Development and Validation of RP-HPLC Method for Simultaneous Estimation of Nimesulide and Diclofenac Sodium in tablet Dosage Form

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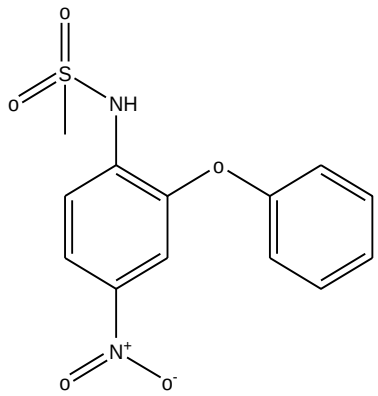
Abstract:
A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Nimesulide and Diclofenac sodium in tablet dosage form. Phenomenex C18 column (250 mm X 4.6 mm i.d., 5 µm particle size) in Isocratic mode with mobile phases containing Acetonitrile and Water (60:40 %v/v, pH 5.5) was used. The flow rate was 1.0mL/min and effluents were monitored at 288 nm.) The retention time of Nimesulide and Diclofenac sodium was 2.93min and 5.37min respectively. The concentration curves of Nimesulide and Diclofenac sodium were linear in the concentration range of 2-10 µg/mL and 1-5 µg/mL of Nimesulide and Diclofenac sodium respectively. The developed method was validated for specificity, precision, linearity, accuracy, LOD, LOQ, robustness. Due to its simplicity, rapidness and high precision, the developed method can be used for routine quality control analysis of titled drugs in pharmaceutical dosage form.

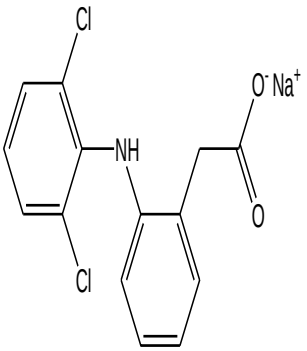
Keywords: *RP-HPLC, Nimesulide, Diclofenac sodium, ICH guideline, Validation*

1. INTRODUCTION

Nimesulide N-(4-nitro-2-phenoxyphenyl) methane sulfonamide is a derivative of p-nitrophenylmethanesulfonamide. It belongs to selective COX-2 inhibitors, with a potent anti-inflammatory and analgesic activity, when administered orally, rectally, or topically. Due to its analgesic and antipyretic properties, it is widely used for the treatment of various inflammatory processes. It is approved for use in treatment of musculoskeletal disorder, thrombophlebitis, dental pain, and inflammation. Diclofenac Sodium is chemically Sodium salt of 2-[{2, 6-dichlorophenyl} amino] benzene acetic acid. It is having anti-inflammatory and analgesic properties.



Structure of Nimesulide



Structure of Diclofenac sodium

Literature review revealed some HPLC and spectrophotometric method have been reported in literature for its estimation. Many methods have been reported in literature for determination of Nimesulide and

Diclofenac sodium with other drugs. The present work describes a validated new reverse phase HPLC method for simultaneous determination of these drugs in combined tablet dosage form.

2. MATERIALS AND METHODS

Instrumentation

A Youngling's HPLC (Acme-9000), UV Detector, Manual injector of 20-µl loop, Column - Phenomenex C_{18} column (250 mm X 4.6 mm i.d., 5 µm particle size) Software – Auto chrome 3000, Digital pH meter (Ecoscan), Corning volumetric flasks (10mL, 50mL and 100 mL), A CP224S analytical balance (Sartorius), Ultrasonic bath (Frontline FS 4 ultrasonic cleaner, Mumbai), Vacuum pump, Pipettes – 1mL, 5mL, 10mL, beakers, measuring cylinder.

Chemicals and Reagents

Authentic samples of Nimesulide and Diclofenac sodium were supplied from Camper Healthcare, Mehsana, India as gift samples. Acetonitrile and Water (HPLC grade, S.D. Fine Chemicals Ltd., Mumbai), 0.22 µm filter (Millipore). The tablet formulations were procured from a local pharmacy.

Chromatographic conditions

Phenomenex C_{18} column (250 mm X 4.6 mm i.d., 5 µm particle size), Mobile phase - Acetonitrile and Water (60:40 %v/v). Flow rate: 1.0 mL/min, Filter: Nylon 0.22 µm membrane filter, Mobile Phase was degassed before use, Detection wavelength: 288 nm, the injection volume: 20 µl, and Temperature: 30 ± 2^{o} C.

Preparation of mobile phase

Acetonitrile and Water in the ratio of 60:40 v/v, respectively (pH 5.5) was mixed. The mobile phase was filtered through nylon 0.22 µm membrane filter and was degassed before use.

Preparation of Solutions

Standard stock solution (100 µg/mL)

Accurately weighed 5 mg of Nimesulide and 5 mg of Diclofenac sodium were transferred to two separate 50 mL volumetric flasks. 25 mL Methanol was added to the flask. The drug was dissolved with sanitations and the final volume was adjusted with Methanol up to the mark to prepare 100 µg/mL stock solutions of both drugs.

Preparation of working standard solution

Accurately measured working standard solutions of Nimesulide(0.2, 0.4, 0.6, 0.8, and 10 mL) and Diclofenac sodium(0.1, 0.2, 0.3, 0.4 and 0.5 mL) were transferred to a series of 10 mL volumetric flasks and the volume in each flask was adjusted to 10 mL with Methanol.

Preparation of Sample solution

Twenty tablets were weighed and powdered. Quantity of the powder equivalent to about 10 mg of Nimesulide and 5 mg of Diclofenac sodium was transferred into 100 mL volumetric flask contain 50 mL of mobile phase and sonicate for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the residue was washed thoroughly with Methanol. The filtrate and washings were combined in a 100 mL volumetric flask and diluted to the mark with Methanol to get a final concentration of 100 µg/mL of Nimesulide and 50 µg/mL of Diclofenac sodium. From this, 0.6 mL of solution was transferred to the 10 mL volumetric flask and diluted to mark with methanol to get final Concentration 6 µg/mL of Nimesulide and 3 µg/mL of Diclofenac sodium.

Determination of Maximum wavelength

The standard solution of Nimesulide and Diclofenac sodium were scanned in the range of 200-400 nm against mobile phase as a blank. Nimesulide and Diclofenac sodium showed maximum absorbance at 288 nm. So the wavelength selected for the determination of Nimesulide and Diclofenac sodium was 288 nm.

Chromatographic method:

Pre-treatment of column:

Phenomenex C_{18} was properly washed with of Acetonitrile (HPLC grade previously filtered with Nylon 0.22 µm membrane filter and degassed properly) for 30 min at 1.0 mL/min of flow rate.

Chromatographic separation:

With the help of micro liter syringe and loop, 20 mL of each working standard solutions or sample solution was injected into the column through loop at 1.0 mL/min flow rate. The Peaks of Nimesulide and Diclofenac sodium were detected at 288 nm and retention times were found to be 2.93 and 5.37 minutes respectively.

Calibration curve of standard Nimesulide and Diclofenac sodium

A calibration curves were plotted over a concentration range of 2- 10µg/mL for Nimesulide and 1-5µg/mL for Diclofenac sodium. Accurately measured standard stock solutions of Nimesulide (0.2, 0.4, 0.6, 0.8, and 1.0 mL) and Diclofenac sodium (0.1, 0.2, 0.3, 0.4 and 0.5 mL) were transferred to a series of 10 mL corning volumetric flasks and the volume in each flask was adjusted to 10 mL with mobile phase. The resulting solution was injected into the column and the peak area obtained at retention time 2.93 and 5.37 minutes and flow rate 1.0 mL/min were measured at 288 nm for Nimesulide and Diclofenac sodium respectively. Calibration curves were constructed for Nimesulide and Diclofenac sodium by plotting peak area versus concentration at 288 nm. Each reading was average of three determinations.

Quantization of Nimesulide and Diclofenac sodium in formulation

Test solution from tablets which contain Nimesulide (6.0 µg/mL) and Diclofenac sodium (3.0 µg/mL) were prepared from the sample solution and solutions were injected into HPLC system and area was measured at 285 nm. Concentrations of both drugs were calculated from respective calibration curve.

METHOD VALIDATION

Solution stability

Sample solutions were kept at 25°C and 2-8°C for 24 hour and three days, respectively. Assay of initial time period was compared with these two time points. The falls in the assay values were evaluated. The difference between assays should not be more than 2 % for formulation, and 0.5% for API.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation proportional to the concentration of analyte in samples within a given range. The range of analytical method is the interval between upper and lower level of analyte including levels that have been demonstrated to be determining with precision and accuracy using the method. The linear response of Nimesulide and Diclofenac sodium were determined by analyzing five independent levels of the calibration curve in the range of 2- 10µg/mL for Nimesulide and 1-5µg/mL for Diclofenac sodium. Result should be expressed in terms of Correlation co-efficient.

Precision

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, Relative standard deviation or coefficient of variance of a series of measurements.

Repeatability (Precision on replication)

It is a precision under a same condition (Same analyst, same apparatus, short interval of time and identical reagents) using same sample. Method precision of experiment was performed by preparing the standard solution of Nimesulide (6µg/mL) and Diclofenac sodium (3µg/mL) for six times and analyzed as per the proposed method.

Intermediate precision (Reproducibility)

It expresses within laboratory variations as on different days analysis or equipment within the laboratory. Intra-day precision of the proposed method was evaluated by assaying freshly prepared solutions of Nimesulide and Diclofenac sodium in triplicate at three different concentrations. Inter-day precision was evaluated by using freshly prepared solutions of Nimesulide and Diclofenac sodium in triplicates at three different days.

Accuracy (% Recovery)

It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the capsules (Nimesulide 6µg/mL and Diclofenac sodium 3µg/mL) with three different concentrations of standards (Nimesulide 4.8, 6, 7.2µg/mL and Diclofenac sodium 2.4, 3, 3.6µg/mL).

Limit of Detection

It is the lowest amount of analyte in sample that can be detected but not necessarily quantitated under the stated experimental conditions. It is expressed as signal to noise ratio of 2:1 or 3:1.

Limit of detection can be calculated using following equation as per ICH guidelines.

$LOD = 3.3 \times N/S$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification

It is the lowest concentration of analyte in the sample that can be determined with the acceptable precision and accuracy condition. It is expressed as signal
to noise ratio of 10:1.

Limit of quantification can be calculated using following equation as per ICH guidelines.

$LOQ = 10 \times N/S$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. In the case of assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

System suitability

System suitability parameter is establishes to ensure that the validity of the analytical method is maintained whenever used. Typical variations are the stability of analytical solution, different equipment, and different analyst. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition, different lots or supplier of columns, the temperature (20 ± 30^{o} C) and flow rate.

4.RESULT AND DISCUSSION

The proposed method can determine Nimesulide and Diclofenac sodium in combined dosage form and the validity of this method was confirmed in accordance with the ICH guidelines. In proposed method retention times were recorded at 2.93 min and 5.37 min at 1.0 mL/min. Flow rate for Nimesulide and Diclofenac sodium respectively. The calibration graphs for Nimesulide and Diclofenac sodium were constructed by plotting the area versus their corresponding concentrations, good linearity was found over the range 2-10 µg/mL for Nimesulide and 1 - 5 µg/mL for Diclofenac sodium. Results obtained by applying the proposed method shown that the concentrations of Nimesulide and Diclofenac sodium can be simultaneously determined in prepared mixtures. The proposed method has been applied to the assay of Nimesulide and Diclofenac sodium in pharmaceutical dosage form. The validity of the method was further assessed by applying the standard addition technique. The results obtained indicate that the additives present do not interfere with analysis of the studied mixtures (Table 1). The optical and

regression characteristics and validation parameters are reported in Table 4. Results of application of proposed method to the pharmaceutical dosage form are shown in Table 5.

Table1 Percentage recovery of Nimesulide and Diclofenac sodium

Drug	Spiked level (%)	Amount taken (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL) ± S.D	% Recovery ± S.D (n=3)
Nimesulide	80	6	4.8	4.805 ± 0.005	100.116 ± 0.113
	100	6	6	6.003 ± 0.009	100.058 ± 0.156
	120	6	7.2	7.213 ± 0.005	100.192 ± 0.075
Diclofenac sodium	80	3	2.4	2.401 ± 0.001	100.039 ± 0.055
	100	3	3	3.002 ± 0.001	100.073 ± 0.044
	120	3	3.6	3.602 ± 0.002	100.073 ± 0.037

Table 2 Repeatability data for analysis of Nimesulide

Concentration	Area (mV*sec)	Amount found	% Amount found
6 µg/mL	652.16	6.009	100.163
	651.28	6.001	100.025
	650.93	5.998	99.970
	650.89	5.997	99.963
	652.72	6.015	100.250
	651.49	6.003	100.058
Mean	651.578	6.004	100.071
SD (n = 6)	0.7256	0.0068	0.1137
%RSD	0.1113	0.1136	0.1136

Table 3 Repeatability data for analysis of Diclofenac sodium

Concentration	Area (mV*sec)	Amount found	% Amount found
3 µg/mL	1288.83	3.004	100.159
	1290.43	3.008	100.284
	1289.67	3.006	100.225
	1288.64	3.004	100.144
	1289.71	3.006	100.228
	1288.49	3.003	100.132
Mean	1289.3	3.005	100.195
SD (n = 6)	0.7608	0.0018	0.0598
%RSD	0.0590	0.0597	0.0597

Table 4 Intermediate precision data for Nimesulide at 288 nm (Intraday & Inter day Precision)

Concentration (µg/mL)	Intraday Precision Mean ± S.D (n=3), %RSD	Inter day Precision Mean ± S.D (n=3), %RSD
2	232.58 ± 0.642, 0.276	232.69 ± 0.722, 0.310
4	433.93 ± 0.438, 0.101	433.62 ± 0.612, 0.141

6	642.82 ± 0.664, 0.103	642.49 ± 0.851, 0.132
8	868.45 ± 0.823, 0.094	867.63 ± 0.977, 0.112
10	1079.83 ± 0.585, 0.054	1079.86 ± 0.770, 0.071

Table 5 Intermediate precision data for Diclofenac sodium at 288 nm (Intraday & Inter day Precision)

Concentration (µg/mL)	Intraday Precision Mean ± S.D (n=3), %RSD	Inter day Precision Mean ± S.D (n=3), %RSD
1	453.46±0.810,0.178	453.58±0.998,0.220
2	888.57±0.776,0.087	888.40±0.873,0.098
3	1289.58±0.734,0.056	1289.54 ±0.791,0.061
4	1754.66±0.692,0.039	1754.7±0.723,0.041
5	2182.53±0.668,0.031	2182.55±0.699,0.0321

Table 6 Robustness data of Omeprazole and Diclofenac sodium

Parameter	Modification	Nimesulide % Recovery ±%RSD (n=3)	Diclofenac sodium %Recovery ± %RSD (n=3)
Flow rate (1 mL/min)	0.9	99.97 ± 0.128	100.01 ± 0.041
	1.1	99.92 ± 0.089	100.16 ± 0.081
Mobile phase	58:42	99.91 ± 0.141	100.01 ± 0.023
	62:38	99.79 ± 0.081	100.15 ± 0.086
Wavelength (288 nm)	287	99.88 ± 0.126	100.15 ± 0.069
	289	99.89 ± 0.114	100.14 ± 0.072
pH (5.5)	5.3	99.95 ± 0.256	100.02 ± 0.016
	5.7	99.91 ± 0.144	100.06 ± 0.057

Table 7 LOD and LOQ data of Nimesulide and Diclofenac sodium

Parameter	Nimesulide	Diclofenac sodium
LOD (µg/mL)	0.021	0.011
LOQ (µg/mL)	0.064	0.031

Table 8 System suitability parameters

Parameter	Diclofenac sodium	Nimesulide
Retention time (Minutes)	2.93	5.37
Theoretical plates (Tp)	3257.58	2149.2
Tailing factor (Tf)	1.25	1.56

Asymmetric factor (Af)	0.872	0.913

Table 9 Method validation parameters for Nimesulide and Diclofenac sodium

Parameters		Nimesulide	Diclofenac sodium
Calibration range		2-10 µg/mL	1-5 µg/mL
Slope		106.32	432.44
Intercept		13.32	16.47
Correlation coefficient		0.9996	0.9996
Precision (%RSD)	Intra-day	0.05 – 0.27	0.030 – 0.178
	Inter-day	0.07 – 0.31	0.032 – 0.220
	Repeatability	0.111	0.059
LOD(µg/mL)		0.021	0.011
LOQ(µg/mL)		0.064	0.031

Table 10 Assay results of Nimesulide and Diclofenac sodium

Formulation	Label Claim (mg/tablet)		Assay (Content in mg)		% Assay (Mean* ± S.D, n=6)	
	NIME	DICLO	NIME	DICLO	NIME	DICLO
DICLOPA NM	100	50	100.06	50.04	100.06 ± 0.16	100.08 ± 0.18

Table 11 Solution Stability Studies of Nimesulide and Diclofenac sodium

Time (hr.)	Peak Area of Nimesulide		Peak Area of Diclofenac sodium	
	Standard	Test	Standard	Test
0	651.14	650.95	1288.43	1287.95
2	651.28	650.09	1286.95	1286.31
4	650.95	649.34	1288.75	1287.99
6	651.23	649.91	1287.94	1287.64
24	650.72	650.12	1287.12	1286.94
Average	651.064	650.082	1287.84	1287.37
SD	0.2298	0.5777	0.7900	0.7249
% RSD	0.0353	0.0888	0.0613	0.0563

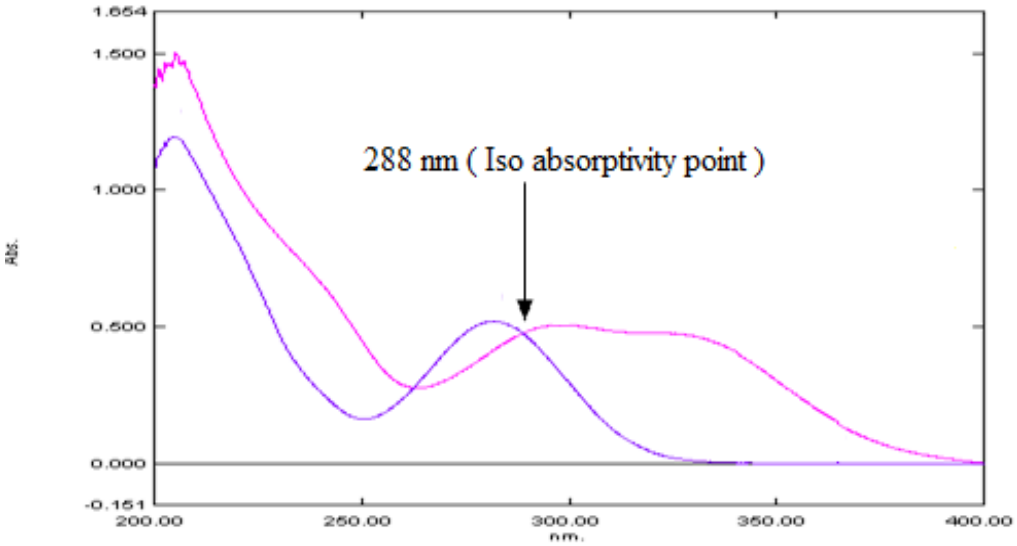


Figure 1 Overlain Chromatogram of Nimesulide and Diclofenac sodium

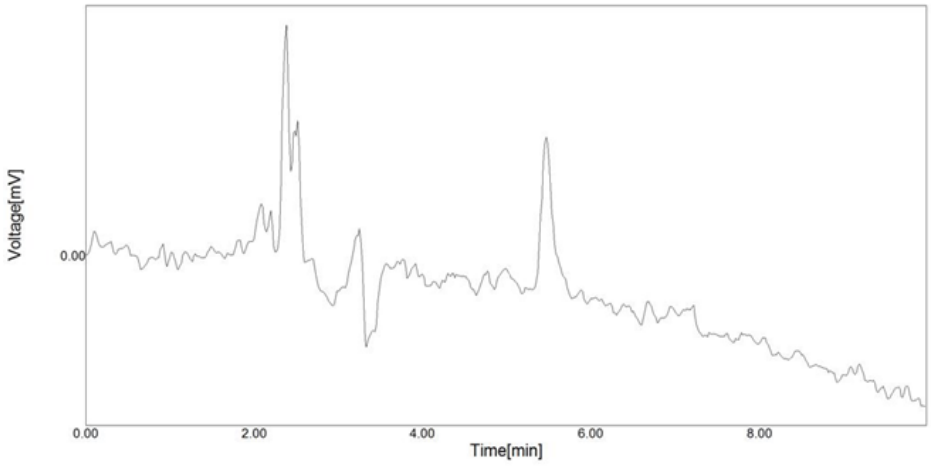


Figure 2 Chromatogram of Blank

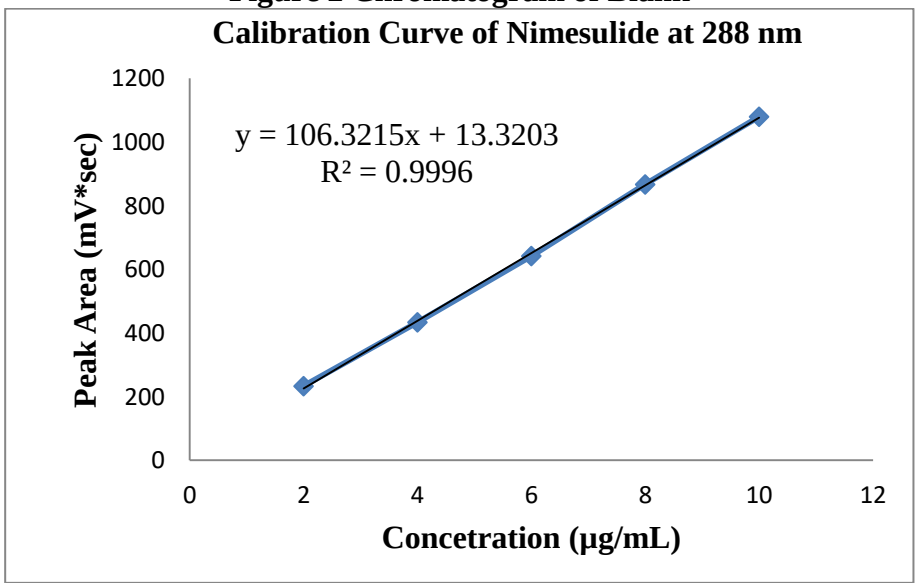


Figure 3 Calibration curve of Nimesulide at 288 nm

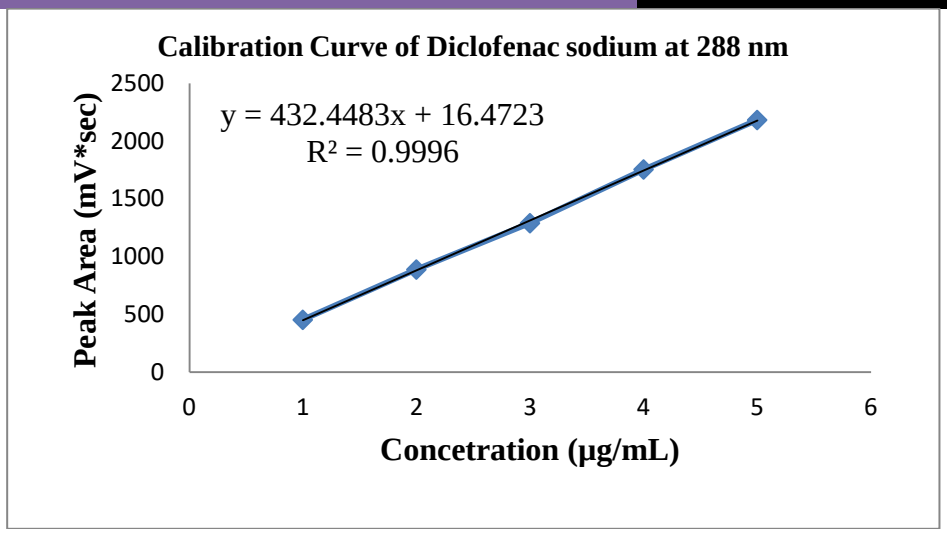


Figure 4 Calibration curve of Diclofenac sodium at 288 nm

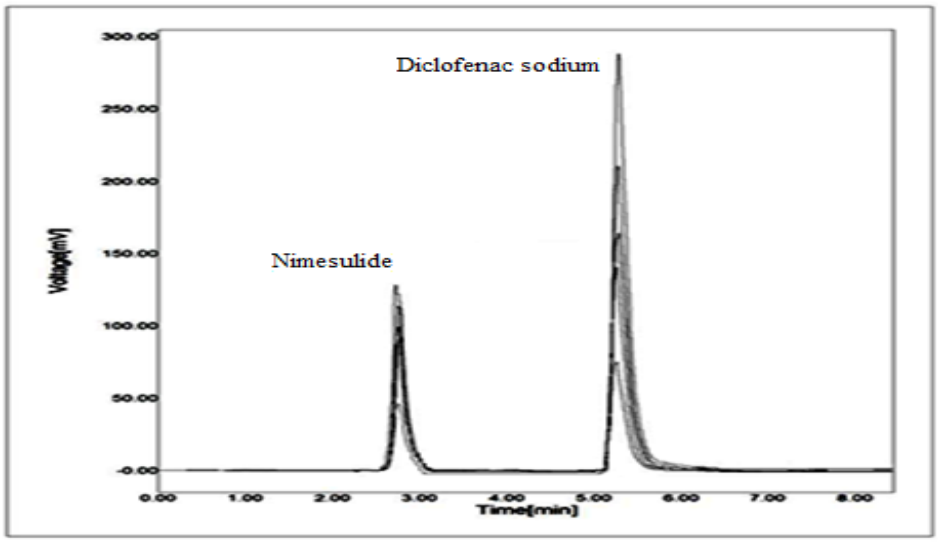


Figure 5 Overlain Chromatogram of Nimesulide and Diclofenac sodium

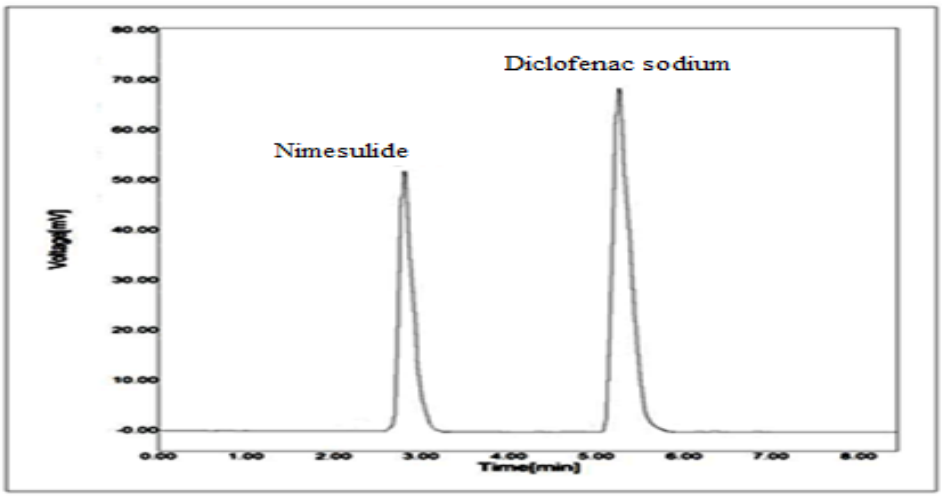


Figure 6 Chromatogram of Sample Nimesulide and Diclofenac sodium

5. CONCLUSION

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, selective, robust and rapid and can be applied successfully in routine analysis for the estimation of Nimesulide and Diclofenac sodium in pharmaceutical formulations without interference from commonly used excipients and additives.

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