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DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROSCOPIC METHOD FOR THE ESTIMATION OF THIOCOLCHICOSIDE IN BULK AND PHARMACEUTICAL DOSAGEFORM

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ABSTRACT

Thiocolchicoside is a semi-synthetic derivative of colchicoside obtained from the plant *Gloriosa superba* and is widely used as a centrally acting skeletal muscle relaxant. It possesses muscle-relaxant, anti-inflammatory, and analgesic properties and is commonly prescribed for the treatment of painful muscular spasms associated with orthopedic, rheumatologic, and traumatic disorders. The present study was aimed at developing and validating a simple, rapid, precise, and accurate UV spectrophotometric method for the quantitative estimation of Thiocolchicoside in bulk drug and pharmaceutical dosage form. In this study, distilled water was selected as the solvent based on solubility studies. The analysis was performed using a Shimadzu 1900i double beam UV-Visible spectrophotometer with matched quartz cells. The standard drug solution was scanned in the wavelength range of 200–400 nm and the maximum absorbance (λmax) of Thiocolchicoside was found to be 260 nm. A calibration curve was constructed using standard solutions in the concentration range of 2–24 µg/ml, which showed good linearity with a correlation coefficient (R^2) of

0.9978 and regression equation of y = 0.0379x + 0.0153. The developed method was validated according to analytical parameters including linearity, precision, accuracy, ruggedness, robustness, limit of detection (LOD), and limit of quantification (LOQ). The LOD and LOQ values were found to be 0.2943 µg/ml and 0.8919 µg/ml respectively. Recovery studies showed percentage recovery within the acceptable range of 98–100.75%, indicating good accuracy of the method. The validated method was successfully applied for the estimation of Thiocolchicoside in a marketed formulation (Myoril 4 mg), and the assay results showed an average drug content of 100.52%. The results indicate that the developed UV spectrophotometric method is simple, economical, accurate, and suitable for routine quality control analysis of Thiocolchicoside in bulk drug and pharmaceutical dosage forms.

KEYWORDS: Thiocolchicoside, UV Spectrophotometry, Method Validation, Pharmaceutical Formulation, Quantitative Analysis.

INTRODUCTION

Thiocolchicoside (TCC) is a partially synthetic compound developed from colchicoside, a natural glucoside found in the *Gloriosa Superba*. It is produced by replacing a methoxy group in colchicoside with a thiomethyl group. Thiocolchicoside (TCC) is commonly used in medical treatment for its muscle-relaxing, pain-relieving, and anti-inflammatory effects. Research has shown that it acts on the central nervous system of rats by interacting with GABA type A receptors (GABAARs) and glycine receptors that are sensitive to strychnine. ThiocolchicosideTHC) chemically, N-[3-(β-D glucopyranoxyloxy)-5,6,7,9-tetrahydro-1,2-imethoxy-10-(methylthio)-9-oxobenzo[a] heptalen-7yl]acetamide. While the compound has been in use since many years in the european countries, the first formulation containing thiocolchicoside was approved in India in the year 2008. Being less sedating than other centrally acting muscle relaxants. Oral, parenteral and topical formulations of thiocolchicoside are available in India. The maximum recommended oral dose is 8 mg every 12 hours for no more than 7 consecutive days. The maximum intramuscular dose should be 4 mg every 12 hours, for up to 5 days. After being taken orally, thiocolchicoside is rapidly absorbed through the digestive system, reaching its highest concentration in the bloodstream in about one hour is used topically for the treatment of muscular spasms and for rheumatologic, orthopaedic and traumatologic disorders.

DRUG PROFILE

❖ DRUG NAME

Thiocolchicoside.

❖ SYNONYMS

- Coltramyl
- Musco-ril
- Prestwick_875
- Thiocol

❖ DESCRPTION

- **Colour** : Pale yellow to yellow
- **Odour** : Characteristic odour
- **Taste** : Unpleasant taste
- **Purity** : >95%
- **Strength** : 4mg & 8mg

❖ CHEMICAL STRUCTURE

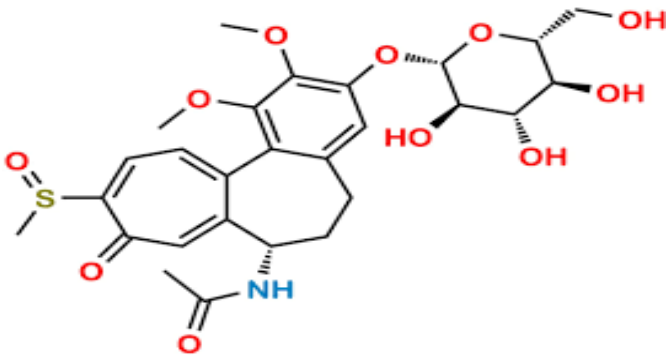

Figure no 1: Structure of Thiocolchicoside.

❖ IUPAC NAME

N-[(7S)-1,2-dimethoxy-10-methylsulfanyl-9-oxo-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-6,7-dihydro-5H-benzo[a]heptalen-7-yl]acetamide

❖ CHEMICAL FORMULA

$C_{27}H_{33}NO_{10}S$

❖ MOLECULAR WEIGHT

563.62 g/mol

❖ **CATEGORY**

It is in a class of medication called centrally acting skeletal muscle relaxant and anti-spasmotic agent.

❖ **DOSE**

Immediate-release (myoril):4mg[IV/PO]

❖ **MELTING POINT**

Range of 190^{o} to 198^{o}C

❖ **HALF LIFE**

The **elimination half-life of thiocolchicoside is about 5–6 hours** (its active metabolite has a half-life of ~7 hours).

❖ **DENSITY**

1.462g/cm^{3}

❖ **SOLUBILITY**

1. Water : Soluble
2. Alcohol : Slightly soluble
3. Ether : Practically insoluble
4. Acetone : Practically insoluble

❖ **STORAGE**

Refrigeration and room temperature.

MECHANISM OF ACTION

Thiocolchicoside is a synthetic derivative of colchicoside from *Colchicum autumnale* that acts as a muscle relaxant by activating inhibitory GABA-A pathways. GABA is the main inhibitory neurotransmitter in the human cortex and is involved in myorelaxation, sedation, and anxiolysis. The drug also interacts with inhibitory glycine receptors, which regulate motor and sensory functions through NMDA receptor modulation. Studies show thiocolchicoside inhibits GABA-A and glycine receptors, partially affects nicotinic acetylcholine receptors at high concentrations, and has no effect on 5-HT3A serotonin receptors.

PHARMACOKINETICS

Thiocolchicoside peaks in plasma around 50 minutes orally and 30 minutes intramuscularly, where it is converted to its active metabolite, 3-desmethylthiocolchicine. It distributes widely in tissues, binds moderately to serum proteins, and is mainly metabolized in the intestine into three metabolites. The drug has a half-life of about 7.7 hours, with metabolites excreted unchanged in feces and urine, and its activity is unaffected by co-administration with other drugs.

USES

- **Uses of centrally acting muscle relaxants:** Treat muscle spasms from injuries, strains, and neurological conditions (MS, cerebral palsy, stroke).
- **Effectiveness:** Safe and effective for acute lower back pain, traumatic, and rheumatologic disorders.
- **Thiocolchicoside in oral submucous fibrosis:** Studied as an adjunct with other muscle relaxants.
- **Potential in bone loss management:** Inhibits osteoclastogenesis via suppression of pro-inflammatory pathways.

ADVERSE EFFECTS

COMMON SIDE EFFECTS

- Drowsiness or Sedation
- Diarrhea
- Flatulence
- Dry mouth

RARE ADVERSE EFFECTS

- Liver injury
- Seizures
- Fertility issues
- Pancreatitis

MATERIALS AND METHODS

MATERIALS USED

Drugs

Thiocolchicoside raw material was bought from pharmaceutical industry.

Formulation

Myoril 4mg was purchased from local pharmacy.

Reagents and chemicals

All the chemicals and solvents used were of analytical grade. The solvent used for this study is Distilled water.

Instruments used

The instruments used for the present work are.

- Digital balance (1 mg sensitive) - **Kingslab.**
- **Shimadzu-1900i** double beam UV-visible spectrophotometer with a pair of matched quartz cells.
- Sonicator.

SPECIFICATION OF INSTRUMENTS:

Instrument No. 1: Digital balance (**1mg** sensitive)

- **Model** **:** KAB220
- **Manufacture** **:** Kingslab.

Instrunent No.2: SHIMADZU- 1900i Double Beam UV-Visible Spectrophotometer.

Table No. 1: Specifications of UV spectrophotometer 1900i.

Light source	20-W halogen lamp and deuterium lamp Built-in light source auto position adjustment.
Monochromator	O-RAY-LIGH grade blazedholographic grating in Czerny-Turner mounting
Detector	Silicon photodiode
Stray light	Less than 0.004% at 220nm (Nal) Less than 0.004% at 340nm ($NaNO_2$) Less than 0.15% at 198nm (KCI)
Wavelength range	190 to 1,100 nm
Spectral bandwidth	1nm (190 to 1,100nm)
Wavelength accuracy	±0.05nm at D2 peak 656.1nm, ±0.3nm for entire range
Wavelength repeatability	±0.025nm
Photometric system	Double beam optics
Photometric range	Absorbance -4 to 4 Abs Transmittance: 0% to 400%

Photometric accuracy	±0.0015 Abs at 0.5 Abs ±0.002 Abs at 1.0 Abs ±0.004 Abs at Abs
Operating temperature/ Humidity	Temperature range: 15-30°C Humidity range: 35-80% (15 to below30°C), 35-70% (30 to below 35°C)

METHOD

In the present work, an attempt was made to develop and validate simple, precise and accurate methods for the estimation of Thiocolchicoside in pure and in tablet dosage form by UV-Visible Spectroscopy.

UV Spectroscopic Method

Selection of solvent

The solubility of Thiocolchicoside was determined in variety of solvents. In this study, from the solubility data distilled water was selected as solvent for the analysis of Thiocolchicoside.

Preparation of standard stock solution

An amount of 0.01g of standard substance Thiocolchicoside was weighted and transferred into 100ml Standard flask, dissolved in 100ml of water, the primary stock solution was prepared. From the primary stock solution 2ml was taken and transferred into 10ml Volumetric flask and made upto the volume with distilled water.

Selection of wavelength

The standard stock solution was further diluted with distilled water to get the concentration of 20µg/ml and the solution was scanned between 200-400nm using distilled water as blank. From the spectra λmax was found.

Preparation of calibration graph

From the standard stock solution 0.2-2.4 ml were transferred into a series of 10ml Volumetric flask and made upto the volume with distilled water. The absorbance of different concentration solution were measured. The calibration curve was constructed by plotting concentration Vs absorbance. Thiocolchicoside was linear with the concentration range of 2-24µg/ml.

Quantification of raw material

From the standard solution of 2ml was taken into a series of six 10ml Volumetric flask and the volume was made upto the mark with distilled water. The absorbance of these solutions was measured. The amount of Thiocolchicoside present was determined by using slope and inercept values from calibration graph.

Assay of formulation

Twenty capsule(Myoril containing 4mg of Thiocolchicoside) were accurately weighed and the average weight was found. The capsule powder of Thiocolchicoside 0.551mg was weighed accurately and transferred into 100ml Standard flask. Added about 100ml of distilled water to dissolve the substance and the solution was sonicated for 10 minutes. The solution was filtered through Whatmann filter paper grade no:1. From the clear solution, further dilutions were made by diluting 2 ml was pipetted out into a series of six 10ml volumetric flask and made upto the mark with distilled water to get the concentration of 20µg/ml of Thiocolchicoside. The absorbance of six solutions were measured and the amount was calculated by using regression equation.

Accuracy

The amount of 0.01g of Thiocolchicoside was accurately weighed and transferred into 100ml Standard flask, 100ml of distilled water was added to dissolve the substance. This solution contains 100µg/ml. To the pre-analyzed formulation, a known quantity of raw material of Thiocolcicoside was added and the procedure was followed RSD were calculated procedure was repeated for three times for each concentration. The % RSD were calculated.

Ruggedness

Ruggedness of method was confirmed by the analysis of formulation was done by using different instruments and different analysts. The amount was calculated. The %RSD were calculated.

Robustness

The robustness method was confirmed by analysis of drug substances by using different conditions such as temperature, solvent ratio, and wavelength. The concentration was calculated. The % RSD was calculated.

LOD and LOQ

The limit of detection (LOD) is the lowest concentration at which the results still satisfy some predetermined acceptance criteria. Below the LOD, the results fail to meet these criteria (analysis is not feasible). It may expressed

LOD = 3.3× σ/S

Where, σ standard deviation of response

S slope of calibration curve

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and degradation products.

LOQ = 10 × σ /S

Where, σ standard deviation of response

RESULTS AND DISCUSSION

LINEARITY

Different aliquots of thiocolchicoside were prepared for this study, the calibration curve was plotted against various concentration vs absorbance prepared by diluting with water. Further the sample solutions are scanned between the range of 200-400nm against water as blank. The absorbance of sample solution is recorbered and to construct the calibration curve by using the readings. On plotting the calibration curve the correlation co-efficient was found to be R^2 = 0.9978., and the regression equation was found to be y =0.0379x+0.0153.

Table no 2: linearity of thiocolchicoside at selected wavelength.

S.NO	CONCENTRATION(µg/ml)	ABSORBANCE at 260 nm
1.	0	0.000
2.	2	0.089
3.	4	0.162
4.	6	0.242
5.	8	0.330
6.	10	0.392
7.	12	0.480
8.	14	0.563
9.	16	0.631
10.	18	0.717

11.	20	0.751
12.	22	0.856
13.	24	0.903

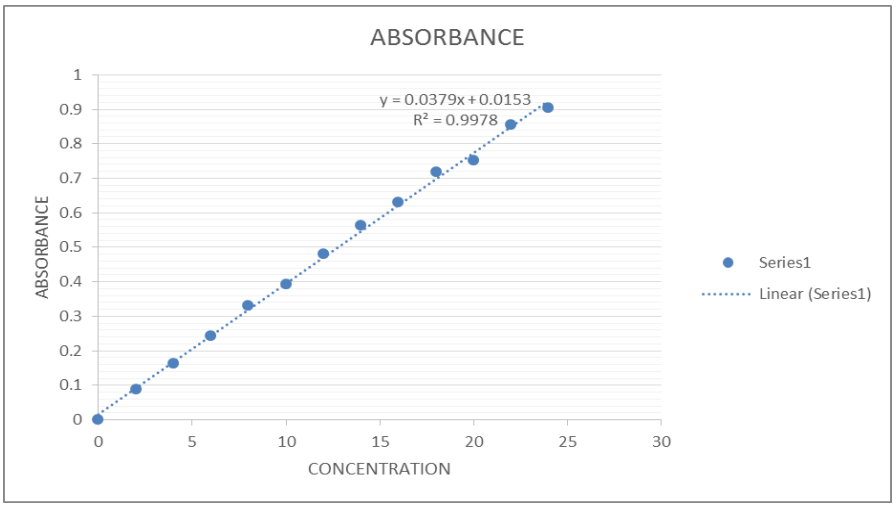

Fig. no. 2: linearity of thiocolchicoside.

PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. For interday precision the samples of thiocolchicoside were analyzed for three times on three consecutive days, while for intraday analysis the samples are taken and analyzed three times within same day. And to validate by calculating mean, SD, and %RSD. The results are shown in table n.o 3,4.

Table no 3: Interday precision of thiocolchicoside.

CONCENTRATION	ABSORBANCE		
	SAMPLE 1	SAMPLE 2	SAMPLE 3
20µg/ml	0.757	0.756	0.758
	0.756	0.769	0.775
	0.758	0.775	0.777
Mean	0.755	0.766	0.770
SD	0.003606	0.0097125	0.01044
%RSD	0.477556	1.2668524	1.355884

Table no 4: intraday precision of thiocolchicoside.

CONCENTRATION	ABSORBANCE		
	SAMPLE 1	SAMPLE 2	SAMPLE 3
20µg/ml	0.753	0.771	0.777
	0.752	0.771	0.781
	0.756	0.773	0.780

Mean	0.75367	0.7716667	0.77933
SD	0.00208	0.0011547	0.00208
%RSD	0.27621	0.14963722	0.26711

LOD and LOQ

Different aliquots of Thiocolchicoside was prepared in the concentration range of 2 to 24µg/ml. The absorbance of solutions were measured at 260nm. The calibration graph was plotted using concentration against absorbance. The preparation of calibration curve was repeated for six times. The optical characteristics like Correlation coefficient, Slope, Intercept, LOD and LOQ were calculated. The correlation coefficient value for the calibration graph was found to be 0.9978. This indicates that the absorbance was linear with the concentration range of 2 - 24 µg/ml. The results are shown in table n.o 5.

Table no 5: LOD & LOQ of thiocolchicoside.

DRUG	PARAMETER	
	LOD	LOQ
Thiocolchicoside	0.2943	0.8919

ASSAY

The assay provide an exact result which allows an accurate statement of the content or potency of the analyte in a sample. The assay of thiocolchicoside was performed by tablet powder of thiocolchicoside 0.551mg was weighed accurately and transferred into 100ml Standard flask. Added about 100ml of ethanol to dissolve the substance and the solution was sonicated for 10 minutes. The solution was filtered through Whatmann filter paper grade no:1. From the clear solution, further dilutions were made by diluting 2ml was pipetted out into a series of six 10ml volumetric flask and made upto the mark with distilled water to get the concentration of 20µg/ml of thiocolchicoside. The absorbance of six solutions were measured and the amount was calculated by using regression equation. The results are shown in table n.o 6.

Table no 6: Assay of thiocolchicoside

SAMPLE	TAKEN AMOUNT (µg/ml)	AMOUNT FOUND (µg/ml)	% obtained	AVERAGE %	S.D.	%RSD
1	20	19.81	99.05			
2	20	20.12	100.6			
3	20	20.18	100.9			
4	20	20.26	101.3			
5	20	20.26	101.3	100.52%	0.87164	0.86708
6	20	20	100			

Ruggedness

Ruggedness of method was confirmed by the analysis of formulation was done by using different instruments and different analysts. The amount was calculated. The %RSD were calculated. The results are shown in table n.o 7.

Table no 7: ruggedness study of thiocolchicoside.

Condition	Labelled Amount (µg/cap)	Amount Found (µg/cap)	Percentage Obtained	Mean (%)	SD	RSD
Analyst 1	20	3.92	98%	0.166003	0.001819	1.095806
		4	100%			
		4.01	100.25%			
Analyst 2	20	3.94	98.5%	0.165131	0.001578	0.955544
		3.92	98%			
		4	100%			

ROBUSTNESS

The developed method was validated for Robustness. It refers to the analysis should be done in different wavelength like 258 nm, 260 nm, 274nm. The percentage RSD value for those three wavelength were found to be 1.521741and 1.357171 and 1.357171 respectively. The low % RSD values indicate that the method was more robusted. The results are shown in table 8

Table no 8: robustness study of thiocolchicoside.

CONCENTRATION	ABSORBANCE		
	SAMPLE 1	SAMPLE 2	SAMPLE 3
258	0.735	0.788	0.784
260	0.744	0.794	0.791
262	0.760	0.809	0.808
MEAN	0.747	0.797	0.794
SD	0.011372	0.010817	0.012342
%RSD	1.521741	1.357171	1.553798

ACCURACY

The accuracy of the method was confirmed by recovery studies. To the pre-analyzed formulation a known quantity of Thiocolchicoside raw material solution was added at three different concentrations. The concentration ofstandard raw material added were 16, 20 and 24µg/ml of the sample concentration. The absorbance was measured and the percentage recovery was calculated. The results were shown in table 9.

Table no. 9: Accuracy study for thiocolchicoside.

%	AMOUNT PRESENT (µg/ml)	AMOUNT ADDED (µg/ml)	AMOUNT ESTIMATED (µg/ml)	AMOUNT RECOVERED (µg/ml)	% RECOVERY	S.D.	%RSD
80%	20	16	36	36.03	100	0.00472	0.340886
	20	16	36	36.27	100.75		
	20	16	36	36.17	100.47		
100%	20	20	40	40.04	100.1	0.00503	0.327187
	20	20	40	40.20	100.5		
	20	20	40	40.31	100.775		
120%	20	24	44	50.04	100.08	0.004	0.208768
	20	24	44	50.26	100.52		
	20	24	44	50.15	100.3		

CONCLUSION

A simple, rapid, precise and accurate UV spectrophotometric method were developed validated for estimation of Thiocolchicoside in bulk and pharmaceutical dosage form. The method employed for the analysis of Thiocolchicoside was UV spectroscopic method.

The solvent selected for solubility was Distilled water. The λ max of Thiocolchicoside was 260nm Thiocolchicoside. was linear with the concentration range of 2 – 24 ug/ml. The correlation coefficientfor the calibration graph was found to be 0.993. The percentage of Thiocolchicoside presentin the prepared raw material solution was found to be 99.90 ± 0.690. Myoril containing 4mg value in Thiocolchicoside were selected for analysis. The percentage label claim present in the tablet confirmed by the repeated analysis of formulation. The precision of the method was confirmed by the repeated analysis of formulation. The %RSD was found to be 1.381377.

Further precision of the method was confirmed by intraday and inter day analysis. The percentage RSD value for inter day analysis of thiocolchicoside was found to be for sample 1(0.003606), sample 2 (0.0097125), sample 3 (0.01044) andintraday sample 1 (0.00208), sample 2 (0.0011547, sample 3 (0.00208) respectively.

The developed method was validated for ruggedness. The percentage RSD value analyst 1 and analyst 2 were found to be 1.0958and 0.9555, respectively. The low % RSD value indicate that the developed method was more rugged.

The percentage RSD value for those three wavelengthwere found to be 1.521741 and 1.357171 and 1.553798 respectively. The low % RSD values indicate that the method was

more robusted. The accuracy of the method was confirmed, the percentage was found to be in the range of 98 to 100.25% of Thiocolchicoside.

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