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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ALOIN AND THIOLCHICOSIDE BY UV-VISIBLE SPECTROPHOTOMETRY

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ABSTRACT

The present study aimed to develop and validate a simple, accurate, precise, and cost-effective UV-Visible spectrophotometric method for the quantitative estimation of Aloin and Thiocolchicoside, two pharmacologically active anti-inflammatory agents. Both drugs were analyzed individually using methanol as a solvent after determining their maximum absorption wavelengths (λ_{max}) at 295 nm for Aloin and 366 nm for Thiocolchicoside. For simultaneous estimation, a common analytical wavelength of 305 nm was selected. The developed method was validated in accordance with ICH Q2(R1) guidelines by evaluating parameters such as linearity, accuracy, precision, specificity, ruggedness, robustness, and sensitivity. Linearity was established within the concentration range of 10–60 $\mu\text{g/mL}$ for Aloin and 5–30 $\mu\text{g/mL}$ for Thiocolchicoside, with correlation coefficients (R^2) of 0.9999 and 0.9992, respectively, indicating excellent linear relationships. The %RSD values for intra-day, inter-day, and repeatability studies were all below 2%, confirming method precision. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 2.01 $\mu\text{g/mL}$ and 7.09 $\mu\text{g/mL}$ for Aloin, and 1.95 $\mu\text{g/mL}$ and 6.10 $\mu\text{g/mL}$ for Thiocolchicoside, respectively, indicating high method sensitivity. Recovery studies demonstrated accuracy within the acceptable range of 98–102%. The developed UV spectrophotometric method proved to be rapid, reliable, and economical, making it suitable for routine quality control analysis and quantitative determination of Aloin and Thiocolchicoside in bulk and pharmaceutical dosage forms.

KEYWORDS: Aloin; Thiocolchicoside; UV-Visible Spectrophotometry; Analytical Method Validation; ICH Q2(R1); Linearity; Precision; Sensitivity.

INTRODUCTION

Analytical method development is a fundamental process in pharmaceutical research that ensures accurate, precise, and reproducible measurement of drug substances in various matrices.^[1] The reliability of analytical results is crucial for quality control, formulation development, and regulatory compliance.^[2] Among different analytical tools, ultraviolet-visible (UV-Vis) spectrophotometry remains one of the most widely employed techniques in pharmaceutical analysis due to its simplicity, cost-effectiveness, sensitivity, and rapid performance without the need for complex instrumentation.^[3] It is especially useful for routine analysis where simultaneous or individual estimation of drug components is required.^[4] Non-steroidal anti-inflammatory drugs (NSAIDs) and muscle relaxants are extensively prescribed for the

management of pain and inflammatory disorders.^[5] Aloin, a naturally occurring anthraquinone glycoside isolated from Aloe species, exhibits notable anti-inflammatory, analgesic, and antioxidant properties.^[6] Thiocolchicoside, a semi-synthetic derivative of colchicoside, possesses potent anti-inflammatory and muscle relaxant activity through its affinity for GABA and glycine receptors. Both drugs are pharmacologically important and frequently used either alone or in combination for treating musculoskeletal and inflammatory conditions.^[7] However, despite their therapeutic relevance, literature reveals limited availability of simple, validated UV spectrophotometric methods for their simultaneous or individual quantification in pharmaceutical dosage forms.^[8] The development of validated analytical methods is essential

to ensure the quality, safety, and efficacy of drug formulations. In this context, the present study focuses on the development and validation of a simple, accurate, precise, and economical UV spectrophotometric method for the quantitative estimation of Aloin and Thiocolchicoside in bulk and pharmaceutical formulations.^[9] Method validation was performed according to ICH Q2(R1) guidelines by evaluating parameters such as linearity, accuracy, precision, specificity, robustness, ruggedness, and sensitivity (LOD and LOQ).^[10] The proposed method aims to provide an efficient analytical tool for routine quality control and formulation analysis in pharmaceutical industries, supporting regulatory and research requirements.

MATERIALS AND METHODS

Materials

Aloin was obtained from Hifenac, and Thiocolchicoside was procured from Sarv Biolabs Pvt. Ltd. Methanol (Methanex Corporation), ethanol (Khaitan India Ltd.), chloroform (Meru Chem Pvt. Ltd.), and dimethyl sulfoxide (Toray Fine Chemicals) were of analytical reagent grade and used without further purification. All glassware used was of Borosil make. The major instruments employed included a Shimadzu UV-Visible double-beam spectrophotometer (Model 1700) with matched quartz cuvettes, an electronic weighing balance (A&D HR 200, Japan), and a digital pH meter (EI, India).

Preformulation Studies

Organoleptic Evaluation

Colour, odour, and physical appearance of Aloin and Thiocolchicoside were examined visually to confirm their characteristic features in accordance with standard Pharmacopoeial monographs.^[11]

Solubility Study

Solubility of both drugs was determined in solvents of varying polarity, including water, methanol, ethanol, chloroform, and dimethyl sulfoxide (DMSO). Small quantities of the drugs were added to 1 mL of each solvent, shaken at room temperature, and visually inspected to assess solubility behaviour.^[12]

pH Determination

Solutions of each drug were prepared in methanol, and their pH was measured using a calibrated digital pH meter to evaluate ionization characteristics and formulation suitability.^[13]

Melting Point Determination

Melting points were determined by the capillary tube method using a digital melting point apparatus. The observed values were compared with reported literature values to confirm drug purity and thermal stability.^[14]

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra of both drugs were recorded using the KBr pellet method in the range of 4000–400 cm^{-1} on a

PerkinElmer FTIR spectrophotometer. The characteristic absorption bands were analyzed to identify functional groups and confirm structural integrity.^[15]

Determination of Maximum Absorption Wavelength (λ_{max})

Standard solutions of Aloin and Thiocolchicoside were prepared by dissolving accurately weighed quantities of each drug in methanol to obtain a concentration of 100 $\mu\text{g/mL}$. These solutions were scanned in the range of 200–400 nm using a UV-Vis spectrophotometer. Aloin exhibited maximum absorbance at 295 nm, while Thiocolchicoside showed maximum absorbance at 366 nm. For simultaneous estimation, a common wavelength of 305 nm was selected.^[16]

Preparation of Standard and Working Solutions

Stock solutions of each drug were prepared at a concentration of 1000 $\mu\text{g/mL}$ in methanol. From these, appropriate dilutions were made to obtain working standard solutions in the range of 10–60 $\mu\text{g/mL}$ for Aloin and 5–30 $\mu\text{g/mL}$ for Thiocolchicoside. Methanol was used as the blank for all absorbance measurements.^[17]

Construction of Calibration Curves (Linearity)

Aliquots of standard drug solutions were transferred into 5 mL volumetric flasks and diluted with methanol to the required concentrations within the specified ranges. The absorbance of each solution was measured at its respective λ_{max} , and calibration curves were constructed by plotting absorbance versus concentration. Linearity was assessed through regression analysis to verify compliance with Beer-Lambert's law.^[18]

Method Validation (According to ICH Q2(R1))

The developed UV spectrophotometric method was validated according to ICH Q2(R1) guidelines by evaluating the following parameters.

Linearity and Range

The linear relationship between concentration and absorbance was confirmed within the selected concentration ranges for both drugs using regression analysis.^[19]

Precision

Precision was assessed to determine the degree of repeatability and reproducibility of the developed UV spectrophotometric method. It was evaluated at three levels: intraday, interday, and repeatability. Intraday precision was performed by analyzing samples three times within a single day using freshly prepared solutions, whereas interday precision was assessed by repeating the same procedure on three consecutive days under identical experimental conditions. Repeatability was determined by performing six replicate measurements at a fixed concentration of each drug using the same instrument and analyst. The precision of the method was expressed as the percent relative standard deviation (%RSD), and values not exceeding

2% were considered acceptable, confirming that the developed method is reliable, reproducible, and suitable for routine quantitative analysis.^[20]

Accuracy (Recovery Studies)

Accuracy was determined by recovery experiments using pre-analyzed samples spiked with known concentrations of each drug corresponding to 80%, 100%, and 120% of the label claim. The mean percentage recovery and %RSD were calculated.^[21]

Specificity

Specificity was established by confirming that no interference occurred from excipients or formulation components at the selected analytical wavelengths.^[22]

Ruggedness

Ruggedness was assessed by performing the analysis under different conditions by two analysts using the same instrument and comparing %RSD values to evaluate reproducibility.^[23]

Robustness

Robustness was evaluated by introducing small deliberate variations in analytical conditions, such as

changes in temperature ($\pm 5^\circ\text{C}$) and wavelength ($\pm 2\text{ nm}$), and observing their effects on absorbance readings.^[22]

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated as per ICH guidelines using the following equations.^[23]

$$\text{LOD} = 3.3 \times \frac{\sigma}{S}, \text{LOQ} = 10 \times \frac{\sigma}{S}$$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

RESULTS AND DISCUSSION

Pre-Formulation Studies

Organoleptic Evaluation

Organoleptic characteristics of both drugs were examined to confirm their identity and physical appearance. Aloin appeared as a yellow-brown, odourless solid powder, while Thiocolchicoside was observed as a light-to-dark yellow crystalline powder with a characteristic odour. These findings were consistent with standard Pharmacopoeial references, indicating the authenticity and purity of the drug samples. The observations are summarized in Table 1.

Table 1: Organoleptic Properties of Aloin and Thiocolchicoside.

Drug	Property	Observation
Aloin	Colour	Yellow-brown
	Odour	Odourless
	Appearance	Powder
	State	Solid powder
Thiocolchicoside	Colour	Light yellow to dark yellow
	odour	Characteristic
	Appearance	Powder
	State	Crystalline powder

Solubility Study

Solubility profiles of both drugs were evaluated in solvents of varying polarity, as summarized in Tables 2 and represented graphically in Figure 1. Aloin was found to be freely soluble in ethanol, methanol, and dimethyl sulfoxide (DMSO), but insoluble in water.

Thiocolchicoside exhibited free solubility in methanol, moderate solubility in water and DMSO, and was insoluble in chloroform. These findings indicated that methanol is the most suitable solvent for UV spectrophotometric analysis due to its superior solubilizing capacity for both analytes.

Table 2: Solubility Study of Aloin and Thiocolchicoside.

Solvent	Aloin	Thiocolchicoside
Water	Insoluble	Soluble
Ethanol	Freely soluble	Slightly soluble
Methanol	Freely soluble	Freely soluble
Chloroform	Soluble	Insoluble
DMSO	Freely soluble	Soluble

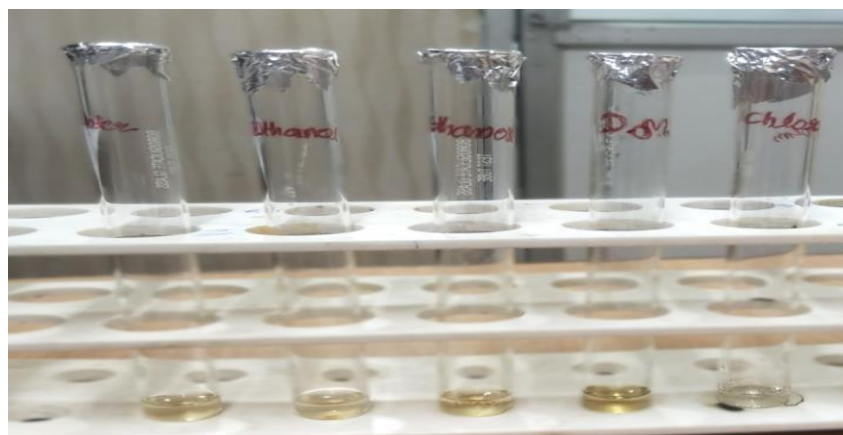


Figure 1: Solubility Profiles of Aloin and Thiocolchicoside.

pH Determination

pH of Aloin and Thiocolchicoside solutions was determined to evaluate their chemical stability and compatibility in formulation media. The measured pH values were 6.1 for Aloin and 6.7 for Thiocolchicoside,

as shown in Table 3. These values indicate that both drugs lie within the slightly acidic to near-neutral range, suggesting good stability and compatibility for pharmaceutical formulation.

Table 3: pH of Aloin and Thiocolchicoside.

Drug	Observed pH
Aloin	6.1
Thiocolchicoside	6.7

Melting Point Determination

Melting point of each drug was determined using the capillary method to assess purity and thermal stability. Aloin exhibited a melting point of 236 °C, while

Thiocolchicoside melted at 196 °C, both of which fall within their respective reported ranges, confirming the purity of the samples (Table 4).

Table 4: Melting Point of Aloin and Thiocolchicoside.

Drug	Observed (°C)	Literature Range (°C)
Aloin	236	233 – 237
Thiocolchicoside	196	190 – 198

Spectroscopic Analysis

Determination of λ_{max}

λ_{max} values obtained from UV absorption spectra were 295.0 nm for Aloin (Figure 2) and 366.0 nm for

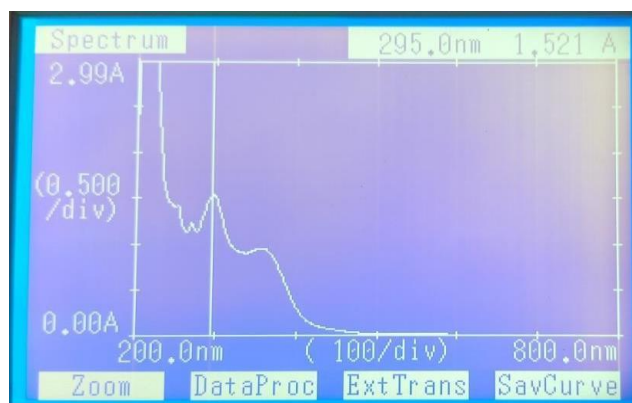
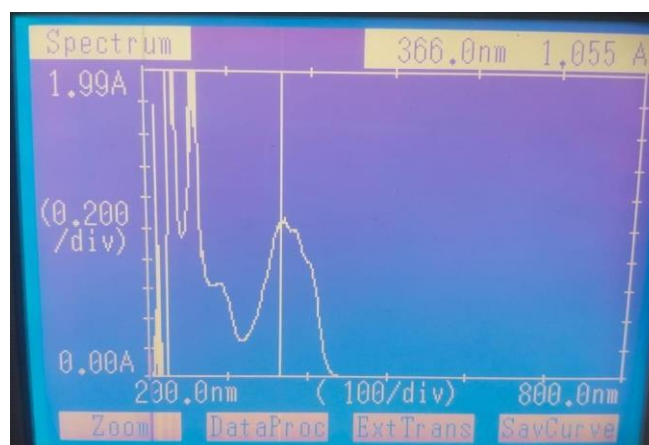
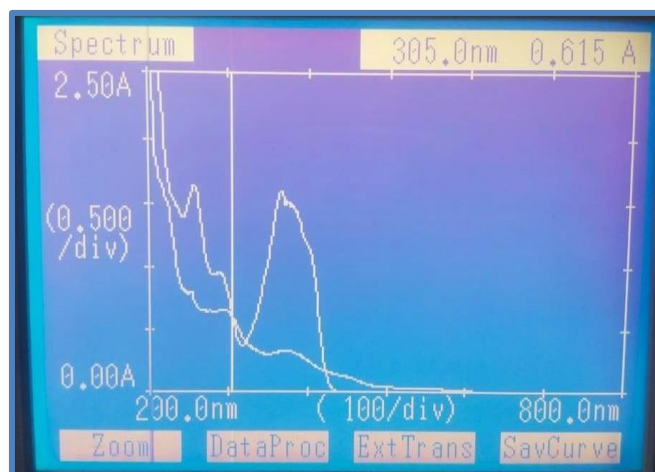
Thiocolchicoside (Figure 3). When analyzed in combination, a common absorption maximum was observed at 305.0 nm (Table 6 and Figure 4), suitable for simultaneous estimation.

Table 5: UV Absorption Maxima (λ_{max}) of Aloin and Thiocolchicoside.

S. No	Drug	UV Absorption Maxima (λ_{max})
1	Aloin	295.0 nm
2	Thiocolchicoside	366.0 nm

Table 6: Combined λ_{max} of Aloin and Thiocolchicoside.

S. No	Drug	UV absorption maxima (Lambda max)
1.	Aloin and Thiocolchicoside	305.0 nm

**Figure 2: UV Spectrum of Aloin.****Figure 3: UV Spectrum of Thiocolchicoside.****Figure 4: UV Spectrum Calibration Curve.**

Calibration Curve and Linearity

Calibration curves were plotted between absorbance and concentration for both drugs. Aloin showed linearity over 10–60 $\mu\text{g/mL}$, and Thiocolchicoside over 5–30 $\mu\text{g/mL}$, with correlation coefficients (R^2) of 0.9999 and 0.9992, respectively (Figures 5 and 6). Although calculated %RSD values from raw data were high due to experimental variation, regression analysis confirmed strong linear relationships, satisfying Beer–Lambert's law.

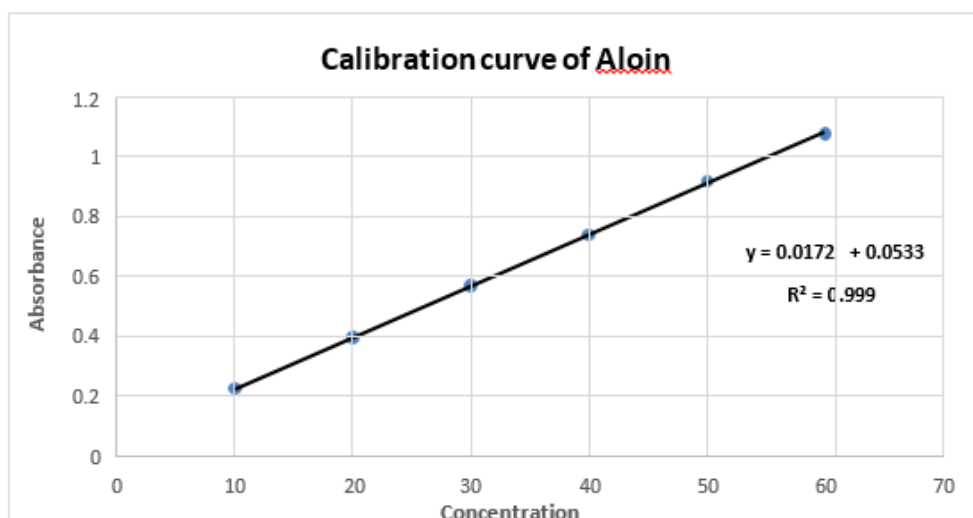


Figure 5: Calibration Curve of Aloin.

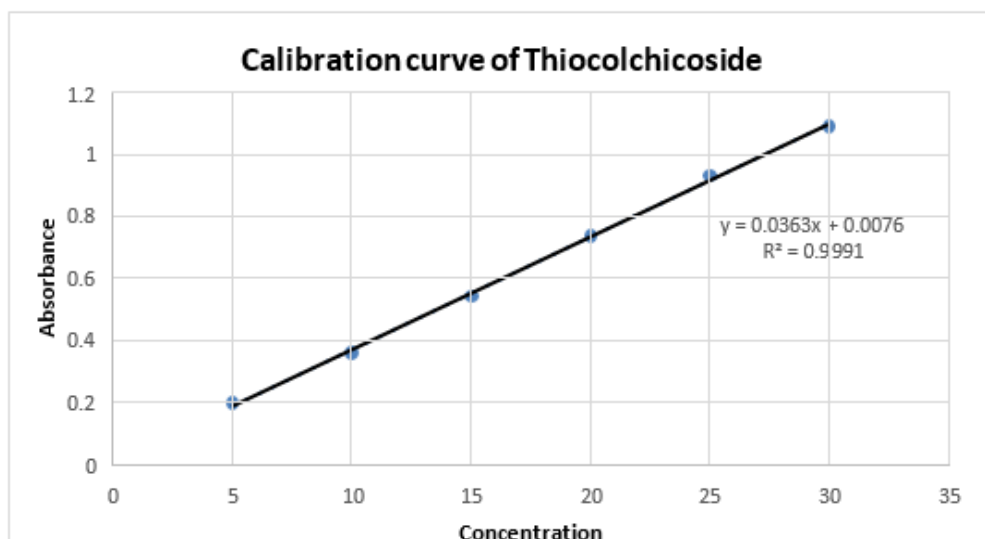


Figure 6: Calibration Curve of Thiocolchicoside.

Fourier Transform Infrared (FTIR) Analysis

FTIR spectra of Aloin and Thiocolchicoside confirmed the presence of characteristic functional groups corresponding to their reported chemical structures (Table 7). Aloin exhibited N-H stretching (3404.40 cm^{-1}), C=C stretching (1647.98 cm^{-1}), S=O stretching (1364.17 and 1154.31 cm^{-1}), C-O stretching (1078.05 cm^{-1}), and C=C bending (861.36 cm^{-1}), indicating primary amine, alkene, sulphonamide, sulfonic acid, and alcohol groups (Figure 7). Thiocolchicoside showed (Figure 8) N-H stretching (3415.69 cm^{-1}), N-H bending (1625.64 cm^{-1}), S=O stretching (1155.92 cm^{-1}), and C-O stretching (1071.58 cm^{-1}), confirming primary amine, sulfone, and alcohol functionalities. These functional groups are consistent with their chemical identities and contribute to their biological activity.

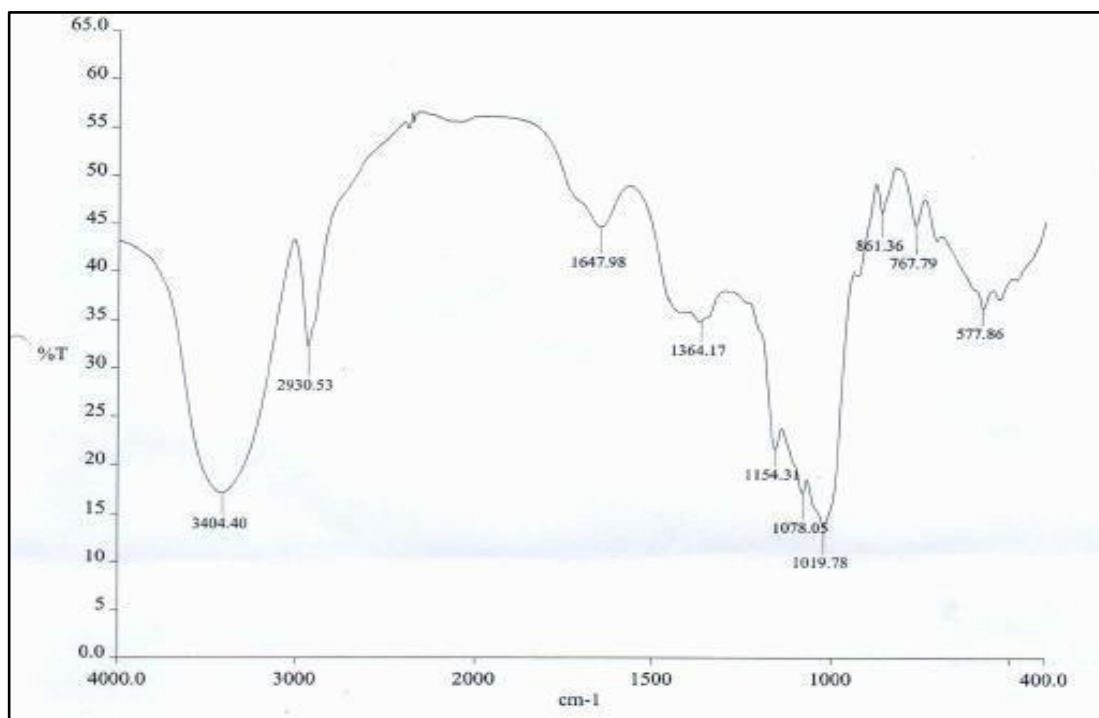


Figure 7: IR study of Aloin.

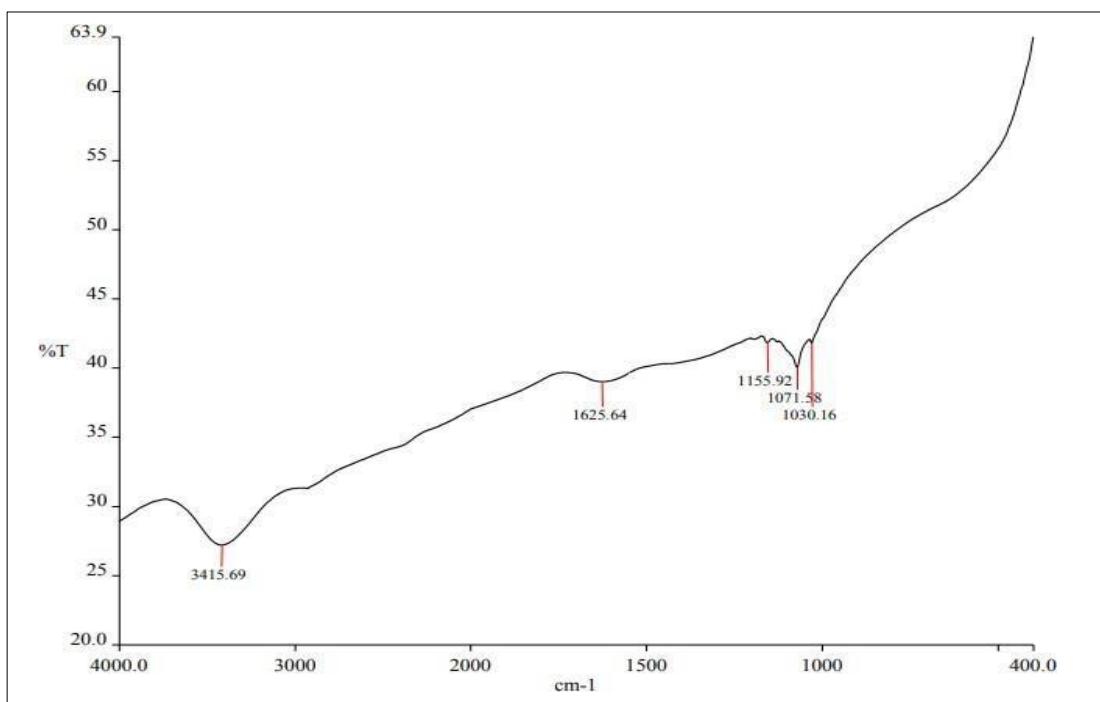


Figure 8: IR study of Thiocolchicoside.

Table 7: Comparative FTIR Analysis of Aloin and Thiocolchicoside.

S. No	Functional Group	Aloin (cm ⁻¹)	Thiocolchicoside (cm ⁻¹)
1	N-H stretching	3404.40	3415.69
2	N-H bending	-	1625.64
3	C=C stretching	1647.98	-
4	S=O stretching	1364.17, 1154.31	1155.92
5	C-O stretching	1078.05	1071.58
6	C=C bending	861.36	-

Method Validation

Precision

Precision was evaluated through intraday, interday, and repeatability studies. Both Aloin and Thiocolchicoside exhibited excellent precision with %RSD values well below 2% (Table 8). These results confirm the reproducibility of the method for repeated measurements under the same and varied conditions.

Ruggedness and Robustness

Ruggedness studies, comparing results from two different analysts, demonstrated consistent readings with %RSD < 2%, while robustness testing under slight temperature variations (25 °C ± 5 °C) showed minimal

variation in absorbance (%RSD < 1.1%) for both drugs (Table 8). These findings indicate that the method is reliable and stable under routine laboratory conditions.

Sensitivity (LOD and LOQ)

Sensitivity of the method was assessed by determining the limits of detection (LOD) and quantification (LOQ). Aloin exhibited an LOD of 2.01 µg/mL and LOQ of 7.09 µg/mL, whereas Thiocolchicoside showed an LOD of 1.95 µg/mL and LOQ of 6.10 µg/mL (Table 8). These results confirm the high sensitivity of the developed method, suitable for accurate quantification of low concentrations of both drugs.

Table 8: Validation Parameters of Aloin and Thiocolchicoside.

Parameter	Aloin	Thiocolchicoside
Intraday Precision (%RSD)	0.646	0.948
Interday Precision (%RSD)	0.532	0.882
Repeatability (%RSD)	0.891	1.217
Ruggedness (%RSD)	0.882–0.888	0.203–0.815
Robustness (%RSD)	0.889–1.062	0.808–1.014
LOD (µg/mL)	2.01	1.95
LOQ (µg/mL)	7.09	6.10

CONCLUSION

The present study successfully established a simple, accurate, precise, and economical UV-Visible spectrophotometric method for the quantitative estimation of Aloin and Thiocolchicoside. The analytical conditions were optimized using methanol as the solvent, and the λ_{max} values were found to be 295 nm and 366 nm for Aloin and Thiocolchicoside, respectively, with a common wavelength of 305 nm selected for simultaneous estimation. Validation of the developed method, performed in accordance with ICH Q2(R1) guidelines, demonstrated excellent linearity ($R^2 > 0.999$), accuracy (recoveries within 98–102%), and precision (%RSD < 2%), along with acceptable sensitivity, robustness, and ruggedness. The low LOD and LOQ values further indicated the method's high sensitivity and reliability. Thus, the developed UV spectrophotometric method is rapid, reproducible, and cost-effective, making it highly suitable for routine quality control, stability studies, and quantitative determination of Aloin and Thiocolchicoside in bulk and pharmaceutical formulations. The method can also serve as a foundation for further analytical advancements, including derivative spectrophotometric or chromatographic studies for complex dosage forms.

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Conflict of Interest

The authors declare that there is no conflict of interest associated with this research work.

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