the treatment of various inflammatory processes due to its analgesic and antipyretic properties. It is

approved for use in treatment of musculoskeletal disorder, thrombophlebitis, dental pain, and inflammation[1-4]. The literature survey reveals that Nimesulide UV methods[5,6], RP-HPLC[7-16] and HPTLC and stability indicating HPTLC[17-22] methods have been reported. The chemical structure of NIM is shown in Figure No.1.

EXPERIMENTAL

Apparatus

Analysis was performed on a chromatographic system of WATERS 2695 separation module connected to WATERS 2487 UV/VIS detector. The system equipped by Empower PC program. The chromatographic separation was achieved on BDS HYPERSIL C18 column (250 x 4.6 mm, 5µ) Detection was performed at 220 nm.

Materials and Reagents

All reagents used were of analytical grade or HPLC grade. Ortho-phosphoric acid was supplied by (Merck, Darmstadt, Germany), Acetonitrile HPLC grade was supplied by (Fischer scientific, U.K.) and Distilled water. Water used in all the experiments was obtained from Milli-RO and Milli-Q systems (Millipore, Bedford, MA). NIM working standard powder was kindly supplied by (Hikma Pharma. Company, 6th of October City).

Pharmaceutical dosage forms

Sulide® tablets labeled to contain 100 mg per tablet. Batch No. 091 (Hikma Pharma. Company, 6th of October City).

Chromatographic condition

The chromatographic separation was achieved on BDS HYPERSIL C18 column (250x4.6 mm, 5µ) Detection was performed at 220 nm using isocratic elution, 50% acetonitrile 50% aqueous contained 0.05% orthophosphoric acid as mobile phase adjust PH at 2.8 and temperature at 40°C.

General procedures

Preparation of stock and standard working solutions

A stock solution of 1 mg / ml of NIM was prepared in methanol. The working standard solutions were prepared by diluting aliquots of each stock solution to obtain concentration 100 µg/ml and. Working solution drug were stable for one week.

Construction of calibration curves

Aliquots of standard solutions, ranging from 1.0 to 200 µg/ml were prepared in a series of 10 ml volumetric flasks, 10 µl were injected to the instrument. Detection was performed at wavelength 220 nm. The calibration graph was constructed by plotting the peak areas obtained versus the corresponding injected concentrations.

Procedure for dosage forms

Assay of Sulide® tablets

Twenty tablets of the formulation were weighted and crushed. An accurately weighted amount of the powdered tablet equivalent to 100 mg of NIM was dissolved in 25 ml methanol, filtered into 50 ml measuring flask and completed to volume with methanol. The procedure was then completed as mentioned above under the general procedure.

Procedure for forced degradation

Acidic Degradation

10 ml of methanolic stock solutions of the drug, 10ml of 2 M HCl, was added. Mixture was refluxed for 4 hours at 80º C on water bath. Degraded samples were then cooled to room temperature and neutralized with 2 M NaOH to pH 7. Suitable aliquot of resultant degraded sample was withdrawn and subjected to analysis after suitable dilutions with methanol.

Alkaline Hydrolysis

10 ml of methanolic stock solutions of the drug and 10 ml of 2 M NaOH was added. Mixture was refluxed for 4 hours at 80 ºC on water bath. Degraded samples were then cooled to room temperature and neutralized with 2 M HCl to pH 7. Suitable aliquot of resultant degraded sample was withdrawn, neutralized and subjected to analysis after suitable dilutions with methanol.

Oxidative Hydrolysis

To 10 ml of methanolic stock solutions of the drugs, 10 ml of 35% V/V H_2O_2 was added. Mixture was refluxed for 4 hours at 80 ºC on water bath. Degraded sample was then cooled to room temperature. Suitable aliquot of resultant

degradation sample was taken and subjected to analysis after suitable dilutions with methanol.

Heat Hydrolysis

Methanolic solution of the drug was refluxed for 4 hours at 80ºC on water bath. Degraded samples were then cooled to room temperature. Suitable aliquot of resultant degraded sample was withdrawn, and subjected to analysis.

RESULTS AND DISCUSSION

Conditions affecting the chromatographic performance of NIM were carefully studied in order to recognize the most suitable chromatographic system. The choice was based on the best resolution in a reasonable time.

METHOD DEVELOPMENT

Type of column

A Hypersil C18 column (250x4.6 mm) 5µm particle size column maintained at 40 ºC and simple isocratic method were used for the determination of NIM and its main degradation products. The column shows excellent symmetrical peak shape and high sensitivity for wide range of analytes using either isocratic or gradient mobile phase at ambient temperature and 40°C. The chromatographic separation was achieved on BDS HYPERSIL C18 column (250x4.6 mm, 5µ) Detection was performed at 220 nm.

Mobile phase composition

Different mobile phase pH-values and acetonitrile contents were applied to reach the best separation conditions. It was found that 50% acetonitrile content and 50% aqueous contained 0.05 % orthophosphric acid, pH-value 2.8 gave rapid elution of NIM and its degradation products. On the other hand, decreasing acetonitrile content to 495 ml and 505 ml of aqueous phase resulted in no significant change of separation of NIM and its degradation products. Column oven temperature was also studied (at room temperature and 40°C) and it was found that good determination could be obtained at 40°C.

Choice of detection wavelength

Analyte peaks were monitored using the three different wavelengths; 254 nm, 230 nm and 220 nm. 220 nm was found to be optimum for detection at which the highest detector response was obtained.

Choice of flow rate

The effect of flow rate was studied to optimize the chromatographic efficiency of the proposed method and improve the resolution of the eluted peaks. The flow rate was changed over the range of 1.0-1.8 ml/min and a flow rate of 1.5 ml/min was optimal for good separation in a reasonable time. So, the optimum chromatographic performances were achieved when using isocratic mobile phase composed of 50% acetonitrile: 50% aqueous contained 0.05 % orthophosphric acid, PH adjusted to pH 2.8, injection volume 10 µl, column temperature 40°C, detection wavelength 220 nm and flow rate 1.5 ml/min. Under the optimized conditions NIM was separated within 2.725 minutes as shown in Figure No.2.

Degradation behavior of Nimesulide

Acidic conditions

Acidic degradation of NIM was observed on heating methanolic solution of the drug and 2 M HCL for 4 hours at 80°C. The acidic degradation product showed a small peak at tR 1.269 min as shown Figure No.3.

Alkaline conditions

Alkaline degradation of NIM observed on heating methanolic solution of the drug and 2 M NaOH for 4 hours at 80°C. The alkaline degradation product showed a small peak at tR 1.269 min as shown in Figure No.4.

Oxidative conditions

Oxidative degradation of NIM was observed on heating methanolic solution of the drug and 35% H_2O_2 for 4 hours at 80°C. The oxidative degradation products showed a small peak at tR 2.049 min. There was a strong peak at 1.049 min due to the presence of hydrogen peroxide, well resolved from main peak of intact drug as shown in Figure No.5.

Heat stability studies

Nimesulide was found to be stable under heat degradation. No degradation product peaks were

observed on exposure of drug solution to heat degradation conditions as shown in Figure No.6.

Method validation

Linearity and range

Six concentrations of NIM solution ranging from 1.0 to 200 µg/ml were analyzed. The graph of the peak area against concentration proved linear in the range of 1.0 to 200 µg/ml as shown in Figure No.7, while determination coefficient (R2) was 0.9998. Limit of detection was found to be 0.98 µg/ml, while limit of quantification was found to be 2.97 µg/ml acc to ICH[23]. Results of analysis are shown in Table No.1.

Selectivity

Selectivity of the method was assessed by sample degradation studies and peak purity evaluation. Acidic degradation conditions and alkaline degradation condition strongly affected NIM, also oxidative degradation condition.

Specificity of the method

Specificity is the ability of the analytical method to discriminate between target analyte and other components which may be expected to be present. The specificity of the assay was determined by the complete chromatographic separation of NIM peak from its degradation product peaks generated under various stress conditions. The comparison between the chromatogram of NIM and that of Sulide® tablets showed in Figure No.2 and 8 which indicate that the excipients in the tablets did not interfere with the determination of the drug.

Repeatability and Precision of the method

High intra- and inter-day precisions are shown in Table No.2 and 3. Intra- day precision was assessed by injection of the standard solution of the drug at three concentrations levels six times during a day. The same was done for inter- day precision test except that the analysis of the samples was done every day for six days.

Robustness of the method

The robustness of the present method was evaluated in terms of flow rate, content of acetonitrile in mobile phase, and wave length. The results are given in Table No.4. The slight variations in the examined factors had no significant effect on the shape of the peak. The results indicate that the method is more sensitive to changes in the flow rate and the content of acetonitrile in mobile phase than to changes in the other factors. The standard parameters of the method are: mixture of 50% acetonitrile and 50% aqueous contained 0.05 % orthophosphoric acid and PH 2.8 as a mobile phase, injection volume 10 µl, column temperature 40°C, detection wavelength 220 nm and flow rate 1.5 ml/min. Recovery studies to check the degree of accuracy of the method were performed in triplicate by standard addition method at 120%, 150% and 200%. Known amounts of standard NIM was added to pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in Table No.5.

Analysis of Nimesulide tablet

A single peak was observed at retention time of NIM when solution of the tablet formulation was chromatographed. There was no interference between NIM and excipients present in the tablets. NIM contents were found to be 99.77% and the RSD values was 0.444%. The low RSD values indicated the suitability of this method for routine analysis of NIM in pharmaceutical dosage forms. Results are shown in Table No.6.

Table No.1: Linearity and calibration parameters data for the stability indicating chromatographic for Nimesulide

S.No	Retention time	2.725
1	Detection limit (Injected concentration (µg/ml)	0.980319
2	Quantitation limit (Injected concentration (µg/ml)	2.970665
3	Range of linearity (µg/ml)	1 - 200
4	Slope	1.492
5	Intercept	0.875
6	Determination coefficient	0.999
7	Number of theoretical plates	2697
8	Capacity factor (K)	2.0267

Table No.2: Evaluation of the intra-day precision and accuracy of Nimesulide

S.No	Method	Drug taken (µg/ml)	Drug found (µg/ml)	Mean	SD	RSD[a] %	Recovery %
1	HPLC	20	20.0095	19.98839	0.94959	0.950142	99.94193
			19.987				
			20.00817				
			19.98713				
			20.00479				
		40	40.0012	40.01591	0.054722	0.054701	100.0398
			39.62478				
			40.00248				
			39.98475				
			40.00248				
		60	59.84125	59.85116	0.390305	0.391275	99.75193
			59.78426				
			60.0014				
			59.84125				
			60.00214				

Table No.3: Evaluation of the inter-day precision and accuracy of Nimesulide using the proposed method

S.No	Drug taken (µg/ml)	20	40	60
		Drug found (µg/ml)		
1	Day 1	20.0095	40.0012	59.84125
		19.987	39.62478	59.78426
		20.00817	40.00248	60.0014
2	Day 2	19.98713	39.98475	59.84125
		20.00479	40.00248	60.00214
		19.987	39.62478	59.78426
3	Day 3	20.00817	40.00248	60.0014
		19.98713	39.98475	59.84125
		20.00479	40.00248	60.00214
Mean		20.07448	40.07799	59.91702
SD		0.51393	0.281565	0.5804
RSD[a] %		0.512024	0.281017	0.581232
Recovery %		100.3724	100.195	99.8617

Table No.4: Robustness of the proposed method

S.No	Slight change in	Wavelength (210, 215 and 220 nm)	flow rate (1.45, and 1.5 ml/min)	Injected volume (10,10.5 and 9.5µl)	Acetonitrile content (45 and 50 %)	
1	Affected parameter	t_R	Peak area	t_R	Peak area	t_R
2	RSD	0.2	0.55	0.13	0.75	0.35

Table No.5: Recovery of Nimesulide in bulk drug using the proposed method

S.No	Drug taken (µg ml^{-1})	Drug formulations	Labeled mg content	Drug found (µg ml^{-1})	Recovery (%)
1	20	Nimesulide tablet.	100	20.00817	100.0409
2	40			39.62559	99.06397
3	80			59.63992	99.39987
4	100			99.9069	99.9069
5	160			160.1882	100.1176
Mean				---	99.70584
SD				---	0.454933
RSD				---	0.456276

Table No.6: Recovery of Nimesulide drug in pharmaceutical formulation using the proposed method

S.No	Drug taken (µg ml^{-1})	Drug formulations	Labeled mg content	Drug found (µg ml^{-1})	Recovery (%)
1	20	Nimesulide tablet	100	20.00817	100.0409
2	40			39.62559	99.06397
3	80			59.63992	99.39987
4	100			99.9069	99.9069
5	160			160.1882	100.1176
Mean				---	99.77067
SD				---	0.44352
RSD				---	0.44454

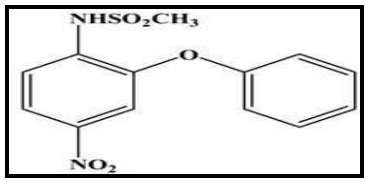

Figure No.1: Chemical structure of Nimesulide

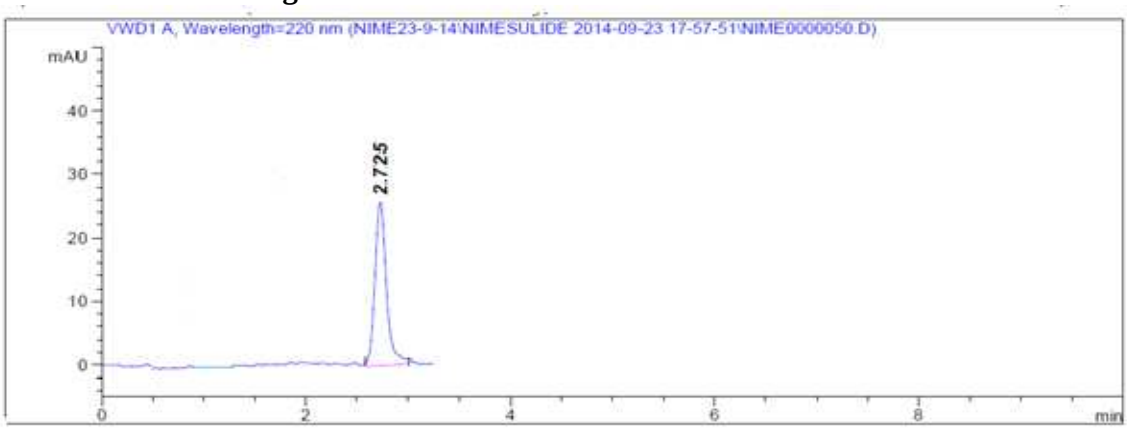

Figure No.2: Chromatogram of 100 µg/ml of Nimesulide (Standard solution)

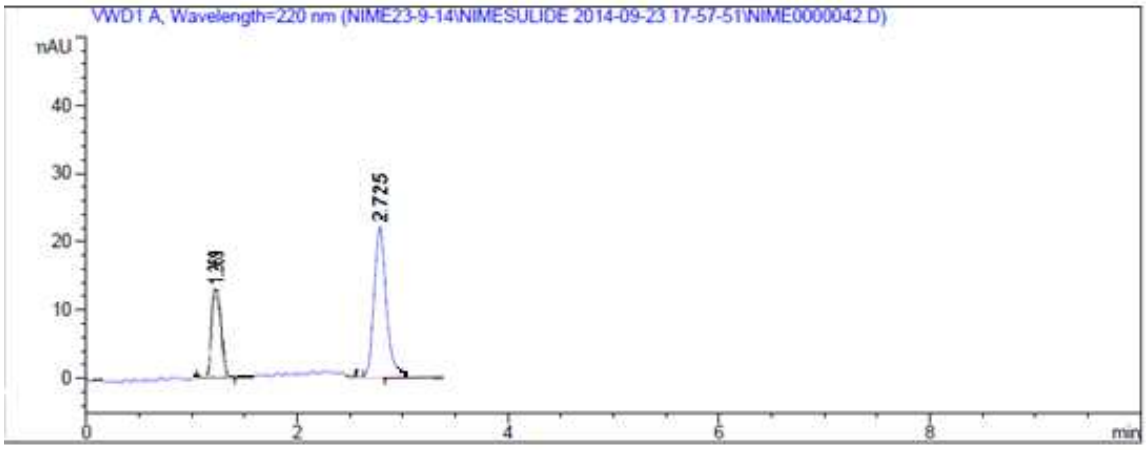

Figure No.3: Chromatogram of 100 µg/ml of Sulide tablet (Sample solution) after acid hydrolysis

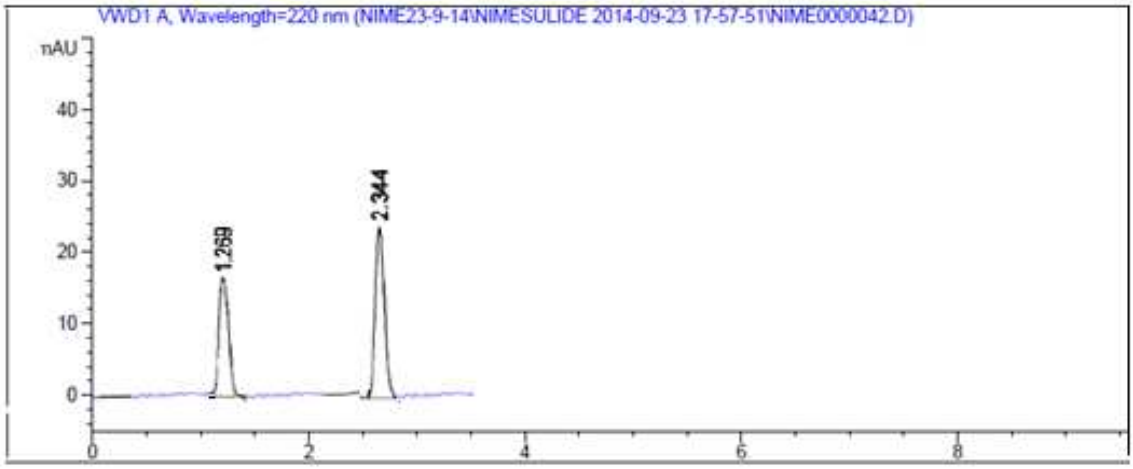

Figure No.4: Chromatogram of 100 µg/ml of Sulide tablet (Sample solution) after base hydrolysis

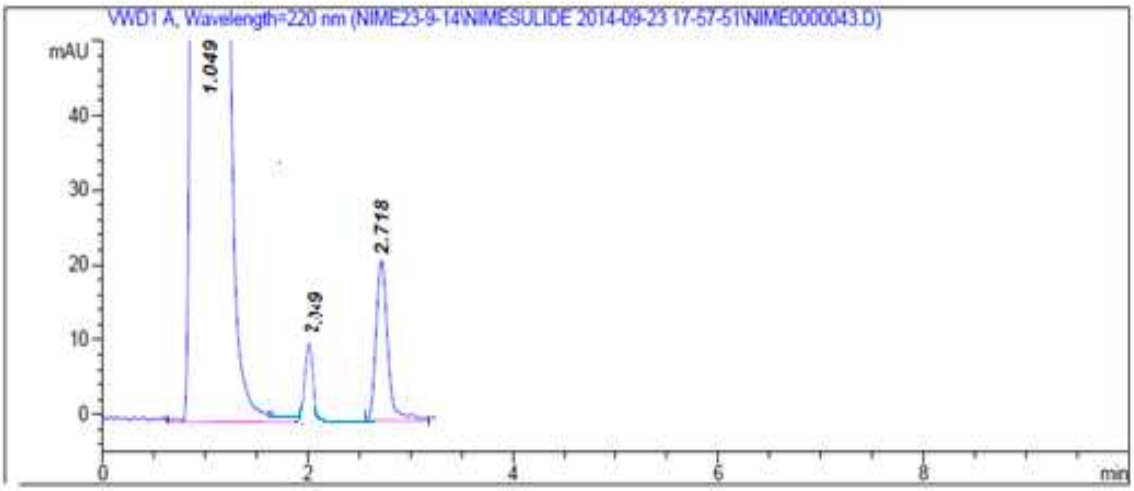

Figure No.5: Chromatogram of 100 µg/ml Nimesulide sample after oxidative degradation

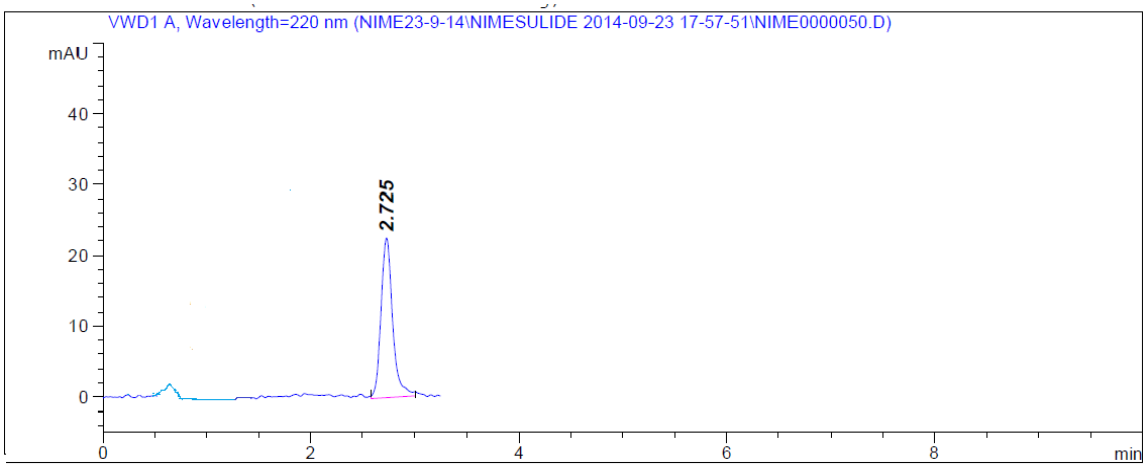

Figure No.6: Chromatogram of 100 µg/ml Nimesulide sample after heat degradation

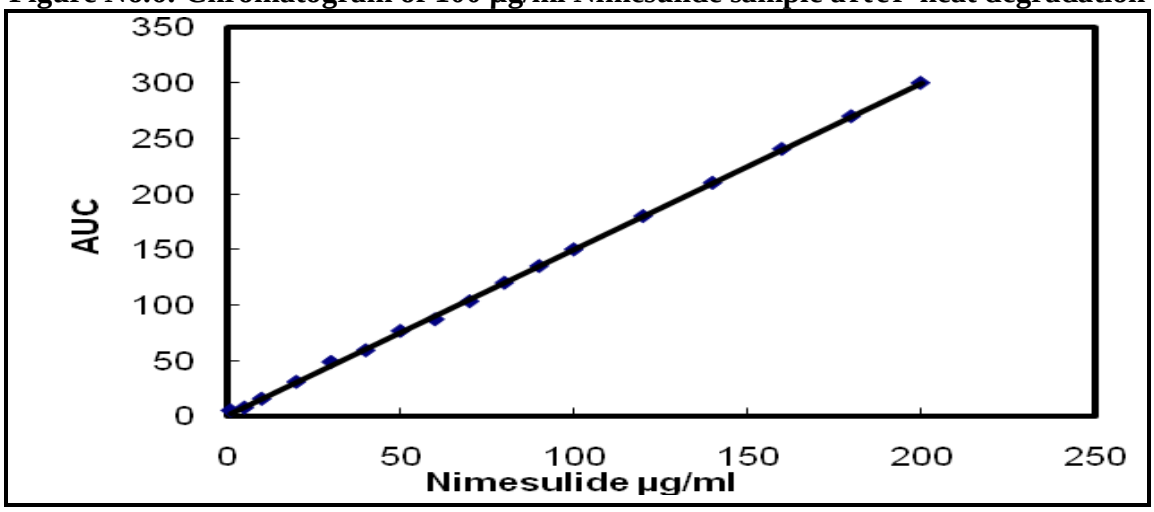

Figure No.7: Calibration curve of Nimesulide

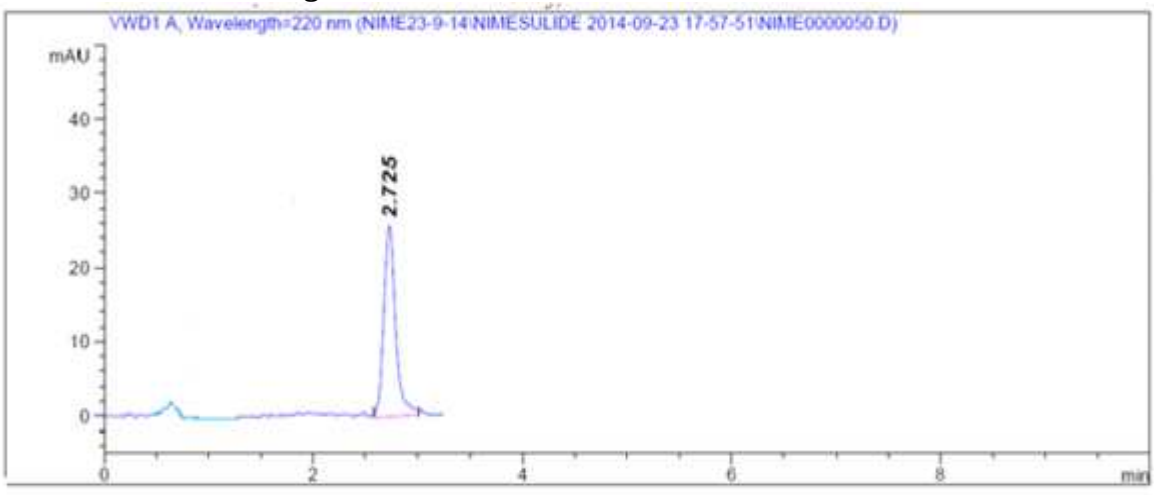

Figure No.8: Chromatogram of 100 µg/ml of Sulide tablet (Sample solution)

CONCLUSION

The proposed reversed phase HPLC method for estimation of NIM in bulk drug and tablet dosage form is simple, precise and accurate. The method is linear in the range reported. Sample preparation is very easy, as it prepared in methanol. It does not suffer from any interference due to common excipients present in pharmaceutical preparations and can be used for routine quality control analysis.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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