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SIMULTANEOUS ESTIMATION AND VALIDATION OF ANALYTICAL METHOD FOR SOME ANTI-INFLAMMATORY DRUG (ACTIVE CONSTITUENTS) BY UV SPECTROPHOTOMETER

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ABSTRACT

A simple, accurate, and precise UV spectrophotometric method was developed and validated for the simultaneous estimation of two anti-inflammatory active constituents, Thiocolchicoside and Andrographolide. The method was based on the measurement of absorbance at selected wavelengths, where Andrographolide showed maximum absorbance (λmax) at 223 nm and Thiocolchicoside at 257nm, with an isobestic point at 263 nm enabling simultaneous analysis. Both drugs obeyed Beer–Lambert's law in the concentration range of 5–25 µg/mL for Andrographolide and 10–50 µg/mL for Thiocolchicoside, showing excellent linearity with correlation coefficients close to 0.99. The method was validated as per standard guidelines and demonstrated high precision with %RSD values less than 2% for intraday, interday, and repeatability studies. Ruggedness and robustness studies confirmed that the method is reproducible under different analysts and slight variations in temperature. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.123µg/mL and 3.404µg/mL for Andrographolide, and 3.067µg/mL and 9.295µg/mL for Thiocolchicoside, indicating good sensitivity. In conclusion, the developed UV spectrophotometric method is simple, economical, accurate, and reliable for the simultaneous estimation of Thiocolchicoside and Andrographolide, making it suitable for routine quality control analysis in pharmaceutical formulations.

KEYWORDS: Thiocolchicoside and Andrographolide.

1. INTRODUCTION

UV spectrophotometric methods are widely applied for the estimation of active pharmaceutical ingredients either alone or in combination dosage forms. The technique is based on the absorption of ultraviolet radiation by molecules containing chromophoric groups **(Kamal *et al.*, 2016).** The amount of radiation absorbed by a substance is directly proportional to its concentration according to Beer–Lambert law. Because of its operational simplicity and low cost compared to sophisticated chromatographic methods, UV spectrophotometry remains a preferred analytical technique in quality control laboratories and research institutions **(Pattebahadur *et al.*, 2025).**

Anti-inflammatory drugs mainly include Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and corticosteroids. NSAIDs are among the most frequently used therapeutic agents worldwide because of their analgesic, antipyretic, and anti-inflammatory properties. Common NSAIDs include diclofenac sodium, ibuprofen, naproxen, aceclofenac, ketoprofen, meloxicam, piroxicam, and aspirin **(Fokunang *et al.*, 2018).** These drugs act primarily by inhibiting cyclooxygenase (COX) enzymes, thereby reducing prostaglandin synthesis responsible for

inflammation and pain. Since anti-inflammatory drugs are widely used in pharmaceutical formulations either individually or in combination therapy, accurate analytical methods are essential for their simultaneous estimation and quality control **(Celia *et al.*, 2020).**

Thiocolchicoside is a semi-synthetic sulfur derivative of colchicoside obtained from the plant *Gloriosa superba*. It is chemically classified as a muscle relaxant with anti-inflammatory and analgesic properties. Thiocolchicoside acts selectively on gamma-aminobutyric acid (GABA) and glycinergic receptors, producing muscle relaxation without causing significant sedation or paralysis **(Bhamburkar *et al.*, 2022).** It is commonly used in the treatment of painful muscular spasms, orthopedic disorders, rheumatologic conditions, low back pain, and neurological disorders associated with muscle stiffness and inflammation. Thiocolchicoside exhibits significant therapeutic benefits because of its ability to reduce muscular tension and associated inflammatory pain **(Karmakar *et al.*, 2025).** It is frequently formulated in combination with anti-inflammatory agents to enhance clinical efficacy. Due to its extensive pharmaceutical use, accurate analytical methods are required for its identification and quantitative estimation in bulk drugs and pharmaceutical dosage forms **(Loos *et al.*, 2016).**

Andrographolide is a naturally occurring diterpenoid lactone isolated from the medicinal plant *Andrographis paniculata*, commonly known as "King of Bitters." It is one of the major bioactive constituents responsible for the pharmacological activities of the plant. Andrographolide possesses potent anti-inflammatory, antioxidant, antimicrobial, antiviral, hepatoprotective, immunomodulatory, and anticancer activities **(Gupta *et al.*, 2017).** The anti-inflammatory activity of andrographolide is mainly attributed to its ability to inhibit inflammatory mediators such as prostaglandins, cytokines, nitric oxide, and nuclear factor-kappa B (NF-κB) pathways. Because of its therapeutic importance, andrographolide is widely used in herbal formulations, nutraceuticals, and pharmaceutical preparations for the management of inflammatory and infectious disorders **(Mishra *et al.*, 2021).**

The present study aims to develop and validate a UV spectrophotometric method for simultaneous estimation of Thiocolchicoside and Andrographolide in bulk and pharmaceutical dosage forms.

2. MATERIALS AND METHODS

2.1 Chemicals

DMSO, Ether, and Methanol, were obtained from Rankem, a reputable supplier of analytical reagents. Qualigens provided the Ethanol and Chloroform. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Himedia, and Rankem.

2.2 Pre-formulation Studies of Andrographolide and Thiocolchicoside

2.2.1 Organoleptic properties

Organoleptic evaluation is a fundamental parameter in assessing the purity and quality of drugs. In terms of pure drugs, it involves the examination such as color, odor, and texture, to confirm identity and detect adulteration or deterioration (**Selvam, 2015).**

2.2.2 Melting point determination

Melting Point Apparatus was used to determine melting point through capillary tube method. Melting point analysis is an essential laboratory technique for identifying pure substances and assessing their purity (**John Young, 2013).**

2.2.3 Solubility Study

Solubility is an important factor in UV–Visible spectroscopic analysis, as accurate absorbance measurement depends on complete dissolution of the analyte in a suitable solvent. According to the Beer–Lambert Law, absorbance is directly proportional to concentration, and any undissolved particles may cause light scattering and inaccurate results. Therefore, appropriate solvents such as water, methanol, or ethanol are selected to ensure complete solubility without interfering at the analyte's λmax. Proper solubility also improves the precision, linearity, and reproducibility of the analytical method by ensuring uniform sample preparation and preventing precipitation or baseline interference **(Árpád Könczöl *et al.*, 2020).**

2.2.4 pH determination

The stability, solubility, and bioavailability of drugs are all significantly impacted by the pH of pharmaceutical solutions by using pH meter.

2.3 Identification of pure compound via FTIR spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy is based on the absorption of infrared radiation by molecules, producing characteristic vibrations of chemical bonds that help identify functional groups and chemical structures. FTIR offers high sensitivity, rapid analysis, and accurate spectral data, making it widely useful in pharmaceutical analysis for drug identification, purity assessment, and compatibility studies. In the present study, FTIR spectra were recorded using a PerkinElmer FTIR spectrometer over the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. The sample was analyzed using the ATR technique after recording a background spectrum, and the obtained spectra were processed through baseline correction and peak analysis for accurate interpretation (**Khan *et al.*, 2018).**

2.4 UV-Vis Spectroscopy Method

Methanol was selected as the solvent for UV–Visible spectroscopic method development due to its excellent solubility for both Andrographolide and Thiocolchicoside, providing clear and stable solutions for

accurate absorbance measurements. Standard stock solutions of each drug were prepared separately at a concentration of 1000 µg/mL in methanol, followed by further dilutions to obtain working solutions in the range of 5–25 µg/mL. The wavelength of maximum absorbance (λmax) for both drugs was determined using a Shimadzu UV–Visible spectrophotometer by scanning solutions between 200–400 nm against methanol as blank. The simultaneous equation method was then applied for quantitative estimation, where absorbance values of both drugs were measured at their respective λmax, and absorptivity values were calculated to enable simultaneous analysis without prior separation **(Moharana *et al.*, 2011).**

The absorptivity values at these particular wave lengths were calculated and substituted in the following equation, to obtain respective concentration in the sample: Thiocolchicoside = (A1*ax2 - A2*ax1) / (ax2*ay1 - ax1*ay2)
Andrographolide = (A1*ax2 - A2*ax1) / (ax2*ay1 - ax1*ay2)

A1 and A2 are absorbance of diluted laboratory mixture at λmax Thiocolchicoside and λmax Andrographolide, respectively. Cx and Cy are concentrations of Thiocolchicoside and Andrographolide respectively (µg/ml). ax1 and ax2 are absorptivities of Thiocolchicoside and Andrographolide at λmax Thiocolchicoside while ay1 and ay2 are absorptivities of Thiocolchicoside and Andrographolide at λmax Andrographolide, respectively.

2.5 Method Validation

The developed UV–Visible Spectrophotometry method was validated according to ICH Q2 (R1) guidelines for the following parameters: specificity, linearity, range, accuracy, precision, robustness, ruggedness, limit of detection (LOD), and limit of quantization (LOQ) (ICH, 2005).

- **Linearity and range**

Linearity studies were performed to demonstrate that the analytical method provides results directly proportional to the concentration of the drugs within a specified range. Standard solutions of both drugs were prepared at concentrations of 5, 10, 15, 20, and 25 µg/mL, and their absorbance values were measured at the respective λmax. Calibration curves of absorbance versus concentration were plotted, and the linear regression equation along with the correlation coefficient (R^2) was determined. An R^2 value of not less than 0.999 was considered indicative of excellent linearity of the method **(Ismail *et al.*, 2014).**

- **Precision**

Precision studies were carried out to evaluate the repeatability and reproducibility of the analytical method under normal operating conditions. Repeatability was assessed by measuring the selected dilution six times for both drugs. Intra-day precision was determined by analyzing samples multiple times within the same day in triplicate, while inter-day precision was evaluated by analyzing the samples on alternate days up to the fifth day. The absorbance values obtained were recorded and compared to determine the consistency and reliability of the method **(Zanobini *et al.*, 2016).**

- **Robustness**

To assess the reliability of the method under small, deliberate variations in experimental conditions. Robustness was evaluated by measuring the absorbance of selected concentrations in triplicate by two different analysts under the same experimental conditions **(Ferreira *et al.*, 2017).**

- **Ruggedness**

To establish ruggedness of the proposed method, absorbance in triplicate were recorded at two varying temperatures **(Biter *et al.*, 2019).**

- **Detection Limit and Quantization limit**

The LOD and LOQ for Thiocolchicoside and Andrographolide by the proposed method were determined via formula (σ/S)*3.3 and (σ/S)*10 respectively. Where S is the slope of the calibration curve, σ is the standard deviation of y-intercept of regression equation (**Tripti Shuklaa *et al.*, 2019)**

3. RESULTS AND DISCUSSION

3.1. Organoleptic properties of Thiocolchicoside and Andrographolide.

Table 1: Organoleptic Properties of Thiocolchicoside and Andrographolide.

Organoleptic Properties	Andrographolide	Thiocolchicoside
Colour	Off-white power	Yellow, Light orange
Odour	Odourless	Odorless
Physical appearance	Flaky crystal	Crystalline powder
State	Solid	Solid

3.1.1 Melting point and pH determination of Thiocolchicoside and Andrographolide.

Table 2: Melting point and pH determination.

Drug	Reference	Observed	Reference	Observed
Thiocolchicoside	190–221 °C	219°C	2.0-6.0. pH	5.6 pH
Andrographolide	229-232°C	231°C	6.0 - 7.5 pH	6.9 pH

3.2 Identification of standard compound by FTIR

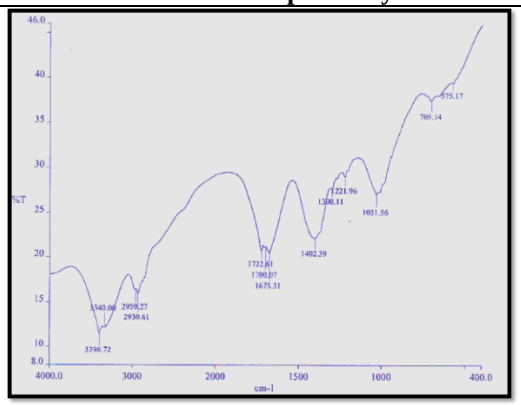

Figure 5: FTIR of pure Andrographolide drug.

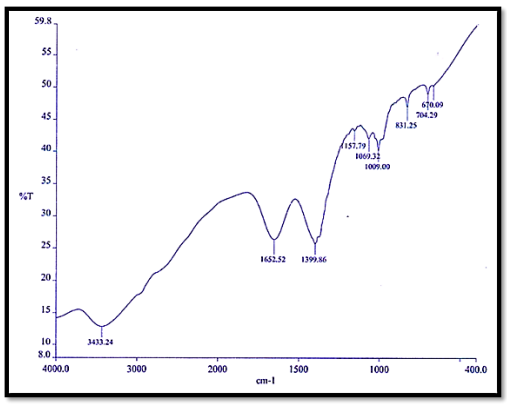

Figure 6: FTIR of pure Thiocolchicoside drug.

Table 3: FTIR Interpretation of Andrographolide.

Range (cm-^{1})	Absorbance	Appearance	Bond	Group
3200-3500	3340	Broad	O-H stretching	Alcohol
2850-2950	2930	Sharp, medium	C-H stretching	Alkane
1680-1750	1722	Broad	C=O stretching	Ester
1375-1400	1402	Broad	C-H Bending	Methyl
1000-1300	1221	Sharp weak	C-O stretching	Alcohol
600-1500	1031	Weak	C-C Stretching	Alkane

Table 4: FTIR Interpretation of Thiocolchicoside.

Frequency Range	Group Absorption (cm^{-1})	Group	Compound Class
3500- 3400 (cm^{-1})	3433.24	N-H stretching	Primary Amine
1658-1648 (cm^{-1})	1652.52	C=C stretching	Alkene
1440-1395 (cm^{-1})	1399.86	O-H bending	Carboxylic Acid
1165-1150 (cm^{-1})	1157.79	S=O stretching	Sulfonic Acid
1210-1163 (cm^{-1})	1069.32	C-O stretching	Ester
730-665 (cm-^{1})	704.29	C=C bending	Alkene

3.2.1 Calibration curve (Linearity)

Table 5: Calibration data of Thiocolchicoside at 257.0nm and Andrographolide at 223.0nm.

Concentration (µg/ml)	Absorbance 1 at 257.0nm	Absorbance 2 at 257.0nm	Absorbance 3 at 257.0nm	Mean Absorbance 257.0nm	Absorbance 1 at 223.0nm	Absorbance 2 at 223.0nm	Absorbance 3 at 223.0nm	Mean Absorbance 223.0nm
	Thiocolchicoside at 257.0nm				Andrographolide at 223.0nm			
10	0.215	0.251	0.265	0.243	0.295	0.259	0.312	0.288
20	0.318	0.301	0.287	0.302	0.371	0.361	0.291	0.341
30	0.381	0.412	0.401	0.398	0.391	0.492	0.481	0.454
40	0.455	0.438	0.461	0.451	0.566	0.551	0.588	0.568
50	0.512	0.525	0.532	0.523	0.678	0.665	0.592	0.645
Mean				**0.3836**				**0.45953**
SD				**0.00668431**				**0.006426**
%RSD				**1.566**				**1.307**

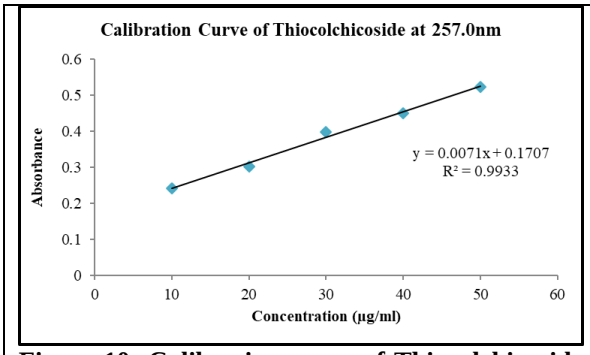

Figure 10: Calibration curve of Thiocolchicoside at 257.0nm

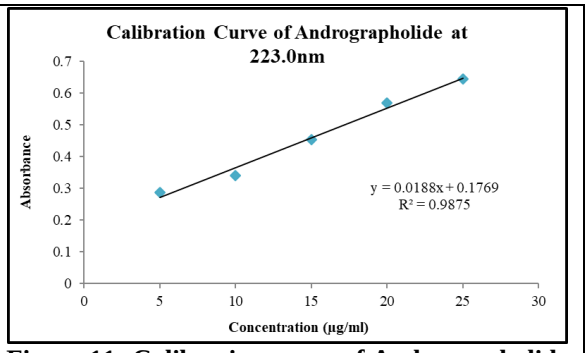

Figure 11: Calibration curve of Andrographolide at 223.0nm

3.2.3 Method Validation via UV spectroscopy for Andrographolide and Thiocolchicoside

3.2.3.1 Precision study

(A) Intraday Precision

Table 6: Result of Intraday Precision (three times on the same day) of Andrographolide at 223 nm and Thiocolchicoside at 257 nm.

Concentration (µg/mL)	Day 1 Absorbance (1) at 223.0 nm	Day 1 Absorbance (2) at 223.0 nm	Day 1 Absorbance (3) at 223.0 nm	Day 1 Absorbance (1) at 257.0 nm	Day 1 Absorbance (2) at 257.0 nm	Day 1 Absorbance (3) at 257.0 nm
10	0.341	0.340	0.341	0.398	0.396	0.397
10	0.340	0.344	0.344	0.395	0.394	0.393
10	0.342	0.343	0.342	0.396	0.397	0.395
Mean	0.341	0.342	0.3423	0.396	0.395	0.395
SD	0.001	0.00208	0.001527	0.00152	0.001528	0.002
%RSD	0.293	0.584	0.292	0.252	0.253	0.506
AVG % R.S.D	0.389			0.337		

(B) Interday Precision

Table 7: Result of Interday Precision (three times on the different day) of Andrographolide at 223 nm and Thiocolchicoside at 257 nm.

Concentration (µg/mL)	Day 1 Absorbance at 223.0 nm	Day 2 Absorbance at 223.0 nm	Day 3 Absorbance at 223.0 nm	Day 1 Absorbance at 257.0 nm	Day 2 Absorbance at 257.0 nm	Day 3 Absorbance at 257.0 nm
10	0.341	0.342	0.346	0.397	0.396	0.392
10	0.344	0.344	0.345	0.399	0.398	0.395
10	0.343	0.346	0.344	0.395	0.391	0.396
Mean	0.34267	0.344	0.345	0.397	0.395	0.394
SD	0.001528	0.002	0.001	0.002	0.0036	0.0020
%RSD	0.292	0.581	0.289	0.503	0.759	0.507
AVG % R.S.D	0.387			0.589		

3.2.3.2 Repeatability

Table 8: Result of repeatability of Andrographolide and Thiocolchicoside.

Concentration (µg/ml)	Absorbance	Statistical analysis		Absorbance		Statistical analysis
	Andrographolide			Thiocolchicoside		
10	0.341	**Mean**	0.398	**Mean**	0.397166667	0.3425
10	0.345	**SD**	0.395	**SD**	0.00147	0.002074
10	0.340	**% RSD**	0.399	**% RSD**	0.251	0.584
10	0.344		0.397			
10	0.344		0.398			
10	0.341		0.396			

3.2.3.3 Ruggedness

Table 9: Result of ruggedness of Andrographolide at 223.0nm and Thiocolchicoside at 257.0nm.

Concentration (µg/ml)	Analyst-1	Analyst-2	Analyst-1	Analyst-2
	Absorbance	Absorbance	Absorbance	Absorbance
	Andrographolide at 223.0nm		Thiocolchicoside at 257.0nm	
10	0.342	0.341	0.398	0.396
10	0.345	0.343	0.395	0.394
10	0.347	0.345	0.397	0.392
Mean	0.344	0.343	0.396	0.394
SD	0.0025	0.002	0.001528	0.002
% RSD	0.581	0.583	0.252	0.507

3.2.3.4 Robustness

Table 10: Results showing robustness of Thiocolchicoside at 257.0nm and Andrographolide at 223.0nm.

Concentration (µg/ml)	Absorbance at 25^0C	Absorbance at 30^0C	Absorbance at 25^0C	Absorbance at 30^0C
	Thiocolchicoside at 257.0nm		Andrographolide at 223.0nm	
10	0.398	0.397	0.341	0.346
10	0.392	0.394	0.345	0.344
10	0.395	0.395	0.347	0.341
Mean	0.395	0.3953	0.3443	0.3436
SD	0.003	0.001528	0.00305	0.00251
% RSD	0.759	0.253	0.872	0.583

3.3 LOD and LOQ of Thiocolchicoside and Andrographolide

Table 11: Results showing LOD and LOQ of Thiocolchicoside and Andrographolide.

Drug name	Wavelength	LOQ (µg/ml)	LOD (µg/ml)
Thiocolchicoside	257.0nm	9.295	3.067
Andrographolide	223.0nm	3.404	1.123

Table 12: Optical Characteristics and Validation Study of Drugs.

Parameters	Thiocolchicoside	Andrographolide
Wavelength λ max nm	257.0nm	223.0nm
Beer's law limit µg/ml	10-50ug/ml	5-25ug/ml
Correlation coefficient (R^2)	0.9933	0.9875
Slope	0.01707	0.01769
Intercept	0.0071	0.0188
SD	0.006684	0.006426
% RSD	1.566	1.307
Precision		
Intraday (% RSD)	0.337	0.389
Interday (% RSD)	0.589	0.387
Repeatability	0.251	0.584
Ruggedness		
Analyst 1 (% RSD)	0.252	0.581
Analyst 2 (% RSD)	0.507	0.583
Robustness		
Temp. 25^0C (% RSD)	0.759	0.872
Temp. 30^0C (% RSD)	0.253	0.583
LOQ (µg/ml)	9.295	3.404
LOD (µg/ml)	3.067	1.123

DISCUSSION

The organoleptic evaluation, melting point determination, solubility studies, and pH analysis of Thiocolchicoside and Andrographolide confirmed their identity, purity, and suitability for analytical method development. Both drugs exhibited characteristic physical properties and good solubility in methanol, supporting its selection as the analytical solvent. FTIR spectral analysis confirmed the presence of characteristic functional groups, validating the chemical structures of both compounds. UV spectrophotometric studies showed distinct λmax values at 223.0 nm for Andrographolide and 257.0 nm for Thiocolchicoside, with sufficient spectral separation for simultaneous estimation. The developed method demonstrated excellent linearity within the selected concentration ranges, with high

correlation coefficients indicating adherence to Beer's law. Precision studies, including repeatability, intraday, and interday analysis, showed low %RSD values below 2%, confirming the accuracy, reproducibility, and reliability of the method. Robustness studies revealed that minor variations in temperature did not significantly affect absorbance, indicating method stability. Furthermore, low LOD and LOQ values demonstrated good sensitivity of the developed UV spectrophotometric method, making it suitable for routine quantitative analysis and quality control of Thiocolchicoside and Andrographolide in pharmaceutical formulations.

4. CONCLUSION

The developed UV spectrophotometric method is validated as accurate, precise, and reliable for simultaneous estimation of both drugs. It showed excellent linearity ($R^2 \approx 0.99$), high precision (%RSD < 2%), and strong sensitivity (low LOD and LOQ values). The method remained robust under small variations (temperature %RSD $\leq$ 0.872%) and rugged between analysts. Therefore, this method is suitable for routine pharmaceutical analysis and quality control of Thiocolchicoside and Andrographolide in bulk and dosage forms.

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