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## Research Article

# Validated UV Spectrophotometric Methods for the Estimation of Aceclofenac in Bulk and Pharmaceutical Formulation

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**Abstract:** Aceclofenac, 2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxyacetic acidis a non-steroidal anti-inflammatory drug (NSAID) used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Simple, rapid, accurate, specific and highly sensitive UV spectroscopic methods were developed and validated for the estimation of Aceclofenac in bulk and pharmaceutical dosage form. The absorption maxima was found to be 274.65nm for the method A(Zero order), 259nm for method B(first order derivative) and for method C(Area under curve) was measured from 269-279nm. The solvent used was methanol: water(40:60) for the preparation of stock solution and distilled water was used for the further dilutions. The methods were found to be linear in the range of 5-30µg/ml and the correlation coefficient values were found to be 0.9994, 0.9991, 0.9995 respectively. The developed methods were validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. The proposed methods can be used for the estimation of Aceclofenac in bulk and Pharmaceutical dosage forms.
**Keywords:** Aceclofenac, Area under curve, First order derivative spectroscopy, Validation.

## INTRODUCTION

Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID) used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Aceclofenac is a cytokine inhibitor and it works by blocking the action of cyclo-oxygenase which is involved in the production of prostaglandins, the chemical which causes pain, swelling and inflammation[1]. Aceclofenac is the glycolic acid ester of diclofenac. Chemically it is 2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxyaceticacid.
Molecular formula is $C_{16}H_{13}C_{12}NO_4$ and it is having the molecular weight of 354.18 g/mol.It is insoluble in water, freely soluble in acetone and alcohol[2]. The solubility of weakly acidic drug, Aceclofenac depends on pH and is highly soluble in basic conditions but relatively soluble in water and acidic pH conditions. The recommended dose is 100 mg twice a day.

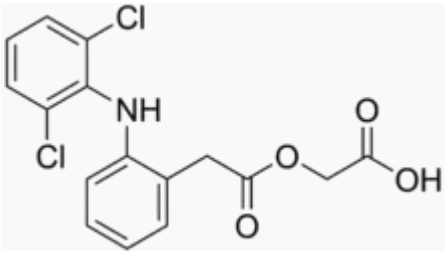


**Fig-1: Structure of Aceclofenac**

Several methods were developed for the estimation of aceclofenac by HPLC, visible and UV spectroscopy [3-17] individually or in combination. So our aim is to develop and validate a new simple, rapid, accurate, specific and highly sensitive UV spectroscopic methods for the estimation of aceclofenac in bulk and pharmaceutical dosage form by using mobile phase which was more economical when compared to other methods.

## MATERIALS AND METHODS:

The instrument used in the present study was double beam UV-VIS spectrophotometer (Evolution 220, Thermo Scientific, Japan) connected to computer loaded with spectra manager software Thermo Insight was employed with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Schimadzu, Japan). Methanol (AR Grade, Merck, India) and double distilled water were used for the study. Working standard Aceclofenac was obtained as a gift sample from Chandra laboratories, Hyderabad, India. Afenak-100 was taken for study which contains Aceclofenac 100mg was purchased from local market.

**METHOD DEVELOPMENT:**

**Selection of solvent:**

Selection of solvent for the drug Aceclofenac was done by testing its solubility in various solvents, which includes distilled water, methanol, ethanol, 0.1N HCl, 0.1N NaOH. Methanol:distilled water(40:60) was chosen as solvent for developing the method (for preparation of stock solution methanol : distilled water(40:60) is used and further dilutions are made with distilled water).

**Determination of $\lambda_{max}$:**

**Preparation of stock solution:**

50mg of working standard Aceclofenac was accurately weighed and transferred to 50ml volumetric flask. Then 20ml of Methanol was added to dissolve the drug by shaking the flask for few seconds. Then the final volume was made upto the mark with distilled water to get the concentration of 1000µg/ml.

**Preparation of working standard solution:**

From the above standard stock solution, 1ml was pipetted out into a 10mL volumetric flask and the volume was made up to the mark with distilled water to get a concentration of 100µg/ml.

**Preparation of calibration curve:**

**Method A: Zero order spectroscopic method**

From the working standard solution, 0.5ml, 1.0ml, 1.5ml,2.0ml, 2.5ml, 3.0ml were pipetted into 10ml volumetric flasks and volume was madeupto the mark with distilled water to produce the concentrations ranging from 5-30µg/ml respectively.The analytical wavelength was selected by scanning 10µg/mlin the wavelength range of 400-200nm using distilled water as a blank and the wavelength corresponding to maximum absorbance ($\lambda_{max}$) was found to be 274.65nm and the corresponding UV spectrum was shown in the figure 2 and the overlay spectrum was shown in figure 3.Then, the calibration curve was plotted in the concentration range of 5-30µg/mlat 274.65nm by taking concentration on X-axis and absorbance on Y-axis. The correlation coefficient ($r^2$) was found to be 0.9994.The calibration curve of aceclofenac was shown in figure 4.

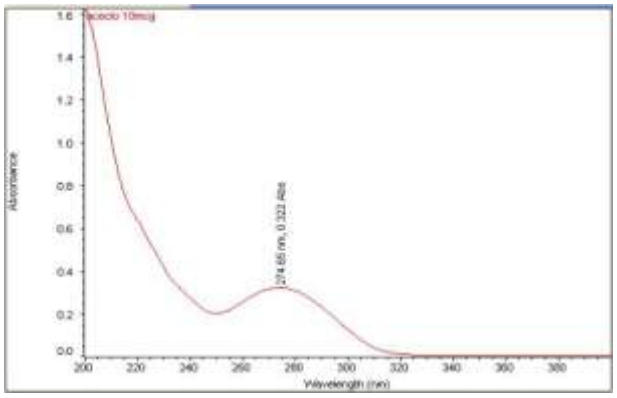

**Fig-2: Absorption maxima of Aceclofenac**

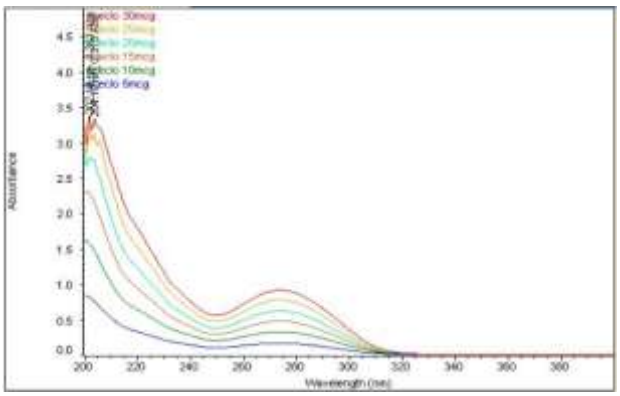

**Fig-3: Overlay spectrum of Aceclofenac(Zero order)**

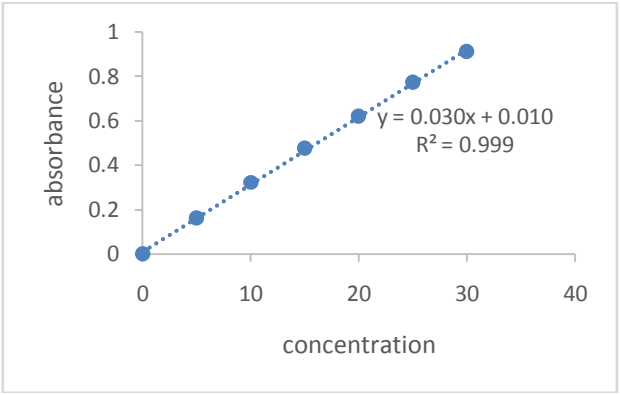


**Fig-4: Calibration curve of Aceclofenac(zero order)**

**Method B: First order derivative spectroscopic method**

For the selection of analytical wavelength solution of 10µg/ml was scanned in the spectrum mode in the wavelength of 200-400nm and the absorption spectra thus obtained was derivatized in the first order. First order derivative spectrum showed a sharp peak at$\lambda_{max}$ at 259nm and the corresponding spectrum was given in the figure 5. The amplitude of absorbance was measured for all solutions in the concentration range of 5-30µg/ml at 259nm and was plotted against concentration for getting the calibration curve and the regression equation was calculated. The calibration curve for the first order derivative spectra was shown in the figure 6.

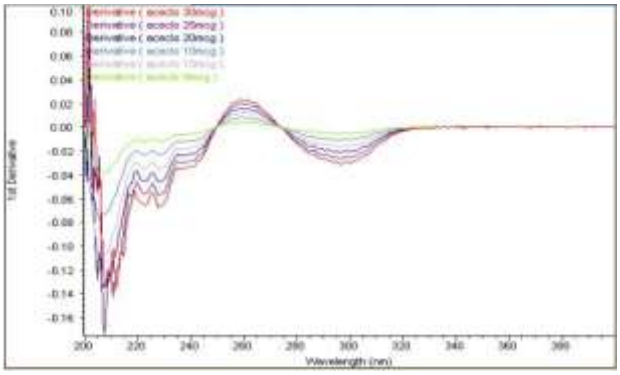

**Fig-5: First order derivative spectrum of Aceclofenac**

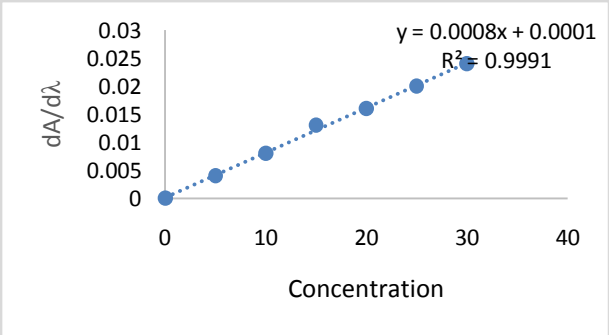


**Fig- 6: Calibration curve of Aceclofenac(first order)**

**Method C: AUC method**

It involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths $\lambda_1$ and $\lambda_2$. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. From the spectrum of the drug, AUC in the wavelength range of 269-279nm was selected for the analysis and the corresponding spectrum was shown in the figure 7. The calibration curve was plotted against concentration v/s α+β and was shown in the figure 8.

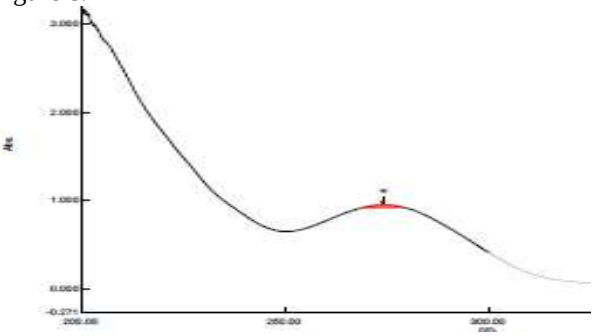

**Fig-7: AUC spectrum of Aceclofenac**

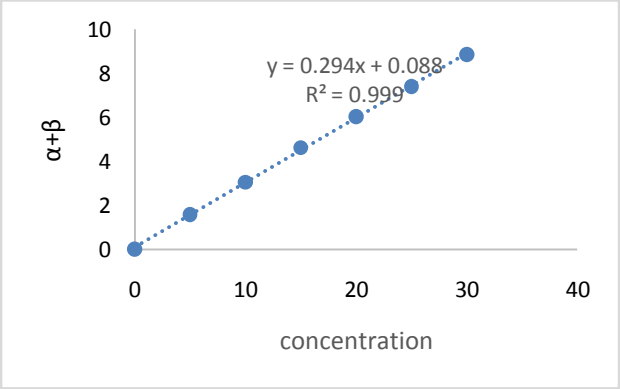


**Fig-8: Calibration curve of Aceclofenac(AUC method)**

**Estimation of Aceclofenac in tablet formulation:**

For estimation of Aceclofenac in tablet formulation by this method 10 tablets of marketed brand of Afenak-100each containing 100mg of aceclofenac was weighed and triturated to fine powder. Amount of powder equivalent to 50 mg drug was taken and dissolved in 20 ml of methanol and made up to the mark with distilled water in 50 ml volumetric flask (1000 µg/ml). It was filtered through whatmann filter paper no. 41. From that stock solution further dilution was made with distilled water to get required concentration. The concentration of Aceclofenac was determined by measuring the absorbance of sample solution at 274.65nm. The assay procedure was repeated six times (n=6).The result of marketed formulation analysis was given in the table 1.

**Table 1: Assay of the Marketed Formulation**

| Analysis method | Label claim (mg) | Amount found(mg) | % Recovery |
|---|---|---|---|
| A | 100 mg | 99.35 | 99.35 |
| B | 100mg | 100.2 | 100.2 |
| C | 100mg | 99.78 | 99.78 |

**VALIDATION OF THE PROPOSED METHOD**

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

The method was validated for different parameters like Linearity, Accuracy and Precision.

**Linearity:**

The linearity of the proposed UVspectroscopic methods were evaluated by plotting absorbances against concentrations of the analyte. Beers law was obeyed for all the three methods in the concentration range of 5-30 µg/ml. The correlation coefficient values were found to be 0.9994, 0.9991,0.9995 respectively. All the results were given in the table 2.

**Table 2: Linearity studies of Aceclofenac by proposed methods**

| S.No | Parameter | Method A | Method B | Method C |
|---|---|---|---|---|
| 1 | Linearity(µg/ml) | 5-30 | 5-30 | 5-30 |
| 2 | Slope | 0.0304 | 0.0008 | 0.2942 |
| 3 | Intercept | 0.0104 | 0.0001 | 0.0882 |
| 4 | Correlation coeffecient | 0.9994 | 0.9991 | 0.9995 |

**Precision:**

The precision of the method was expressed in terms of % relative standard deviation (% RSD). To check the intra-day and inter-day variation of the methods, solutions containing 15, 20, 25µg/ml concentration of Aceclofenac were subjected to the proposed spectroscopic methods of analysis .The % RSD values were found to be less than 2 for intraday and inter day precision, the precision results showed good reproducibility. The results are expressed in tables 3,4,5 respectively for the three methods.

**Table 3: Intra-day and Inter-day Precision data of Aceclofenac(Zero order)**

| Concentration taken (µg/ml) | Intra-day precision | | Inter-day precision | |
|---|---|---|---|---|
| | Mean ± SD (n=3) | % RSD | Mean ± SD (n=3) | % RSD |
| 15 | 0.473±0.002 | 0.42 | 0.471±0.004 | 0.84 |
| 20 | 0.618±0.001 | 0.16 | 0.615±0.004 | 0.65 |
| 25 | 0.772±0.002 | 0.25 | 0.769±0.003 | 0.39 |

**Table 4: Intra-day and Inter-day Precision data of Aceclofenac(First order)**

| Concentration taken (µg/ml) | Intra-day precision | | Inter-day precision | |
|---|---|---|---|---|
| | Mean ± SD (n=3) | % RSD | Mean ± SD (n=3) | % RSD |
| 15 | 0.01506±0.0002 | 1.32 | 0.01532±0.0002 | 1.36 |
| 20 | 0.0163±0.0001 | 0.61 | 0.0172±0.00015 | 0.87 |
| 25 | 0.0208±0.0002 | 0.96 | 0.0212±0.0003 | 1.41 |

**Table 5: Intra-day and Inter-day Precision data of Aceclofenac(AUC method)**

| Concentration taken (µg/ml) | Intra-day precision | | Inter-day precision | |
|---|---|---|---|---|
| | Mean ± SD (n=3) | % RSD | Mean ± SD (n=3) | % RSD |
| 15 | 4.617±0.001 | 0.021 | 4.624±0.011 | 0.237 |
| 20 | 6.0125±0.003 | 0.049 | 6.092±0.005 | 0.082 |
| 25 | 7.3602±0.002 | 0.027 | 7.426±0.022 | 0.296 |

**Accuracy:**

Accuracy for the methods was established at 80, 100, 120% levels by the addition of standard drug of Aceclofeanc to the pre-quantified sample solution. Each dilution was observed six times and the percentage recovery of the drug was measured and the results were given in the table no.6.

**Table 6: Recovery studies of Aceclofenac by proposed methods**

| Concentration taken(µg/ml) | Spiked level(%) | Amount added(mg) | Amount found(mg) | | | %Recovery | | |
|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | A | B | C |
| 10 | 80 | 8 | 17.89 | 18.04 | 17.98 | 99.38 | 100.22 | 99.88 |
| 10 | 100 | 10 | 19.84 | 20.06 | 19.97 | 99.20 | 100.3 | 99.85 |
| 10 | 120 | 12 | 21.86 | 22.09 | 21.98 | 99.36 | 100.4 | 99.91 |

**RESULTS AND DISCUSSION**

The methods discussed in the present work provided a convenient and accurate way for the analysis of Aceclofenac in bulk and in pharmaceutical dosage form. The absorbance maxima of Aceclofenac was found to be 274.65 nm for the method A, the absorption maxima of first order derivative spectra was found to be 259nm for method B and for method C the area under curve in the range of 269-279nm was selected for the analysis. Linearity for all the three methods was observed in the concentration range of 5-30µg/ml as shown in the table 2. The assay of the three methods were found to be within the range of 98-102% as shown in the table 1. The developed method was validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. In all the three methods both the intra-day and inter-day precision study the %RSD were found to be less than 2.0 indicating the good precision as shown in the tables 3,4,5 respectively. The validation of proposed methods were further confirmed by recovery studies, the %recovery values vary from 98- 102% as shown in the table 6. The summary of the optical characteristics of validation parameters were given in the table 7.Based on results obtained, it was found that the proposed methods were found to be accurate, precise and reproducible and can be employed for routine quality control analysis of Aceclofenac in tablet dosage form.

**Table 7: Summary of Optical characteristics and Validation parameters**

| S.No | Parameter | Method A | Method B | Method C |
|---|---|---|---|---|
| 1 | Absorption maxima($\lambda_{max}$) | 274.65 | 259 | 269-279 |
| 2 | Beer's limit(µg/ml) | 5-30 | 5-30 | 5-30 |
| 3 | Linearity indicated by correlation coefficient | 0.9994 | 0.9991 | 0.9995 |
| 4 | Regression Equation | y = 0.0304x + 0.0104 | y = 0.0008x + 0.0001 | y = 0.2942x + 0.0882 |
| 5 | Accuracy indicated by %Recovery | 99.20-99.38 | 100.22-100.3 | 99.85-99.91 |
| 6 | Precision<br>Intraday(%RSD) (n=6)<br>Interday (% RSD) (n=6) | <br>0.16-0.42<br>0.39-0.84 | <br>0.61-1.32<br>0.87-1.41 | <br>0.021-0.049<br>0.082-0.296 |

## CONCLUSION

The proposed methods were found to be simple, sensitive, accurate and precise and showed no interference from the common additives and excipients. The developed method was validated in terms of linearity, accuracy, precision in accordance with the ICH guidelines. Hence the proposed methods can be routinely used for the estimation of Aceclofenac in bulk and pharmaceutical dosage forms.

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