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# Area under Curve method for Simultaneous Estimation of Nimesulide and Diclofenac sodium in Combined Pharmaceutical Dosage Form

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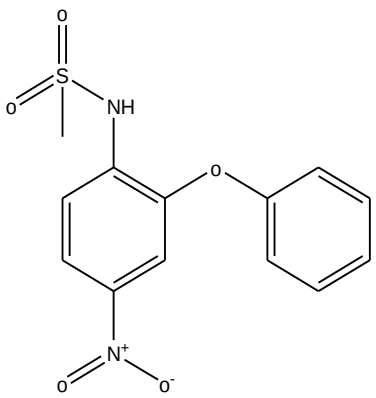
**Abstract:**
*The objective of this work was to develop and validate a new UV spectrophotometric Area under curve method was developed and validated for the simultaneous estimation of Nimesulide and Diclofenac sodium respectively. UV spectrophotometric method involves AUC method using Wavelength 293-303nm for Nimesulide and 277-287nm for Diclofenac sodium. For spectrophotometric method, Methanol was used as a solvent. Both the drug and their mix obeyed Beer- Lamberts law at selected wavelength was observed in concentration range of 4-20 µg/ml of Nimesulide and 2-10 µg/ml of Diclofenac sodium. The results of analysis have been validated statistically and by recovery studies as per ICH guidelines. This method was found to be simple, precise, accurate, selective and rapid and can be successfully applied for the determination of pure laboratory mixtures and tablet formulations.*

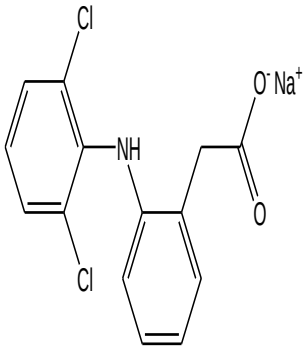
**Keywords:** *Area under Curve Method, Nimesulide, Diclofenac sodium, ICH guideline, Validation*

## 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

Simple, Precise, Accurate, Rapid & Economical UV- Spectrophotometric method for simultaneous determination of these drugs in combined tablet dosage form. To validate the developed analytical method as per ICH guideline ($Q_2$R1) for various parameters like accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), linearity, range, etc.

## 2. Materials and Methods

### *2.1 Instrumentation*

UV 1800 double beam UV Visible Spectrophotometer (Shimadzu) with a pair of 10mm path length matched quartz cells were used for the study. The UV solutions 2.42 software was used. An electronic balance used for weighing purpose was Shimadzu. Volumetric flasks and pipettes used in the study were of borosilicate glass. All the statistical calculations were carried out by using Microsoft Excel 2007.

### *2.2 Chemicals and Reagents*

The analytical samples of Nimesulide and Diclofenac sodium were received from Camper Healthcare, Mehsana, India as gift samples. All the chemicals used were of analytical grade. The tablet formulations were procured from a local pharmacy.

### *2.3 Preparation of Solutions*

#### *2.3.1 Standard stock solution (100 µg/ml)*

Accurately weighed 10 mg of Nimesulide and 10 mg of Diclofenac sodium were transferred to two separate 100 ml volumetric flask. Make up the final volume with water up to the mark to prepare a 100 µg/ml stock solution of both drugs.

#### *2.3.2 Preparation of working standard solution*

Standard stock solution (100µg/ml) of Nimesulide and Diclofenac sodium were used as working standard solutions. Accurately measured standard stock solution of Nimesulide (0.4, 0.8, 1.2, 1.6, & 2.0 ml) and standard stock solution of Diclofenac sodium (0.2, 0.4, 0.6, 0.8, & 1.0 ml) were transferred to a separate series of 10 ml of volumetric flasks and diluted to the mark with methanol.

#### *2.3.3 Preparation of Sample solution*

Twenty Tablets were weighed accurately. Quantity of the powder equivalent to about 10 mg of Nimesulide and 5 mg of Diclofenac sodium into 100 ml measuring flask 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 concentration of 100 µg/ml Nimesulide and 50 µg/ml of Diclofenac sodium. Take 1.2 ml from the flask and transfer into 10ml of volumetric flask and diluted up to the mark with methanol to get final conc. of 12 µg/ml Nimesulide 6 µg/ml of Diclofenac sodium.

## 3.Result and Discussion

### *3.1 Spectral characteristic of Nimesulide and Diclofenac sodium*

Nimesulide and Diclofenac sodium working solutions (2.0 ml of Nimesulide and 1.0 ml of Diclofenac sodium) were separately transferred into a 10 ml volumetric flask and dilute to volume with methanol. The Area under curve values was measured at 293-303 nm (For Nimesulide) and 277-287 nm (For Diclofenac sodium). The absorption spectra of Nimesulide and Diclofenac sodium are shown in Figure 1and 2.

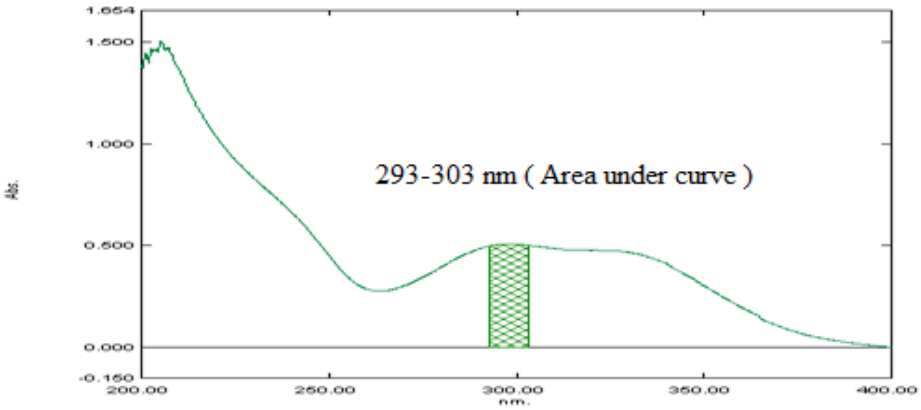



**Figure 1 Absorption spectra of Nimesulide**

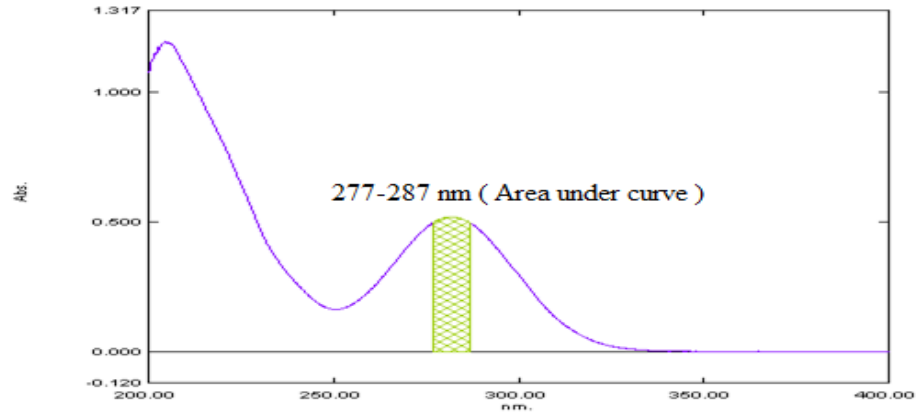



**Figure 2 Absorption spectra of Diclofenac sodium**

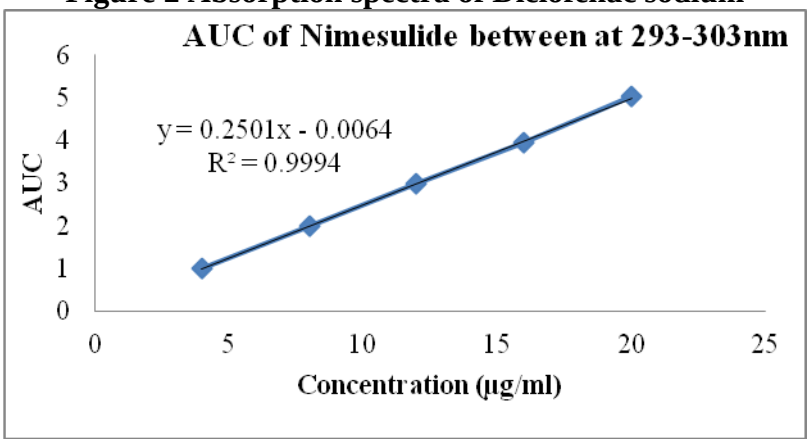



**Figure 3 Calibration Curve of Nimesulide at 293-303 nm**

A linear correlation was obtained between peak amplitude and the corresponding concentration in the range of 4-20 µg/ml for Nimesulide (Figure 5.3.3), from which the linear regression equation was computed and found to be:

Y = 0.2501x – 0.0064, r2 = 0.9994 at 293-303 nm

Where Y is peak amplitude at 293-303/277-287 nm, X is the concentration in µg/ml and r2 is the correlation coefficient.

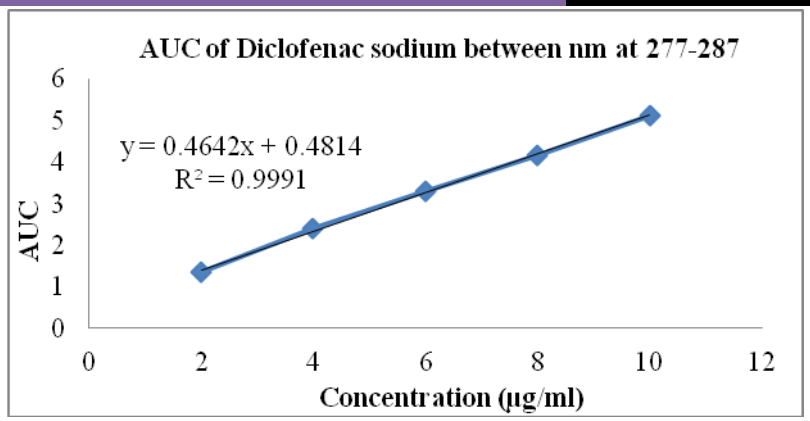



**Figure 4 Calibration Curve of Diclofenac sodium at 277-287 nm**

A linear correlation was obtained between peak amplitude and the corresponding concentration in the range of 2-10 µg/ml for Diclofenac sodium (Figure 5.3.4), from which the linear regression equation was computed and found to be:

Y = 0.4642x+0.4814, r2 = 0.9991 at 277-287 nm

Where Y is peak amplitude at 293-303/277-287 nm, X is the concentration in µg/ml and r2 is the correlation coefficient.

### *3.1.1 Accuracy (% Recovery)*

Accuracy of the method was assured by use of the standard addition technique, involving analysis of formulation of the samples containing 12 µg/ml of Nimesulide and 6 µg/ml of Diclofenac sodium to which certain amounts of authentic drugs were added. The resulting mixtures were assayed and the results obtained for both drugs were compared to those expected. Good recoveries with the standard addition method (Table 4) prove the good accuracy of the proposed method.

#### *3.1.1.1 Precision*

**Method precision (Repeatability)**

The precision of the instrument was checked by repeatedly (n=6) measuring the absorbance of Nimesulide (12 µg/ml) and Diclofenac sodium (6 µg/ml). The results of repeatability experiment are shown in Table 7 and 8. the developed method was found to be precise as the %RSD values for repeatability study were found to be less than 1.0%

### *3.1.2 Intermediate precision (Reproducibility)*

Results of intermediate precision for both intraday and interday are shown in Table 5 and 6. Replicate analyses of entire concentrations range of both Nimesulide (4-20 µg/ml) and Diclofenac sodium (2-10 µg/ml) were evaluated by three replicate determinations to estimate intraday variation and inter day variation. The developed method was found to be precise as the %RSD values for reproducibility study were found to be less than 2.0%. Low %RSD values of intraday and interday precision reveal that the proposed method is precise.

### *3.1.3 Limit of Detection (LOD) and Limit of Quantification (LOQ)*

LOD and LOQ for both drugs were calculated theoretically. These data show that the method is sensitive for the simultaneous determination of Nimesulide and Diclofenac sodium.

**Table 1 Linearity Data for Nimesulide**

| Concentration (µg/ml) | Mean AUC at 293-303nm ± SD (n=3) | % RSD |
|---|---|---|
| 4 | 1.014 ± 0.0035 | 0.346 |
| 8 | 1.999 ± 0.0045 | 0.229 |

| | | |
|---|---|---|
| 12 | 2.973 ± 0.0051 | 0.172 |
| 16 | 3.943 ± 0.0045 | 0.116 |
| 20 | 5.045 ± 0.0036 | 0.071 |

**Table 2 Linearity Data for Diclofenac sodium**

| Concentration (µg/ml) | Mean AUC at 277-287nm ± SD (n=3) | % RSD |
|---|---|---|
| 2 | 1.363 ± 0.0055 | 0.403 |
| 4 | 2.392 ± 0.006 | 0.251 |
| 6 | 3.297 ± 0.0070 | 0.212 |
| 8 | 4.157 ± 0.0055 | 0.133 |
| 10 | 5.123 ± 0.0045 | 0.088 |

**Table 3 LOD and LOQ data of Nimesulide and Diclofenac sodium.**

| Parameter | Nimesulide | Diclofenac sodium |
|---|---|---|
| | 293-303nm | 277-287nm |
| LOD (µg/ml) | 0.062 | 0.045 |
| LOQ (µg/ml) | 0.189 | 0.139 |

**Table 4 Accuracy (% Recovery) of Nimesulide and Diclofenac sodium**

| Drug | Amount taken (µg/ml) | Amount Added (µg/ml) | Amount found (µg/ml) ± S.D (n=3) | % Recovery ± S.D (n=3) |
|---|---|---|---|---|
| Nimesulide | 12 | 9.6 | 9.596 ± 0.006 | 99.962 ± 0.063 |
| | 12 | 12 | 11.994 ± 0.005 | 99.951 ± 0.038 |
| | 12 | 14.4 | 14.394 ± 0.006 | 99.961 ± 0.042 |
| Diclofenac sodium | 6 | 4.8 | 4.796 ± 0.004 | 99.93 ± 0.089 |
| | 6 | 6 | 5.998 ± 0.004 | 99.978 ± 0.071 |
| | 6 | 7.2 | 7.197 ± 0.003 | 99.971 ± 0.045 |

**Table 5 Intermediate precision data for Nimesulide at 293-303nm(Intraday & Inter day Precision)**

| Concentration (µg/ml) | Intraday Precision Mean ± S.D (n=3), %RSD | Inter day Precision Mean ± S.D (n=3), %RSD |
|---|---|---|
| 4 | 1.018 ± 0.006, 0.644 | 1.017 ± 0.009, 0.891 |
| 8 | 2.002 ± 0.009, 0.453 | 2.003 ± 0.011, 0.551 |
| 12 | 2.975 ± 0.011, 0.355 | 2.974 ± 0.013, 0.457 |
| 16 | 3.942 ± 0.008, 0.215 | 3.943 ± 0.012, 0.323 |
| 20 | 5.043 ± 0.006, 0.127 | 5.042 ± 0.009, 0.183 |

**Table 6 Intermediate precision data for Diclofenac sodium at 277-287nm (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 | 0.784 ± 0.004, 0.515 | 0.784 ± 0.005, 0.674 |
| 4 | 1.515 ± 0.006, 0.403 | 1.515 ± 0.007, 0.512 |
| 6 | 2.101 ± 0.006, 0.323 | 2.099 ± 0.009, 0.432 |
| 8 | 2.837 ± 0.006, 0.211 | 2.838 ± 0.008, 0.307 |
| 10 | 3.482 ± 0.004, 0.142 | 3.483 ± 0.006, 0.173 |

**Table 7 Repeatability Data for Nimesulide**

| Concentration | AUC | Amount found | %Amount found |
|---|---|---|---|
| 12 µg/ml | 2.992 | 11.988 | 99.906 |
| | 2.998 | 12.012 | 100.107 |
| | 2.997 | 12.008 | 100.073 |
| | 3.004 | 12.036 | 100.307 |
| | 2.995 | 12.001 | 100.007 |
| | 2.987 | 11.968 | 99.740 |
| Mean | 2.995 | 12.002 | 100.023 |
| SD (n = 6) | 0.0052 | 0.023 | 0.1917 |
| %RSD | 0.1753 | 0.1916 | 0.1916 |

**Table 8 Repeatability Data for Diclofenac sodium**

| Concentration | AUC | Amount found | %Amount found |
|---|---|---|---|
| 6 µg/ml | 3.265 | 5.996 | 99.942 |
| | 3.272 | 6.011 | 100.194 |
| | 3.268 | 6.003 | 100.05 |
| | 3.266 | 5.998 | 99.978 |
| | 3.267 | 6.001 | 100.014 |
| | 3.264 | 5.994 | 99.906 |
| Mean | 3.267 | 6.001 | 100.014 |
| SD (n = 6) | 0.0025 | 0.0061 | 0.1015 |
| %RSD | 0.0790 | 0.1015 | 0.1015 |

### *3.2 Estimation of Nimesulide and Diclofenac sodium in formulation*

Test solution from Tablets which contain Nimesulide 10 µg/ml and Diclofenac sodium 5 µg/ml were prepared and solutions were analyzed at 293-303nm and 277-287nm of Nimesulide and Diclofenac

sodium. The Absorptivity coefficients of these two drugs were determined using calibration curve equation. The concentration of Nimesulide and Diclofenac sodium were determined using following equations.

$$Y1 = mX1 + C$$
$$Y2 = mX2 + C$$

Where X1 and X2 are concentrations of Nimesulide and Diclofenac sodium, respectively in gm/100 ml sample solution. Y1 and Y2 are the Area under curve of the mixture at 293-303 nm and 277-287 nm respectively.

**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 |
| DICLOPANM | 100 | 50 | 100.084 | 50.025 | 100.084 ± 0.169 | 100.05 ± 0.122 |

**Table 11 Summary of Validation parameters**

| Parameters | | Nimesulide | Diclofenac sodium |
|---|---|---|---|
| Calibration Range (µg/ml) | | 4 - 20 | 2 - 10 |
| Slope (m) | | 0.2501 | 0.4642 |
| Intercept (c) | | 0.0064 | 0.4814 |
| Correlation Coefficient($r^2$) | | 0.9994 | 0.9991 |
| Precision (%RSD) | Repeatability | 0.175 | 0.079 |
| | Intra day | 0.127 – 0.664 | 0.141 – 0.515 |
| | Inter-day | 0.183 – 0.891 | 0.173 – 0.674 |
| LOD (µg/ml) | | 0.062 | 0.045 |
| LOQ (µg/ml) | | 0.189 | 0.139 |

## 4. Conclusion

Area under curve method was developed for the determination of Nimesulide and Diclofenac sodium in combined pharmaceutical dosage form. The proposed method is Simple, Accurate, Precise and this method is suitable for routine analysis of Nimesulide and Diclofenac sodium in combined pharmaceutical dosage form. Detection and Quantification limits achieved, describe that the method is sensitive. High recoveries and acceptable %RSD values confirms accuracy and precision of developed method. Assay results show that the method can be successfully applied for routine analysis of Nimesulide and Diclofenac sodium in combined pharmaceutical dosage form.

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