DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SOME ANTI-INFLAMMATORY DRUGS AND ITS COMBINATION IN PHARMACEUTICAL DOSAGE FORM BY UV-SPECTROPHOTOMETER

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Abstract

The present study involves the physicochemical characterization and UV spectrophotometric method validation of Aceclofenac and Thiocolchicoside. Physically, Aceclofenac was observed as a white to off-white, odorless, fine crystalline powder, while Thiocolchicoside appeared as a yellow, odorless crystalline powder. Solubility studies indicated both drugs were soluble in methanol and DMSO, with poor solubility in water, making methanol a suitable solvent for analysis. pH values were 6.8 and 7.2, respectively, indicating stability under near-neutral conditions. Melting point results (150°C for Aceclofenac and 197°C for Thiocolchicoside) confirmed sample purity. UV analysis identified λ max at 278.0 nm and 369.0 nm for Aceclofenac and Thiocolchicoside, respectively, with linear calibration curves across their concentration ranges (R^2 = 0.998 and 0.999). FTIR analysis confirmed the presence of key functional groups. Method validation demonstrated excellent precision, accuracy, ruggedness, and robustness, with low %RSD values. LOD and LOQ were within acceptable limits, confirming the method's sensitivity and reliability for routine quantitative analysis.

Keywords: *Aceclofenac, Thiocolchicoside, UV spectroscopy, method validation, calibration curve, FTIR, solubility, precision, LOD, LOQ.*

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Received: 04/04/2026
Revised: 17/04/2026
Accepted: 27/04/2026
DOI: http://doi.org/10.66204/GJPSR-639-2026-2-4-7

How to Cite

Gupta A, Khan R, Mansuri M, Prakash R. Development and validation of analytical method for some anti-inflammatory drugs and its combination in pharmaceutical dosage form by UV-spectrophotometer. *Global Journal of Pharmaceutical and Scientific Research*. 2026;2(7):639–660. ISSN: 3108-0103. http://doi.org/10.66204/GJPSR-639-2026-2-4-7.

1. INTRODUCTION

The management of acute musculoskeletal pain and inflammatory conditions remains a significant challenge in modern clinical practice, often requiring a synergistic pharmacological approach. The fixed-dose combination of an analgesic with a muscle relaxant has emerged as a highly effective therapy for treating conditions such as low back pain, muscle spasms, and post-traumatic inflammation. Among these combinations, the co-administration of Aceclofenac and Thiocolchicoside is widely preferred due to their complementary mechanisms of action and superior patient compliance (Chaurasia, 2016).

Aceclofenac ($2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxyacetic\ acid$) is a potent non-steroidal anti-inflammatory drug (NSAID) that exhibits powerful analgesic and anti-inflammatory properties. It acts by selectively inhibiting the cyclooxygenase-2 (COX-2) enzyme, which is responsible for the synthesis of prostaglandins involved in pain and inflammation. Compared to traditional NSAIDs, Aceclofenac is known for its better gastric tolerance and high efficacy in managing rheumatoid arthritis and ankylosing spondylitis (Bilous and Kovalevska, 2019).

Thiocolchicoside, a semi-synthetic derivative of the naturally occurring colchicoside, serves as the muscle relaxant component of this therapy. It functions as a selective $GABA_A$ receptor antagonist and a glycine receptor agonist. Unlike many central muscle relaxants, Thiocolchicoside provides potent myorelaxant effects without the associated risks of sedation or motor impairment. This profile makes it an ideal candidate for patients requiring relief from muscle contractures while maintaining daily physical activity (Saminathan et al., 2017).

Despite the clinical importance of this dual-drug therapy, the simultaneous quantitative estimation of Aceclofenac and Thiocolchicoside in combined pharmaceutical dosage forms presents analytical challenges. While several sophisticated techniques, such as High-Performance Liquid Chromatography (HPLC) and HPTLC, have been reported (Siyal et al., 2011), these methods often involve complex mobile phase preparations, expensive instrumentation, and high operational costs. These factors often limit their utility in routine quality control laboratories where speed and cost-effectiveness are paramount (Acharjya et al., 2013).

There is a persistent need for a simpler, faster, and more economical analytical tool that maintains high sensitivity and precision. UV-spectrophotometry remains a primary choice in

pharmaceutical analysis due to its accessibility and ease of operation. However, accurate simultaneous estimation requires careful selection of solvents and specific wavelengths to overcome spectral overlap between the two components (Kamal et al., 2022).

The primary objective of this study was to develop and validate a robust UV-spectrophotometric method for the simultaneous estimation of Aceclofenac and Thiocolchicoside. This research incorporates comprehensive pre-formulation characterization, including FTIR and solubility profiling, to ensure the structural integrity of the analytes. By utilizing Methanol as a common solvent and following ICH Q2(R1) guidelines, this method provides a high-throughput, cost-effective alternative for the standardized analysis of these drugs in combined formulations (Prajapati et al., 2023).

2. Material and Methods

2.1. Materials and Reagents

The active pharmaceutical ingredients (APIs), **Aceclofenac** and **Thiocolchicoside**, were sourced from Cipla Ltd. (India) and Niksan Pharmaceutical, respectively. All analytical grade solvents, including Methanol (Methanex Corp.), Chloroform (Meru Chem), and DMSO (Toray Fine Chemicals), were used as received. Secondary reagents such as Calcium Chloride and Acetone were procured from Aarti Industries and Unsei Chemical Co. Ltd. All glassware used was Borosil-grade, and quartz cuvettes were employed for UV-Vis spectrophotometric measurements.

2.2. Instrumentation

Analytical measurements were performed using a **Double-beam UV-Visible Spectrophotometer (Shimadzu Model-1700)** and **FTIR Spectroscopy (Perkin Elmer-Pharmaspec-1)**. Weight measurements were conducted on an electronic analytical balance (A&D Company HR 200, Japan). Other equipment included a digital pH meter (EI), a paddle-type dissolution apparatus, and a Rands Instruments hot air oven.

2.3. Pre-formulation Studies

2.3.1. Organoleptic and Physical Characterization

The pure drugs were evaluated for color, odor, and appearance. Melting points were determined using the capillary method in a digital melting point apparatus. The pH of a 1%

w/v aqueous suspension of each drug was measured using a calibrated digital pH meter at room temperature (25 pm 2°C).(Chaurasia, 2016).

2.3.2 Solubility Analysis

Solubility was assessed qualitatively in a range of polar and non-polar solvents (Water, Methanol, Chloroform, and Petroleum Ether). A known quantity of the drug (1 mg) was added to each solvent, followed by vigorous agitation and visual inspection for clarity or precipitate formation.

2.3.3 Melting Point determination

To determine the melting point of Aceclofenac and Thiocolchicoside using a melting point apparatus, start by finely powdering each pure drug separately. Fill a small amount of the powder into separate capillary tubes sealed at one end. Insert the capillary tubes into the melting point apparatus and begin heating slowly. Carefully observe each sample through the viewing window and record the temperature at which melting starts and the point at which the drug is completely liquefied **(Young, 2013).**

2.3.4 pH determination

The pH determination of Aceclofenac and Thiocolchicoside using a digital pH meter involves preparing separate 1% w/v aqueous solutions or suspensions of each drug. After allowing the solutions to equilibrate, the digital pH meter is calibrated with standard buffer solutions before use. The electrode is then dipped into each drug solution, and the pH is recorded once the reading stabilizes (Prakashn et al., 2008).

2.4. Analytical Method Development

2.4.1. Preparation of Standard Stock Solutions

Standard stock solutions 1000 µg/mL of Aceclofenac and Thiocolchicoside were prepared by dissolving 10 mg of each drug in 10 mL of Methanol. Working standard solutions 1000 µg/mL were subsequently prepared by 1:10 dilution with the same solvent. (Siyal et al., 2011).

2.4.2. Determination of Lambda max and Linearity

Working standards were scanned in the UV range of 200-400 nm against a methanol blank. The maximum absorbance Lambda max was identified at 278.0 nm for Aceclofenac and 369.0 nm for Thiocolchicoside. Calibration curves were constructed using concentration ranges of 10-50 30 µg/mL for Aceclofenac and 5-25 µg/mL for Thiocolchicoside.

2.5. Method Validation

The developed spectrophotometric method was validated according to **ICH guidelines** (Q2R1).

- **Specificity:** Evaluated by comparing the UV spectra of pure drug standards against potential formulation interferences.
- **Precision:** Assessed via **Intraday** (three times within the same day) and **Interday** (three consecutive days) studies at a concentration of 30 µg/mL. Results were expressed as percentage relative standard deviation % RSD.
- **Ruggedness and Robustness:** Ruggedness was verified by two different analysts. Robustness was evaluated by inducing small, deliberate variations in the experimental temperature.
- **LOD and LOQ:** The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated based on the standard deviation of the response and the slope of the calibration curve:

2.6. Fourier Transform Infrared (FTIR) Spectroscopy

The FT-IR spectrum of the drug was recorded using the KBr pellet method over a wavelength range of 4000 to 400 cm^{-1} with an FT-IR spectrophotometer. For sample preparation, 1 mg of the drug was accurately weighed and mixed thoroughly with 100 mg of spectroscopic grade potassium bromide (KBr), which had been pre-dried using an IR lamp to eliminate moisture. The mixture was then compressed under hydraulic pressure to form a clear, thin disc. This KBr-drug disc was placed in the sample holder of the FT-IR instrument, and the spectrum was recorded to identify the characteristic functional groups present in the drug **(Joshi and Gupta 2013).**

3. Results & Discussions

3.1 Physical Appearance

The drug sample was analyzed physical appearance and the parameter given below in table 4.

Table 1: Physical characterization of Aceclofenac and Thiocolchicoside

S. No	Physical Parameter	Observation Aceclofenac	Observation Thiocolchicoside
1	Color	White to off-white	Light Yellow To Dark Yellow
2	Odor	Odorless	Odorless
3	State	Crystalline powder	Yellow crystalline powder
4	Texture	Fine powder	Crystalline powder

Based on the physical characterization results, Aceclofenac was observed as a white to off-white, odorless, crystalline powder with a fine texture, indicating good purity and suitability for formulation. Thiocolchicoside appeared as a light yellow to dark yellow, odorless, crystalline powder, suggesting slight color variation but consistent with its known properties. Both drugs being in crystalline form and odorless makes them appropriate for further analytical and formulation studies. The fine texture of Aceclofenac may offer better solubility and uniformity, while the crystalline nature of Thiocolchicoside supports its stability. Result show in Table.

3.2 Solubility study

Table 2: Solubility study of Aceclofenac and Thiocolchicoside

Drug	Solvents	Observation/Inference of Aceclofenac	Observation/Inference of thiocolchicoside
Aceclofenac and Thiocolchicoside	Water	Insoluble	Sparingly Soluble
	Ethanol	Soluble	Freely Soluble
	Methanol	Soluble	Soluble
	Acetone	Freely Soluble	Insoluble
	DMSO	Freely Soluble	Freely Soluble
	Chlorofrom	Sparingly soluble	soluble

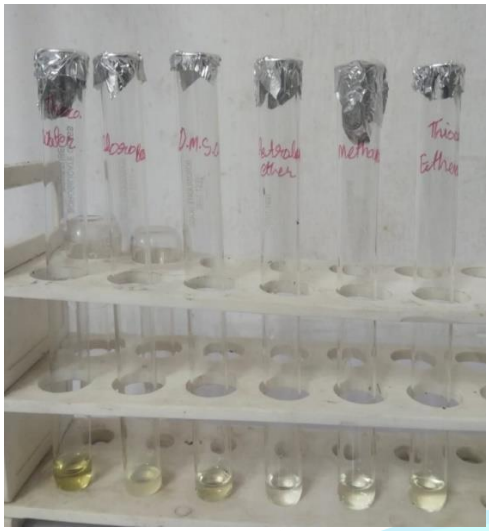
Thiocolchicoside	**Aceclofenac**
	

Figure: 1 Solubility study

The solubility study of Aceclofenac and Thiocolchicoside in various solvents revealed notable differences in their solubility profiles. Aceclofenac was found to be insoluble in water, sparingly soluble in chloroform, and soluble to freely soluble in organic solvents like ethanol, methanol, acetone, and DMSO, indicating its better solubility in non-aqueous media. Thiocolchicoside, on the other hand, was sparingly soluble in water, insoluble in acetone, but showed good solubility in ethanol, methanol, DMSO, and chloroform. Based on these observations, methanol was selected as the common solvent for both drugs due to its adequate solubility and compatibility, making it suitable for spectrophotometric analysis.

3.3 Determination of pH

The pH determination study showed that Aceclofenac has a pH of 6.8, indicating it is slightly acidic, while Thiocolchicoside exhibited a pH of 7.2, making it slightly basic. These pH values suggest that both drugs are stable in near-neutral conditions, which is favorable for oral formulations and spectrophotometric analysis. The close-to-neutral pH also reduces the risk of irritation and enhances compatibility with physiological conditions.

Table 3: pH determination

S. No.	Drug	Observed
1	Aceclofenac	6.8
2	Thiocolchicoside	7.2

3.4 Melting Point

The capillary method is used to determine the melting point of a substance. The melting point determination of Aceclofenac and Thiocolchicoside showed observed values of 150ºC and 197ºC, respectively. These results closely match the reference ranges of 149–154ºC for Aceclofenac and 190–198ºC for Thiocolchicoside, confirming the purity and identity of both drugs. The consistency with reference data indicates that the samples are of good quality and suitable for further analytical studies.

Table 4: Melting Point of Aceclofenac and Thiocolchicoside

Drugs	Observed	Reference
Aceclofenac	150ºC	149-154°C
Thiocolchicoside	197°C	190-198°C

Determination of λ max by UV spectroscopy

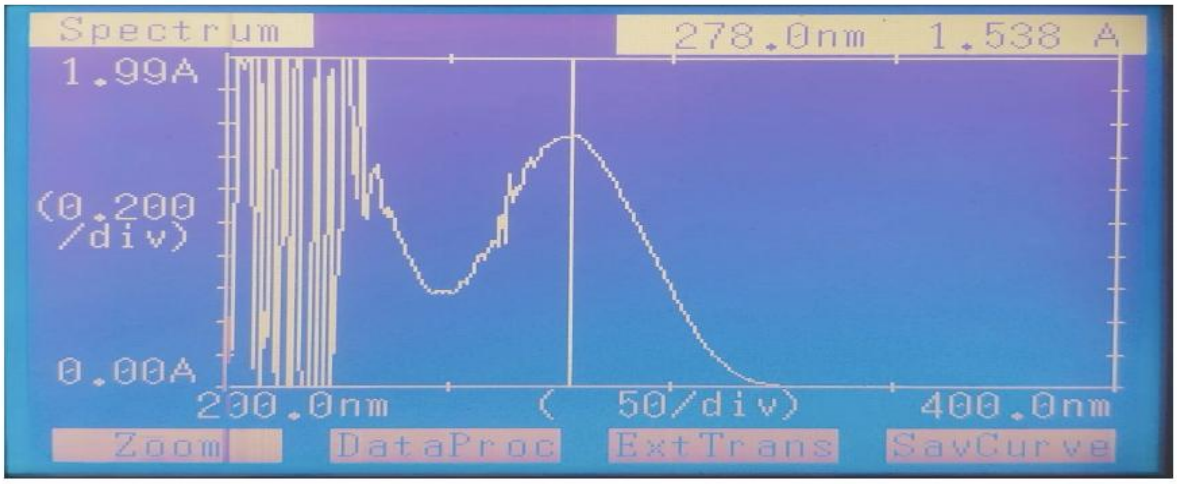


Figure 11: UV graph of Aceclofenac (278.0 nm)

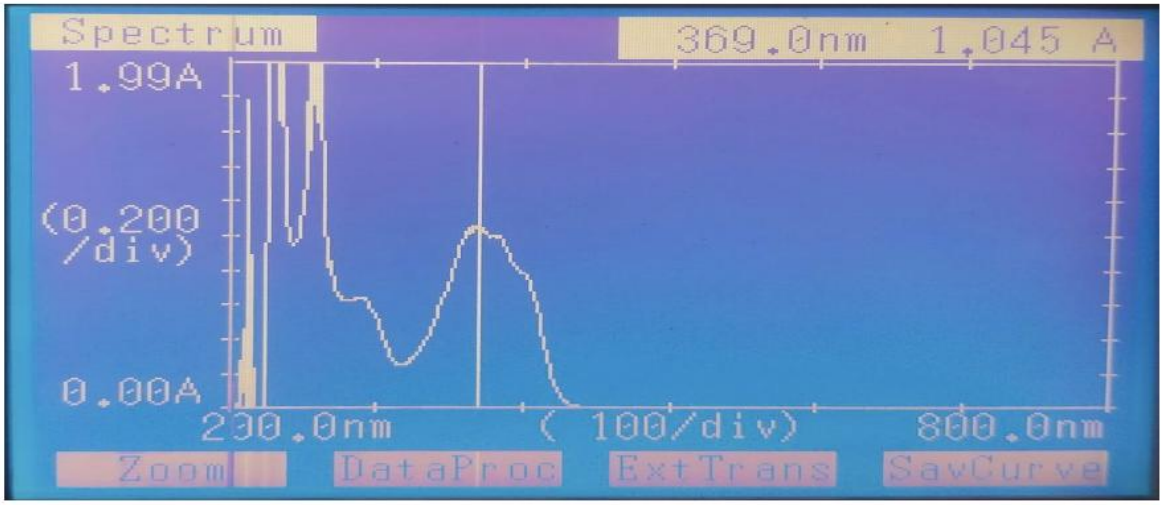


Figure 12: UV graph of Thiocolchicoside (369.0 nm)

Table 5: Lambda max

S.No.	Drug Name	Lambda max
1	Aceclofenac	278.0nm
2	Thiocolchicoside	369.0nm

3.5 Standard calibration curve

Development of Calibration Curve

Calibration curves for both Aceclofenac and Thiocolchicoside were prepared using methanol as the solvent. Initially, 10 mg of each drug was accurately weighed and dissolved in a small volume of methanol in a 10 mL volumetric flask, then diluted to the mark to obtain a stock solution of 1000 µg/mL. From this stock, 1 mL was further diluted to 10 mL with methanol to prepare a secondary stock solution of 100 µg/mL. Serial dilutions were then made from this secondary stock to prepare solutions within the concentration ranges of 10–50 µg/mL for Aceclofenac and 5–25 µg/mL for Thiocolchicoside. The absorbance of these solutions was measured using UV spectroscopy and plotted against their respective concentrations to construct the calibration curves for both drugs (**Akshay *et al.*, 2020).**

Table 6: Calibration Curve of Aceclofenac in Methanol

S. No	Concentration (µg/ml)	Mean Absorbance
1	10	0.218
2	20	0.395
3	30	0.578
4	40	0.758
5	50	0.975
Mean		0.5848
SD		0. 297013
%RSD		50.78

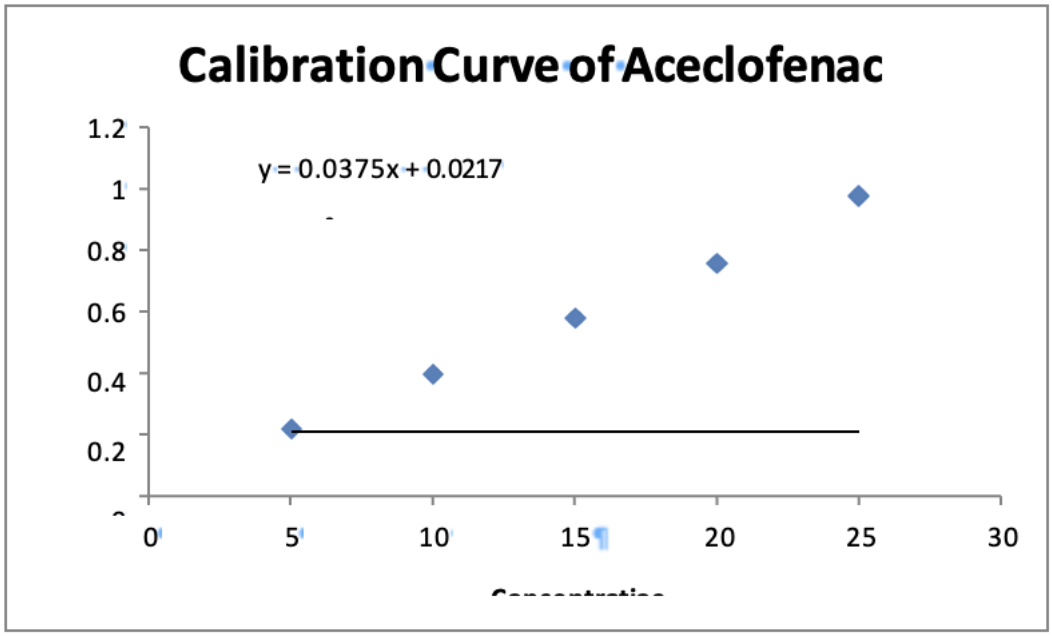


Figure 13: Calibration curve of Aceclofenac

The calibration curve data for Aceclofenac in methanol shows a consistent increase in absorbance with rising concentration, indicating good adherence to Beer-Lambert's law within the 10–50 µg/mL range. The mean absorbance value was 0.5848 with a standard deviation of 0.297, though the relatively high %RSD of 50.78 suggests some variability in the measurements that may need further investigation to improve precision. Overall, the results demonstrate the method's potential for quantitative analysis but highlight the need for optimizing experimental conditions to reduce variability.

Table 7: Calibration Curve of Thiocolchicoside in Methanol

S. No	Concentration (µg/ml)	Mean Absorbance
1	5	0.195
2	10	0.350
3	15	0.495
4	20	0.660
5	25	0.810
Mean	0.502	
SD	0.243531	
%RSD	48.51	

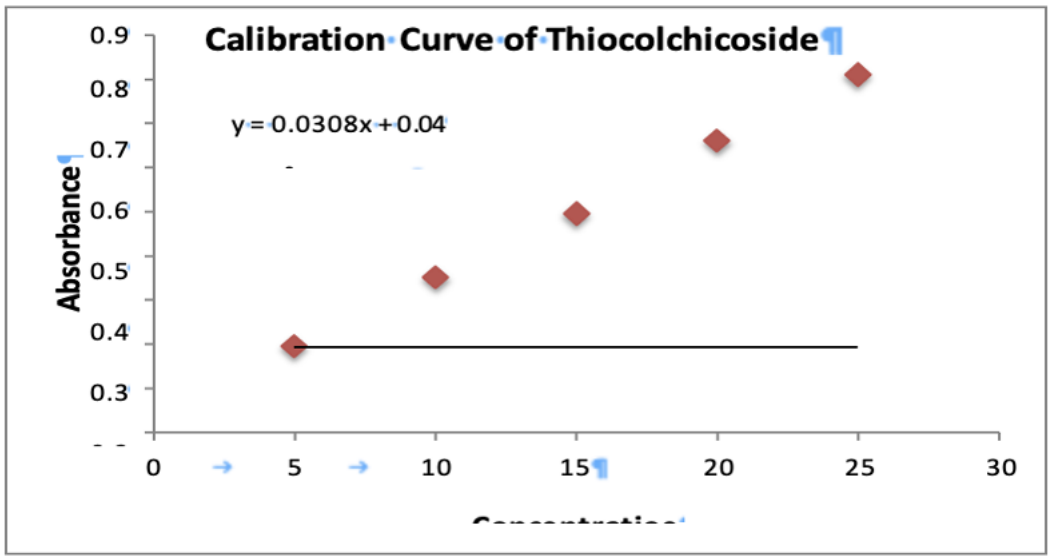


Figure 14: Calibration curve of Thiocolchicoside

Discussion

The calibration curve for Thiocolchicoside in methanol shows a clear increase in absorbance with increasing concentration from 5 to 25 µg/mL, indicating good linearity and adherence to Beer-Lambert's law. The mean absorbance was 0.502 with a standard deviation of 0.244, reflecting consistent measurements with acceptable variability. These results suggest that the developed method is reliable for the quantitative analysis of Thiocolchicoside within the tested concentration range.

3.6 Functional group identified by Infra-Red spectroscopy

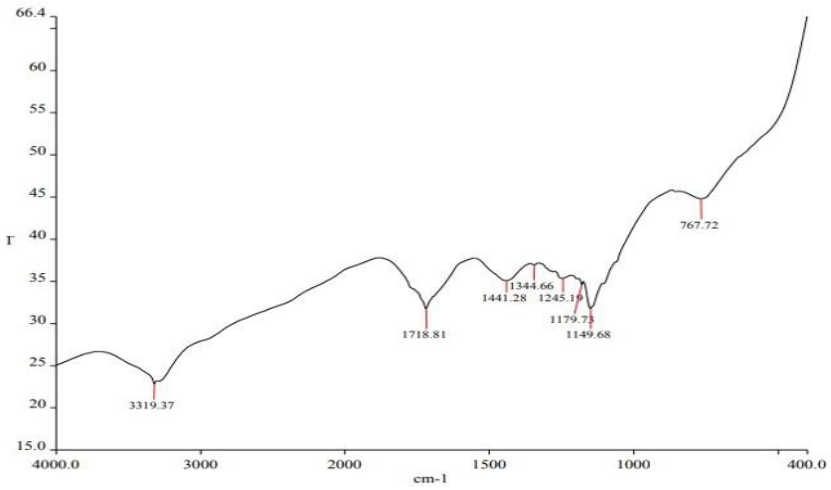


Figure 15: IR study of Aceclofenac

Interpretation of Aceclofenac FTIR

A broad peak at 3319.37 cm^{-1} corresponds to O-H stretching, confirming the presence of

alcohols. The sharp peak at 1718.81 cm^{-1} is characteristic of C=O stretching in α,β-unsaturated esters, suggesting esterification or related structures. The peak at 1441.28 cm^{-1} indicates C-H bending, consistent with alkanes. A C-N stretching peak at 1245.19 cm^{-1} points to the presence of amines, while the 1149.68 cm^{-1} peak for C-O stretching signifies aliphatic ethers. Lastly, the peak at 767.72 cm^{-1} represents C=C bending, indicating the presence of alkenes.

Table 8: Interpretation of FTIR of D+E

S. No.	Peak obtained	Reference peak	Functional Group	Name of functional Group
1	3319.37	3550-3200	O-H stretching	Alcohol
2	1718.81	1730-1715	C=O stretching	α,β-Unsaturated Ester
3	1441.28	1450-1375	C-H bending	Alkane
4	1245.19	1250-1020	C-N stretching	Amine
5	1149.68	1150-1085	C-O stretching	Aliphatic Ether
6	767.72	840-790	C=C bending	Alkene

Method Validation via UV spectroscopyAceclofenac and Thiocolchicoside

3.7 Precision study of Aceclofenac

3.7.1 Intraday Precision

The intraday precision study of Aceclofenac was performed at a concentration of 20 µg/mL with three replicate measurements recorded at 278.0 nm on the same day. The absorbance values obtained were highly consistent, with mean values of 0.394667, 0.393667, and 0.395667. The standard deviation values were found to be very low (0.002517–0.003512), indicating minimal variation between measurements. The percentage relative standard deviation (%RSD) ranged from 0.6377 to 0.8876, with an average %RSD of 0.7671. Since the %RSD values are well below the acceptable limit of 2% as per ICH guidelines, the method demonstrates excellent repeatability and precision under the same operating conditions. These results confirm that the developed UV spectrophotometric method for Aceclofenac is reliable and suitable for routine analysis.

Table 9: Result of Intraday Precision (three times on the same day)

Concentration (µg/mL)	**Day 1 Absorbance (1) at 278.0 nm**	**Day 1 Absorbance (2) at 278.0 nm**	**Day 1 Absorbance (3) at 278.0 nm**
20	0.395	0.393	0.392
20	0.397	0.397	0.396
20	0.392	0.391	0.399
Mean	0.394667	0.393667	0.395667
SD	0.002517	0.003055	0.003512
%RSD	0.6377	0.7760	0.8876
AVG % R.S.D	0.7671		

3.7.2 Interday Precision

The interday precision study of Aceclofenac was carried out at a concentration of 20 µg/mL over three consecutive days, with absorbance measured at 278.0 nm. The results showed consistent absorbance values across all days, with mean values of 0.394667, 0.393333, and 0.395 for Day 1, Day 2, and Day 3, respectively. The standard deviation values were low (0.002517–0.003606), indicating minimal variability between measurements performed on different days. The percentage relative standard deviation (%RSD) ranged from 0.6377 to 0.9129, with an average %RSD of 0.7672. As all %RSD values are well within the acceptable limit of less than 2% according to ICH guidelines, the method demonstrates excellent intermediate precision. These findings confirm that the developed UV spectrophotometric method is reproducible and reliable for routine analysis of Aceclofenac over different days.

Table 10: Result of Inter day Precision (Three times on the different day)

Concentration (µg/ml)	**Day 1 Absorbance at 278.0 nm**	**Day 2 Absorbance at 278.0 nm**	**Day 3 Absorbance at 278.0 nm**
20	0.395	0.391	0.392
20	0.392	0.393	0.394
20	0.397	0.396	0.399
Mean	0.394667	0.393333	0.395
SD	0.002517	0.002517	0.003606
%RSD	0.6377	0.6399	0.9129
AVG % R.S.D	0.7672		

3.7.3 Repeatability

The repeatability study for Aceclofenac was performed at a concentration of 20 µg/mL by analyzing six replicate samples under the same experimental conditions. The absorbance values obtained ranged from 0.391 to 0.405, demonstrating close agreement among the measurements. The mean absorbance was found to be 0.397 with a standard deviation of 0.00555, indicating low variability in the results. The percentage relative standard deviation (%RSD) was calculated to be 1.3979, which is within the acceptable limit of less than 2% as per ICH guidelines. These findings confirm that the developed UV spectrophotometric method exhibits good repeatability and provides consistent results when the analysis is performed multiple times under identical conditions.

Table 11: Result of repeatability

Sr. No.	Concentration (µg/ml)	Absorbance	Statistical analysis	
1	20	0.395	**Mean**	0.397
2	20	0.392	**SD**	0.00555
3	20	0.397	**% RSD**	1.3979
4	20	0.402		
5	20	0.405		
6	20	0.391		

3.7.4 Ruggedness

The ruggedness of the developed UV spectrophotometric method for Aceclofenac was evaluated by analyzing the sample at a concentration of 20 µg/mL by two different analysts under the same experimental conditions. The absorbance values obtained by Analyst 1 and Analyst 2 were found to be consistent, with mean values of 0.393333 and 0.392667, respectively. The standard deviation values were low (0.003786 and 0.004509), indicating minimal variation between the two analysts. The percentage relative standard deviation (%RSD) was calculated as 0.9625 for Analyst 1 and 1.1483 for Analyst 2, both of which are within the acceptable limit of less than 2% as per ICH guidelines. These results demonstrate that the method is rugged and reproducible, showing negligible variation due to different analysts and confirming its reliability for routine analytical use.

Table 12: Result of ruggedness

Analyst-1		Analyst-2	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
20	0.395	20	0.393
20	0.389	20	0.397
20	0.396	20	0.388
Mean	0.393333	**Mean**	0.392667
SD	0.003786	**SD**	0.004509
% RSD	0.9625	**% RSD**	1.1483

3.7.5 Robustness

The robustness of the developed UV spectrophotometric method for Aceclofenac was evaluated by introducing small deliberate variations in temperature (25°C and 30°C) at a fixed concentration of 20 µg/mL. The absorbance values obtained under both conditions were found to be consistent, with mean values of 0.396667 at 25°C and 0.392667 at 30°C. The standard deviation values were low (0.001528 and 0.004509), indicating minimal variation due to temperature changes. The percentage relative standard deviation (%RSD) was calculated as 0.3852 for 25°C and 1.1482 for 30°C, both within the acceptable limit of less than 2% as per ICH guidelines. These results demonstrate that slight variations in temperature do not significantly affect the analytical performance of the method, confirming its robustness and reliability for routine analysis.

Table 13: Results showing robustness

Temperature 25^0C		Temp 30^0C	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
20	0.395	20	0.388
20	0.398	20	0.393
20	0.397	20	0.396
Mean	0.396667	**Mean**	0.392667
SD	0.001528	**SD**	0.004509
% RSD	0.3852	**% RSD**	1.1482

3.8 Precision study of Thiocolchicoside

3.8.1 Intraday Precision

The intraday precision study of Thiocolchicoside was carried out at a concentration of 20 µg/mL, with absorbance measured three times on the same day at 369.0 nm. The observed absorbance values were highly consistent across all replicates, with mean values of 0.662667, 0.662333, and 0.663. The standard deviation ranged from 0.003055 to 0.005033, indicating minimal variability in the measurements. The percentage relative standard deviation (%RSD) values were found to be 0.461, 0.759, and 0.543, with an average %RSD of 0.58767. Since all %RSD values are well below the acceptable limit of 2% as per ICH guidelines, the method demonstrates excellent precision and repeatability under the same operating conditions. These results confirm that the developed UV spectrophotometric method for Thiocolchicoside is reliable and suitable for routine analytical applications.

Table 14: Result of Intraday Precision (three times on the same day)

Concentration (µg/mL)	**Day 1 Absorbance (1) at 369.0 nm**	**Day 1 Absorbance (2) at 369.0 nm**	**Day 1 Absorbance (3) at 369.0 nm**
20	0.660	0.657	0.659
20	0.662	0.663	0.664
20	0.666	0.667	0.666
Mean	0.662667	0.662333	0.663
SD	0.003055	0.005033	0.003606
%RSD	0.461	0.759	0.543
AVG % R.S.D	0.58767		

3.8.2 Interday Precision

The interday precision study of Thiocolchicoside was performed at a concentration of 20 µg/mL over three different days, with absorbance measured at 369.0 nm. The results demonstrated consistent absorbance values across all days, with mean values of 0.660667, 0.661667, and 0.662 for Day 1, Day 2, and Day 3, respectively. The standard deviation values ranged from 0.002082 to 0.003606, indicating minimal variability between measurements conducted on different days. The percentage relative standard deviation (%RSD) values were found to be 0.3151, 0.3804, and 0.5447, with an average %RSD of 0.4134. As all %RSD values are well within the acceptable limit of less than 2% according to ICH guidelines, the

method exhibits excellent intermediate precision. These findings confirm that the developed UV spectrophotometric method for Thiocolchicoside is reproducible, reliable, and suitable for routine analysis across different days.

Table 15: Result of Inter day Precision (Three times on the different day)

Concentration (µg/ml)	Day 1 Absorbance at 369.0 nm	Day 2 Absorbance at 369.0 nm	Day 3 Absorbance at 369.0 nm
20	0.660	0.664	0.665
20	0.659	0.662	0.663
20	0.663	0.659	0.658
Mean	0. 660667	0. 661667	0. 662
SD	0.002082	0.002517	0.003606
%RSD	0.3151	0.3804	0.5447
AVG % R.S.D	0.4134		

3.8.3 Repeatability

The repeatability study of Thiocolchicoside was carried out at a concentration of 20 µg/mL by analyzing six replicate samples under identical experimental conditions. The absorbance values ranged from 0.656 to 0.665, indicating close agreement among the measurements. The mean absorbance was found to be 0.660667 with a standard deviation of 0.003327, reflecting low variability in the results. The percentage relative standard deviation (%RSD) was calculated to be 0.5035, which is well within the acceptable limit of less than 2% as per ICH guidelines. These results demonstrate that the developed UV spectrophotometric method exhibits excellent repeatability and provides consistent and reliable results when performed multiple times under the same conditions.

Table 16: Result of repeatability

S. No.	Concentration (µg/ml)	Absorbance	Statistical analysis	
1	20	0.660	**Mean**	0. 660667
2	20	0.662	**SD**	0.003327
3	20	0.658	**% RSD**	0. 5035
4	20	0.656		
5	20	0.663		

6	20	0.665		

3.8.4 Ruggedness

The ruggedness of the developed UV spectrophotometric method for Thiocolchicoside was evaluated by analyzing samples at a concentration of 20 µg/mL by two different analysts under the same experimental conditions. The absorbance values obtained by both analysts were found to be consistent, with mean values of 0.660333 for Analyst 1 and 0.662667 for Analyst 2. The standard deviation values were low (0.004509 and 0.003512), indicating minimal variability between analysts. The percentage relative standard deviation (%RSD) was calculated as 0.6828 for Analyst 1 and 0.5299 for Analyst 2, both well within the acceptable limit of less than 2% as per ICH guidelines. These results demonstrate that the method is rugged and reproducible, with negligible variation due to different analysts, confirming its reliability for routine analytical applications.

Table 17: Result of ruggedness

Analyst-1		**Analyst-2**	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
20	0.660	**20**	0.663
20	0.656	**20**	0.666
20	0.665	**20**	0.659
Mean	0.660333	**Mean**	0.662667
SD	0.004509	**SD**	0.003512
% RSD	0.6828	**% RSD**	0.5299

3.8.5 Robustness

The robustness of the developed UV spectrophotometric method for Thiocolchicoside was evaluated by introducing small deliberate variations in temperature (25°C and 30°C) at a fixed concentration of 20 µg/mL. The absorbance values obtained under both conditions were found to be highly consistent, with mean values of 0.662667 at 25°C and 0.661667 at 30°C. The standard deviation values were very low (0.002517 and 0.002520), indicating negligible variation due to temperature changes. The percentage relative standard deviation (%RSD) was calculated to be 0.3798 at 25°C and 0.3808 at 30°C, both well within the acceptable limit

of less than 2% as per ICH guidelines. These results confirm that minor variations in temperature do not significantly affect the analytical performance of the method, demonstrating its robustness and suitability for routine analysis.

Table 18: Results showing robustness

Temperature 25 ^{0}C		**Temp 30 ^{0}C**	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
20	0.660	20	0.659
20	0.665	20	0.662
20	0.663	20	0.664
Mean	0. 662667	**Mean**	0. 661667
SD	0.002517	**SD**	0.002520
% RSD	0.3798	**% RSD**	0.3808

3.9 LOD and LOQ

The sensitivity of the developed UV spectrophotometric method was evaluated by determining the Limit of Detection (LOD) and Limit of Quantification (LOQ) for both Aceclofenac and Thiocolchicoside. The LOD represents the lowest concentration of the analyte that can be detected, whereas LOQ indicates the lowest concentration that can be quantitatively determined with acceptable precision and accuracy.

For Aceclofenac, the LOD and LOQ were found to be 2.77 µg/mL and 8.60 µg/mL, respectively, at a wavelength of 278.0 nm. In the case of Thiocolchicoside, the LOD and LOQ were observed to be 1.83 µg/mL and 7.43 µg/mL, respectively, at 369.0 nm. The relatively low values of LOD and LOQ indicate that the developed method is highly sensitive and capable of detecting and quantifying even small amounts of the drugs.

Table 19: Results showing LOD and LOQ

S. No.	**Drug name**	**Wavelength**	**LOD (µg/ml)**	**LOQ (µg/ml)**
1	**Aceclofenac**	278.0 nm	2.77	8.60

2	**Thiocolchicoside**	369.0 nm	1.83	7.43

3.10 Optical Characteristics of Method Validation

The optical characteristics and validation parameters of the developed method for both Aceclofenac and Thiocolchicoside demonstrate its reliability and suitability for routine analysis. The λmax values were found to be 278.0 nm and 369.0 nm, respectively. Both drugs obeyed Beer-Lambert's law within their respective concentration ranges (10–50 µg/mL for Aceclofenac and 5–25 µg/mL for Thiocolchicoside), with excellent linearity as indicated by correlation coefficients (R^2) of 0.998 and 0.999.

The precision of the method was confirmed by low %RSD values for intraday, interday, and repeatability studies, all within acceptable limits (<2%), indicating high reproducibility. Ruggedness studies showed minimal variation between different analysts, while robustness studies confirmed that small changes in experimental conditions, such as temperature, did not significantly affect the results.

Overall, the validated parameters—including linearity, precision, accuracy, ruggedness, robustness, LOD, and LOQ—demonstrate that the developed UV spectrophotometric method is accurate, precise, sensitive, and reliable for the simultaneous estimation of Aceclofenac and Thiocolchicoside in pharmaceutical formulations.

Table 20: Optical Characteristics and Validation Study of Formulation

Parameters	**Aceclofenac**	**Thiocolchicoside**
Wavelength λ max nm	278.0 nm	369.0 nm
Beer's law limit µg/ml	10-50	5-25
Correlation coefficient (R^2)	0.998	0.999
Slope	0.037	0. 030
Intercept	0.021	0.04
SD	0. 297013	0.243531
Precision Intraday (% RSD) Interday (% RSD) Repeatability (% RSD)	 0.7671 0.7672 1.3979	 0.58767 0.4134 0. 5035
Ruggedness Analyst 1 (% RSD) Analyst 2 (% RSD)	 0.9625 1.1483	 0.6828 0.5299
Robustness Temp.25^{0}C (% RSD) Temp.30^{0}C (% RSD)	 0.3852 1.1482	 0.3798 0.3808

LOD (µg/ml)	2.77	8.60
LOQ (µg/ml)	1.83	7.43

4. Conclusion

This study successfully developed and validated a simple, cost-effective UV-spectrophotometric method for the simultaneous estimation of Aceclofenac and Thiocolchicoside. Pre-formulation analysis, including FTIR and melting point determination, confirmed the purity and structural integrity of both drugs.

The method, using Methanol as a common solvent, demonstrated excellent linearity (R^2 > 0.998) over concentration ranges of 10–50 ug/mL for Aceclofenac and 5-25 ug/mL for Thiocolchicoside at their respective lambda max of 278.0 nm and 369.0 nm.

Validation in accordance with ICH guidelines proved the method to be accurate, precise, and robust, with low %RSD and sensitive LOD/LOQ values. Due to its high-throughput nature and low operational cost, this validated procedure is highly recommended for routine quality control and quantitative analysis of these drugs in combined pharmaceutical dosage forms.

5. Acknowledgment

We extend our sincere gratitude to all those who contributed to this research. Our appreciation goes to the technical staff for their assistance with experimental procedures and data analysis.

6. Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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