Advances in Bioresearch
Adv. Biores., Vol 15 (5) September 2024: 207-216

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.5.207216

Advances in Bioresearch

ORIGINAL ARTICLE

Advanced Analytical Techniques, and Stringent Validation Procedures of Aceclofenac

Bhavika Neeraj, Sarika Saini, Praveen kumar, Mehtab Ali
Department of Pharmaceutical Chemistry, Himalayan Institute of Pharmacy & Research, Rajawala Dehradun, India
*Corresponding author email: tyagi.bhavika@gmail.com

ABSTRACT
This study aims to synthesize, develop, and validate a spectrophotometric method for the accurate and selective quantification of Aceclofenac. The methodology adhered to the validation requirements outlined by the International Council for Harmonization (ICH). Key validation parameters such as linearity, precision (intraday and interday), robustness, ruggedness, accuracy (% recovery), limit of detection (LOD), and limit of quantitation (LOQ) were thoroughly evaluated and found to be reliable. The procedure showed no interference when excipients were used. Cost-effective solvents—distilled water, ethanol, and methanol—were chosen based on their accessibility and the drug's solubility in each solvent type. The % RSD values for all methods were below 2%, indicating successful processes. This analytical method is suitable for routine laboratory analysis. The assay methods employed include titration method, ordinary method, and modified methods. Method A (Zero Order) measures the direct absorbance at 274.65 nm, providing a straightforward analysis. Method B (First Order Derivative) utilizes the first derivative of the absorbance spectrum to determine the maxima at 259 nm, offering better resolution and sensitivity. Method C (AUC) involves calculating the area under the curve between 269 and 279 nm, providing a comprehensive measurement over a range of wavelengths. These techniques ensure accurate and reliable determination of Aceclofenac concentration, making them suitable for routine analysis and quality control in pharmaceutical formulations.
Keywords: Aceclofenac, Spectroscopy, Assay, Method and validation

Received 12.06.2024 Revised 24.08.2024 Accepted 16.09.2024

How to cite this article:
Bhavika N, Sarika S, Praveen K, Mehtab A. Advanced Analytical Techniques, and Stringent Validation Procedures of Aceclofenac. Adv. Biores. Vol 15 [5] September 2024. 207-216

INTRODUCTION

Spectroscopy is the branch of science dealing with the study of the interaction of electromagnetic radiation with matter. The most important consequence of such interaction is that energy is absorbed or emitted by the matter in discrete amounts called quanta. The absorption or emission processes are known throughout the electromagnetic spectrum ranging from the gamma region to the radio region [1]. The data that is obtained from spectroscopy is called a spectrum. A spectrum is a plot of the intensity of energy detected versus the wavelength (or mass or momentum or frequency, etc.) of the energy. A spectrum can be used to obtain information about atomic and molecular energy levels, molecular geometries, chemical bonds, interactions of molecules, and related processes [2,3]. Often, spectra are used to identify the components of a sample (qualitative analysis). Spectra may also be used to measure the amount of material in a sample (quantitative analysis).

Analytical Method Validation is "the collection and evaluation of data, from the process design stage throughout production, which establishes scientific evidence that a process is capable of consistently delivering quality products" [4]. When extended to an analytical procedure, depending upon the application, it means that a method works reproducibly, when carried out by the same or different persons, in the same or different laboratories, using different reagents, different equipment, etc [5,6].

Aceclofenac is a non-steroidal anti-inflammatory drug with good analgesic and anti-rheumatic properties. Chemically it is [[[2-[(2, 6-Dichlorophenyl) amino] phenyl] acetyl] oxy] acetic acid. It is used in various pain conditions like rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. Several

analytical techniques like titrimetric, colorimetric, spectrofluorimetric, densitometric, PLC, RP–HPLC spectrophotometric, and stripping voltammetric have been reported for assay of Aceclofenac. However, some of these methods are costlier and time-consuming [7]. To overcome these difficulties, spectrophotometric analysis serves to be the quickest, most promising, and reliable method for routine analytical needs. The overall aim is to synthesize aceclofenac and to develop, validate, and optimize analytical methods for its assay to ensure effective treatment of inflammatory diseases with precise and reliable techniques.

MATERIAL AND METHODS

Materials:

2-[(2,6-Dichlorophenyl) amine] phenylaceticacid was purchased from the Tokyo chemicalindustry (TCI). Tetrahydrofuran (THF) was obtained from Gayatri Industries. Diisopropylethylamine, tert-butyl-bromoacetate, and Sodium hydroxide were acquired from Central Drug House(CDH).

Methods:

Synthesis of 2-[(2,6-Dichloro- Phenyl)Amino]Phenylacetoxyacetic Acid

It involves a multi-step process with the formation of key intermediates. The synthetic route comprises two primary steps:

Initial functionalization: The reaction of diclofenac acid with N, N-diisopropylethylamine (DIPEA) in an appropriate aprotic solvent under controlled temperature conditions yields the first intermediate.

Acetylation and deprotection: The intermediate undergo acetylation via nucleophilic substitution with a suitable α-haloacetic acid ester, forming an acetate derivative. Subsequent deprotection of the acetate moiety is achieved through acidic hydrolysis.

The final product is obtained after workup and purification. The overall synthetic scheme can be represented as:

Diclofenac acid → Intermediate (step 1) → Acetate derivative (step 2a) → 2-[(2,6-dichlorophenyl) amino] phenylacetoxyacetic acid (step 2b)

This methodology leverages the reactivity of the carboxylic acid group in diclofenac and employs strategic protection-deprotection strategies to achieve the desired functionalization.

Melting point determination

A small amount of the purified, dry 2-[(2,6-chlorophenyl) amino] phenylacetoxyacetic acid was finely ground using a mortar and pestle to ensure uniformity. The powdered sample was packed into a capillary tube to a depth of approximately 2-3 mm. The capillary tube was placed in a digital melting point apparatus [8]. The temperature was increased at a rate of 1°C/min starting from 20°C below the expected melting point. The temperature at which the sample began to melt (onset) and the temperature at which it completely melted (clear point) were recorded. The measurement was repeated three times with fresh samples to ensure reproducibility.

Assay of Aceclofenac

The assay may be defined as the process in which the substance is analyzed to check the impurity, concentration, and quality of the particular substance. Different methods were used for the estimation of Aceclofenac bulk. One method used for the analysis was the titration method and the other method involved for the assay was the UV method [9].

Assay of Aceclofenac bulk by Titration Method

An initial 0.1 m NaOH solution, which will serve as the estimated reagent, was made for the test [10].

Assay of Aceclofenac bulk by UV Spectroscopy (Ordinary Method)

Accurately weigh 50.0 mg (±0.1 mg) of Aceclofenac ($C_{16}H_{13}Cl_2NO_4$) using an analytical balance. Transfer the weighed Aceclofenac to a 100 mL class A volumetric flask. Add approximately 50 mL of distilled water to the flask. Sonicate the mixture in an ultrasonic bath until complete dissolution is achieved, ensuring a homogeneous dispersion of the analyte. Allow the solution to cool to room temperature if heating occurs during sonication [11]. Dilute to volume with HPLC-grade methanol, creating a final concentration of 0.5 mg/mL Aceclofenac. Seal the flask with a ground glass stopper and homogenize the solution by inverting and gently shaking the flask several times.

Preparation of Standard Calibration Curve

Method A: Zero order spectroscopic method

Prepare a series of standard solutions by diluting aliquots of the aceclofenac stock solution. Create solutions ranging from 5 to 40 µg/mL by transferring 0.5 to 4.0 mL of the stock solution to separate 10 mL volumetric flasks [8]. For example, pipette 0.5 mL of the stock solution into a 10 mL volumetric flask and dilute to volume with deionized water to achieve a final concentration of 5 µg/mL. Repeat this process to prepare the entire concentration range up to 40 µg/mL. Perform a UV spectral scan of a 10

µg/mL standard solution over the wavelength range of 200-400 nm, using deionized water as the blank. The λmax was determined to be 274.6 nm, corresponding to the maximum absorbance of aceclofenac in this spectral region. Based on the calibration curve, the recommended dosage of Aceclofenac was determined.

Method B: First-order derivative spectroscopic method

Perform a first-order derivative spectral scan of a 10 µg/mL aceclofenac solution over the wavelength range of 200-400 nm to determine the optimal analytical wavelength. Measure the amplitude of the first-order derivative spectrum at 259 nm for each standard solution in the concentration range of 5-40 µg/mL [12]. Construct a calibration curve by plotting the derivative amplitude against concentration. Calculate the coefficient of determination (R^2) to assess the linearity of the response.

Method C: AUC Method

The AUC method involves calculating the integral of absorbance concerning wavelength between two selected wavelengths, λ1 and λ2. The optimal wavelength range for integration is determined empirically to ensure a linear relationship between AUC and analyte concentration. Based on spectral analysis, the AUC integration limits were established as λ1 = 269 nm and λ2 = 279 nm. A calibration curve was constructed by plotting the calculated AUC (α+β) against the corresponding aceclofenac concentration.

Analytical Method Validation

The reliability and suitability of an analytical procedure for its intended purpose can only be established through a process of method validation. This systematic approach ensures the quality, consistency, and integrity of analytical data [13]. The validation parameters assess the method's performance characteristics and provide evidence of its reliability. Key validation parameters include:

Linearity: The ability of the method to elicit test results that are directly proportional to the concentration of analyte in samples within a given range.

Precision: The closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions.

Intraday Precision (Repeatability): Precision under the same operating conditions over a short interval of time.

Interday Precision (Intermediate Precision): Precision under different conditions, such as different days, analysts, or equipment, within the same laboratory [14].

Robustness: The capacity of the method to remain unaffected by small, deliberate variations in method parameters.

Ruggedness: The degree of reproducibility of test results obtained by analyzing the same samples under various conditions, such as different laboratories, analysts, instruments, or reagent lots

Limit of Detection (LOD): The lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, under the stated experimental conditions.

Limit of Quantification (LOQ): The lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

Linearity

The aceclofenac calibration curve has six points, with concentrations ranging from 5 to 40µg/ml (table:7). The drug's reaction was determined to be linear over the studied range, and the following linear regression equation and correlation coefficient were established:

Method A: y=0.022x+0.005 Correlation Coefficient: 0.998
Method B: y=0.022x+0.001 Correlation Coefficient: 0.999
Method C: y=0.022x+0.001 Correlation Coefficient: 0.999

Precision

To ensure the reliability and reproducibility of analytical results, adherence to standardized validation protocols is essential. The method's precision is evaluated through replicate analyses, typically performed in hexaplicate (n=6). From these replicates, the following statistical parameters are calculated [15]:

Where, mean ($\bar{x}$); standard deviation (s); relative standard deviation, (%RSD or Coefficient of Variation, CV)

$$RSD = \frac{S.D}{Mean} X\ 100$$

Accuracy

The reliability of the approach is calculated in three stages using a conventional adding method. The spiked levels are taken as 80,100,120% and added to the sample. The accuracy was indicated by the % recovery studies.

$$\% \text{ Recovery} = \frac{\text{Conc. (spiked sample} - \text{Conc. (µg/ml)}}{\text{Conc. (added)}} \text{ X } 100$$

LOD & LOQ

LOD is stated as Limit of Detection and LOQ is stated as Limit of Quantification. These terms describe the smallest concentration of an analyte that the analytical technique can consistently identify.

$$LOD = \frac{S.D}{Slope} X\, 10$$

$$LOD = \frac{S.D}{Slope} X\, 3.3$$

RESULTS

Melting Point: The observed melting point of the product was 31.5°C, consistent with the literature value for pure aceclofenac.

Assay of Aceclofenac

The titration procedure revealed that the purity of aceclofenac was 100.03%, confirming the high quality of the synthesized compound

Table 1: Reading of Aceclofenac by titration

S.No.	Initial reading of burettecontaining titrant (0.1 M NaOH)	Final reading of burettecontaining titrant (0.1 MNaOH)	The volume of 0.1 MNaOH used (Final reading – Initial reading)
1.	0 mL	10.4 mL	10.4 mL
2.	0 mL	10.2 mL	10.2 mL
3.	0 mL	10.6 mL	10.6 mL

The assay of aceclofenac using the ordinary method showed a highdegree of accuracy, with a result of 99.72% at a wavelength of 275 nm

Table 2: Concentration and standard-test absorbance of Aceclofenac

S.No.	Concentration(µg/ml)	Standard absorbance at 275 nm	Test absorbance at 275 nm
1.	2	0.593	0.562
2.	2	0.690	0.663
3.	2	0.642	0.687
4.	2	0.529	0.695
5.	2	0.623	0.598
6.	2	0.583	0.679

Assay of Aceclofenac by UV spectroscopy(modified method)

It was found that R^2, or the correlation coefficient, was 0.9980. The modified method was also found to be highly accurate, with correlation coefficients of 0.9988, 0.999, and 0.999 for methods A, B, and C, respectively, at wavelengths of 274.6, 259, and 269-279 nm, respectively, across a concentration range of 5-40 µg/ml

Method 1: Overlay spectrum of aceclofenac by the zero-order spectrometric method

Table 3: Reading by Zero order method

S. No.	Conc. (µg/ml)	Absorbance at 274.6 nm
1.	5	0.112999
2.	10	0.229009
3.	15	0.359432
4.	20	0.469321
5.	25	0.599264
6.	30	0.697708
7.	35	0.783188
8.	40	0.91794

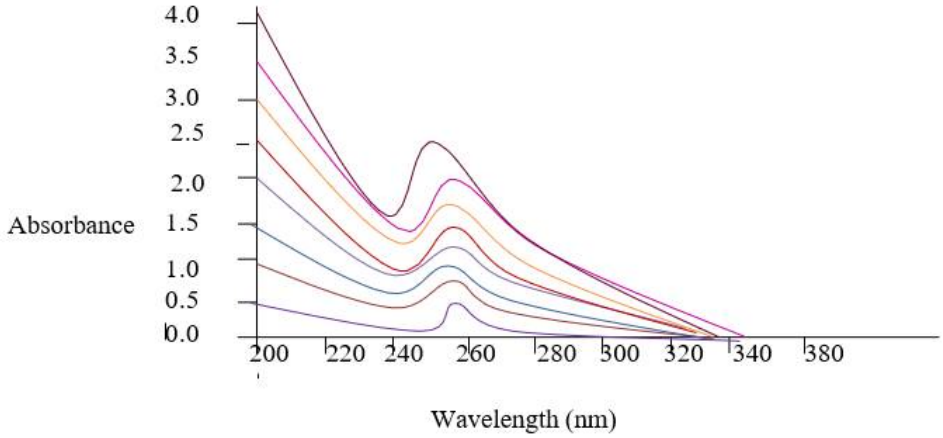

Fig 1: Spectrum by zero-order method

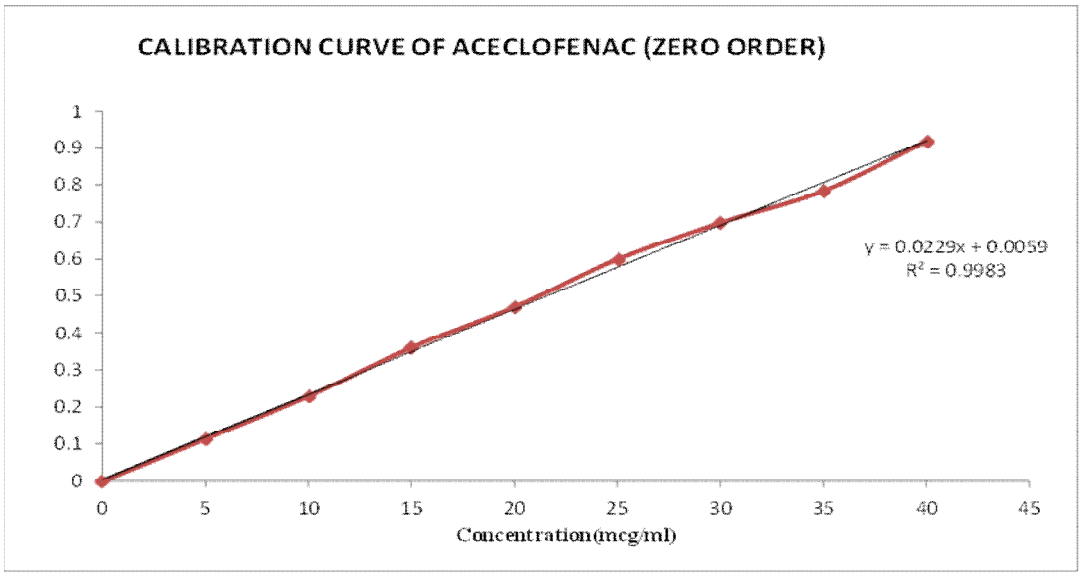

Fig: 2 Calibration curve of Aceclofenac Zero-order

Method 2: Overlay spectrum of aceclofenac by the first-order spectrometric method

Table 4: Reading by first order method

S. No.	Conc. (µg/ml)	Absorbance at 259 nm
1.	5	0.121989
2.	10	0.239007
3.	15	0.339432
4.	20	0.469217
5.	25	0.579264
6.	30	0.675708
7.	35	0.794188
8.	40	0.927946

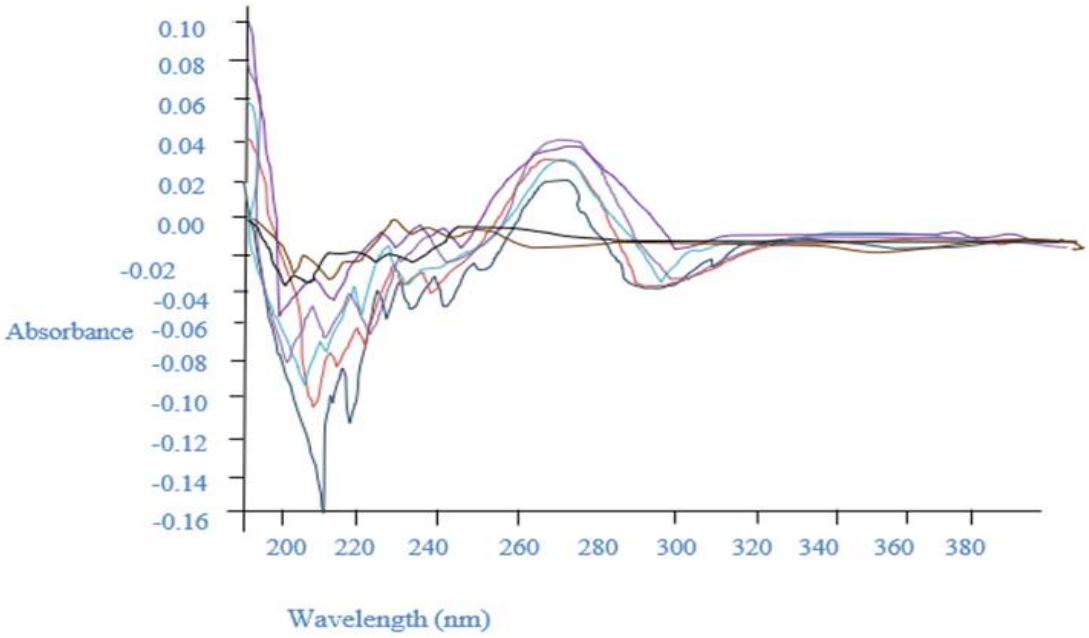

Fig 3: Spectrum by first-order method

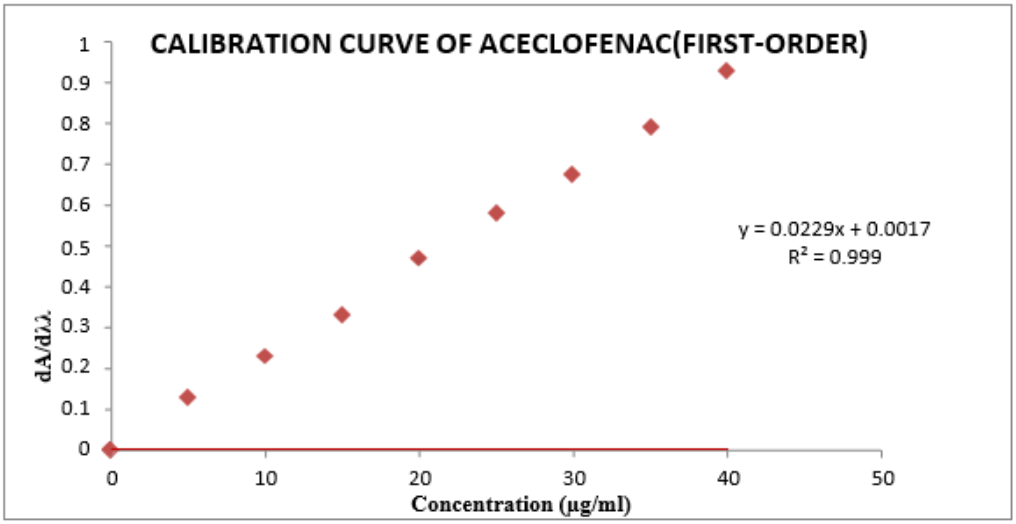

Fig 4: Calibration curve of Aceclofenac first-order

The correlation coefficient value (R^2) was found to be 0.9990.

Method 3: Overlay spectrum of Aceclofenac byAUC Method

Table 5: Reading by AUC method

S. No.	Conc. (µg/ml)	Absorbance at 269-279 nm
1.	5	0.125989
2.	10	0.229007
3.	15	0.329432
4.	20	0.469217
5.	25	0.579264
6.	30	0.675708
7.	35	0.794188
8.	40	0.927946

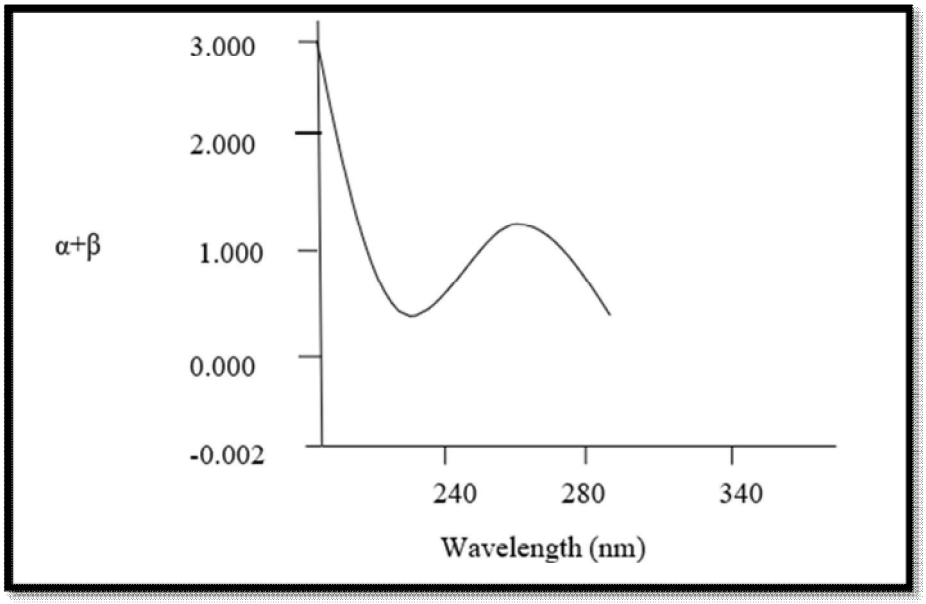

Fig 5: Spectrum by AUC method

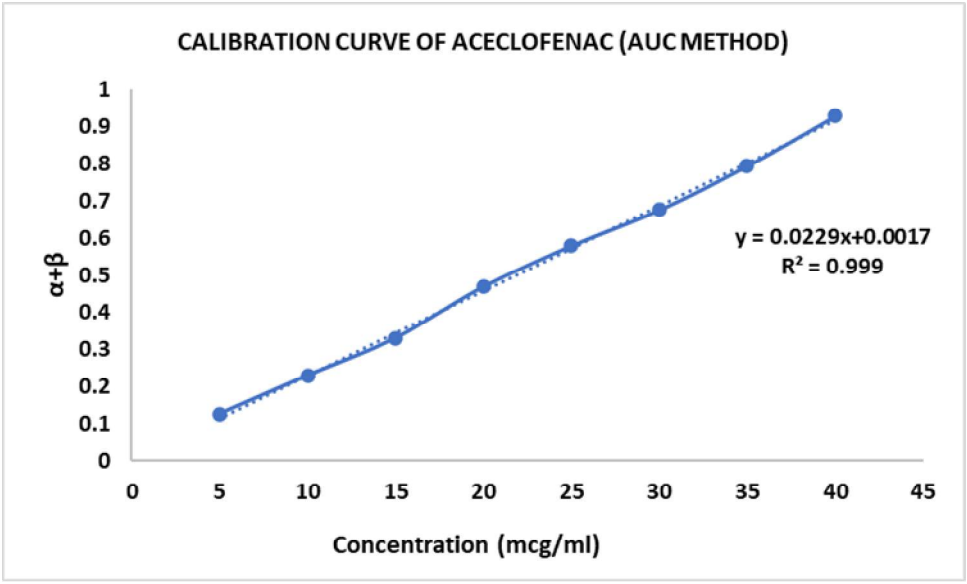

Fig 6: Calibration curve of Aceclofenac by AUC method

VALIDATION OF METHODS

LINEARITY

Notably, all methods employed in this study adhered to Beer's Lambert Law, which describes the relationship between the absorbance of light and the concentration of a substance. The proposed UV spectroscopic method's linearity was evaluated by plotting absorbance versus analyte concentration. The Beers law obeyed the method at the conc. range of 5- 40µg/ml. "The correlation coefficient values were found to be" 0.9990, 0.9990, and 0.9990 respectivel

Table 6: Linearity studies of Aceclofenac by proposed methods

S. No.	Parameter	Method A "(Zero order)"	Method B "(First order)"	Method C"(AUC method)"
1.	Linearity(µg/ml	5-40 µg/ml	5-40 µg/ml	5-40 µg/ml
2.	Slope	0.022	0.022	0.022
3.	Intercept	0.005	0.001	0.001
4.	Correlation Coefficient	0.9980	0.9990	0.9990

PRECISION STUDIES

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values found to be less than 2 that indicate this method precise

Table 7: Result of precision studies

S. No.	Parameters	Method A		Method B		Method C	
		SD	%RSD	SD	%RSD	SD	%RSD
1.	Precision	0.015355	1.48069	0.012237	1.17675	0.015631	1.50490
2.	Intraday precision	0.018660	1.80501	0.017509	1.69297	0.017616	1.70437
3.	Interday precision	0.016006	1.55279	0.016251	1.56538	0.012867	1.24236
4.	Robustness	0.013964	1.36061	0.014414	1.38663	0.015460	1.49118
5.	Ruggedness	0.015137	1.46084	0.014301	1.38207	0.015172	1.46090

ACCURACY

The solutions were reanalyzed by the proposed method; results of recovery studies are reported in table 8 which showed that the % amount found was between 99.30 - 99.91 for method 1 , 100.25 -100.70 method B and 99.66-100.30 for method C with % RSD > 2.

Table 8: Recovery studies of Aceclofenac by the proposed method

S. No.	Conc. (µg/ml)	Spikedlevel (%)	Added amount(mg)	Amount found (mg)			% Recovery		
				A	B	C	A	B	C
1.	10	80	8	17.97	18.02	17.99	99.62	100.25	99.87
2.	10	100	10	19.93	20.07	20.03	99.30	100.70	100.30
3.	10	120	12	21.99	22.03	21.96	99.91	100.25	99.66

LOD and LOQ

LOD and LOQ were calculated as 6.879 µg/ml and 20303µg/ml for method A ,5.562µg/ml and 1.835µg/ml for method B ,7.105 and 2.344 µg/ml for method C

Table 9: LOD & LOQ Values

S .No.	Method	LOD value	LOQ value
1.	A	6.979	2.303
2.	B	5.562	1.835
3.	C	7.105	2.344

SUMMARY

Table 10: Summary of method validation

S.No.	Parameters	Method A	Method B	Method C
1.	Absorption maxima (λmax)	274.6nm	259nm	269-279nm
2.	Beers law limit	5-40 µg/ml	5-40 µg/ml	5-40 µg/ml
3.	Regression equation	y=0.022x+0.005	y=0.022x+0.001	y=0.022x+0.001
4.	Slope	0.022	0.022	0.022
5.	Intercept	0.005	0.001	0.001
6.	Linearity indicated bycorrelation coefficient	0.998	0.999	0.999
7.	Accuracy indicated by % Recovery	99.30-99.91	100.25-100.70	99.87-100.30
8.	Precision(%RSD)	1.48069	1.17675	1.50490
9.	Intraday precision(%RSD)	1.80501	1.69297	1.70437
10.	Interday precision(%RSD)	1.55279	1.56538	1.24236
11.	Robustness	1.36061	1.38663	1.49118
12.	Ruggedness	1.46084	1.38207	1.46090
13.	LOD	6.979	5.562	7.105
14.	LOQ	2.303	1.835	2.344

DISCUSSION

The observed melting point of the product was 31.5°C, consistent with the literature value for pure aceclofenac. The titration procedure revealed that the purity of aceclofenac was 100.03%, confirming the high quality of the synthesized compound. The assay of aceclofenac using the ordinary method showed a

high degree of accuracy, with a result of 99.72% at a wavelength of 275 nm. It was found that R^2, or the correlation coefficient, was 0.9980. The modified method was also found to be highly accurate, with correlation coefficients of 0.9988, 0.999, and 0.999 for methods A, B, and C, respectively, at wavelengths of 274.6, 259, and 269-279 nm, respectively, across a concentration range of 5-40 µg/ml. Notably, all methods employed in this study adhered to Beer's Lambert Law, which describes the relationship between the absorbance of light and the concentration of a substance. The proposed UV spectroscopic method's linearity was evaluated by plotting absorbance versus analyte concentration. The Beers law obeyed the method at the conc. range of 5- 40µg/ml. "The correlation coefficient values were found to be" 0.9990, 0.9990, and 0.9990 respectively.

CONCLUSION

The present study successfully demonstrated convenient, accurate, and cost-effective methods for synthesizing, analyzing, and validating aceclofenac. Notably, all methods employed in this study adhered to Beer's Lambert Law, which describes the relationship between the absorbance of light and the concentration of a substance. Two methods were used to estimate aceclofenac using UV spectroscopy: the ordinary method and the modified method. Both methods demonstrated high specificity and precision, providing reliable results. To ensure the accuracy of the results, various parameters were checked during the validation of aceclofenac. The results obtained were compiled in a tabular form to facilitate easy interpretation and rectification. Overall, this study demonstrates the effectiveness of the proposed methods for the synthesis, analysis, and validation of aceclofenac, and highlights their potential for application in pharmaceutical and biomedical research.

References

1. Parvati B. Patel and Tejas K. Patel (2017). Efficacy and safety of aceclofenac in osteoarthritis: A meta-analysis of randomized controlled trials, European Journal of Rheumatology, 4(1): 11–18.
2. A Bose, PP Dash, and MK Sahoo (2010). Simple spectrophotometric methods for estimation of aceclofenac from bulk and formulations, Pharmaceutical Methods, 1(1), 57–60.
3. D. Nikam, Sampada S. Pawar, and S. V. Gandhi (2008). Estimation of Paracetamol and Aceclofenac in Tablet Formulation by Ratio Spectra Derivative Spectroscopy, Indian Journal of Pharmaceutical Sciences, 70(5), 635–637.
4. P. R. Mahaparale, J. N. Sangshetti and B. S. Kuchekar (2007). Simultaneous spectrophotometric estimation of aceclofenac and paracetamol in tablet dosage form, Indian Journal of Pharmaceutical Sciences, 69 (2), 289- 292.
5. K. S. Kokilambigai and K. S. Lakshmi (2021). Utilization of green analytical chemistry principles for the simultaneous estimation of paracetamol, aceclofenac and thiocolchicoside by UV spectrophotometry, Green Chemistry Letters and Reviews, 14(1),99-107.
6. Rathinam S, Karunanidhi Santhana L (2021). Ecofriendly Simple UV Spectrophotometric and Chemometric Methods for Simultaneous Estimation of ParacetamolAceclofenac and Eperisone Hydrochloride in Pharmaceutical Formulation: Assessment of Greenness Profile. Processes, 9(8), 1272.
7. M.V. Bhure, A.T. Hemke and K.R. Gupta (2010). UV-Spectrophotometric Methods for Determination of Aceclofenac and Diacerein in Pharmaceutical Formulation, Journal of pharmaceutical sciences and research, Journal of Pharmaceutical Sciences & Research, (7),426-432.
8. Deepali Gharge, Chandrakant Raut, Pandurang Dhabale (2010). Simultaneous Estimation of Aceclofenac and Paracetamol in Solid Dosage Form by UV Spectrophotometry. Research J. Pharm. and Tech, 3(1), 247-250.
9. S.M. Ashraful Islam, Sharif Md. Abuzar and Pijush Kumar Paul (2011). Validation of UV-Spectrophotometric and RP-HPLC methods forthe simultaneous analysis of Paracetamol and Aceclofenac in marketed tablets, International journal of pharmacy & life sciences, 2(12), 1267-1275.
10. Shailendra Suryawanshi Sanjay, Zaranappa, Chaluvaraju K C, Veena M K, Rajani S (2016). Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Aceclofenac and Pantoprazole in Bulk and Tablet Dosage Forms Using Hydrotropic Solvent, International journal of pharmacy and pharmaceutical research, 6 (3), 331- 344.
11. Ram BabuDurgam, Sireesha. D, V. V. L. N Prasad, Prakash V Diwan (2013). Analytical method development and validation of Drotaverine Hydrochloride and Aceclofenac in bulk and pharmaceutical dosage forms by UV-Spectrophotometer, International Journal of Drug Development & Reserch, 5(4), 268-272.
12. Suchithra TJ and Gurupadayya BM (2018). Simultaneous Estimation of Aceclofenac and Pregabalin in Combined Dosage Form by Solubility Based Separation Method, Acta Scientific Pharmaceutical Sciences,2(12),20-26.
13. S. D. Jadhav, V.S. Madankar, P. S. Chaudhari (2018). Analytical method development and validation of Aceclofenac in Bulk and Marketed Formulation, Journal of Emerging Technologies and Innovative Research, (5),428-437.
14. H. Basnett, A. Singha, D. Roy, B. Shrestha, A. Pradhan (2019). Estimation of paracetamol and aceclofenac in tablets by a novel ratio difference method, Life Science Informatics Publications, 5(1), 187-194.

15. S. D. Harugade, M. A. Nagras (2013). Development and validation of stability indicating RP HPLC assay method for simultaneous estimation of rabeprazole sodium and aceclofenac in capsule dosage form, Asian Journal of Pharmaceutical Research and Development, 1(4), 98-107.