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## Development and Validation of Area Under Curve Method for Simultaneous Estimation of Thiocolchicoside and Lornoxicam in Tablet Dosage Form

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### ABSTRACT:

Area Under Curve Method has been developed for the quantitative estimation of Thiocolchicoside (THC) and Lornoxicam (LNC) in combined tablet dosage form. The wavelength ranges selected for estimation of THC were 365 - 375 nm ( $\lambda_1$ - $\lambda_2$ ) and for LNC wavelength ranges selected were 285 - 295 nm ( $\lambda_3$ - $\lambda_4$ ) and area were integrated between these selected wavelength ranges for both drugs. The method was linear in the range of 4-20  $\mu\text{g/ml}$  ( $r_2 = 0.9998$ ) for THC and 4-20  $\mu\text{g/ml}$  ( $r_2 = 0.9994$ ) for LNC. The results of the precision study indicate that the proposed method showed good repeatability for THC and LNC with % CV of 0.16 % and 0.28 % respectively. The % CV for intraday precision was found to be 0.11 - 0.27 % for THC and 0.16 - 0.44 % for LNC. Similarly % CV from the interday precision data were found to be 0.16 - 0.54 % for THC and 0.28 - 0.62 % for LNC. The % recoveries for THC and LNC obtained in the accuracy study were 99.25-100.41% and 99.43-100.27% respectively. The LOD for THC and LNC was found to be 0.0330  $\mu\text{g/ml}$  and 0.3588  $\mu\text{g/ml}$  respectively. Similarly LOQ for THC and LNC was found to be 0.1002  $\mu\text{g/ml}$  and 1.0875  $\mu\text{g/ml}$  respectively. The % assay results of 99.20% for THC and 99.42% for LNC indicate that the developed method was successfully utilised for the estimation of THC and LNC in their tablet formulation.

**KEY WORDS:** Thiocolchicoside, Lornoxicam, Area Under Curve method, simultaneous estimation.

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### INTRODUCTION:

The Area under curve method usually applicable when there is no sharp peak or when broad spectra are obtained. It concerns with the calculation of integrated value of absorbance in terms of area with respect to the wavelength range between the two selected wavelengths  $\lambda_1$  and  $\lambda_2$ . The area covered by the curve and the horizontal axis is calculated. The wavelength ranges to be entered over which area has to be calculated between two horizontal axes. The wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration.

THIOLCHICOSIDE is a muscle relaxant agent with anti-inflammatory and analgesic actions. It is a colchicines derivative (3-demethyl- thiocolchicoside glucoside). Thiocolchicoside acts as a competitive GABA-A receptor antagonist and also inhibits glycine receptors with similar potency and nicotinic acetylcholine receptors to a much lesser extent. It has powerful convulsant activity. Thiocolchicoside's activity is ascribed to its ability to interact with the strychnine sensitive glycine receptor and therefore being endowed with glycino-mimetic activity and produce myorelaxant effect.

LORNOXICAM (chlortenoxicam) is a non-steroidal anti-inflammatory drug (NSAIDs) of the oxicam class with analgesic, anti-inflammatory and antipyretic properties.

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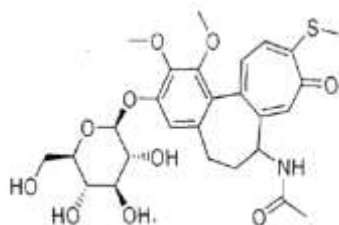
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Lornoxicam differs from other oxycam compounds in its potent inhibition of prostaglandin biosynthesis. CDSCO has approved Thiocolchicoside IP 8/16mg and Lornoxicam 8/16mg on 08/04/13 for the treatment of patients with acute painful musculoskeletal conditions. Thiocolchicoside IP 8/16mg + Lornoxicam 8/16mg Sustained release Tablets are manufactured by "Indoco Remedies Ltd" under the brand name Lorchek MR, "Genera Hindustan Inc" under the brand name Dolgen MR.



Thiocolchicoside



Lornoxicam

Thiocolchicoside is official in IP 2014[1] and includes HPLC method for estimation of THC. Lornoxicam is not official in any pharmacopoeia.[1-4] The combination of these two drugs is not official in any pharmacopoeia.[1-4] Literature review shows that numbers of analytical methods are available for estimation of both the drugs either alone or in combination with other drugs.[5-14] Based on our current and ongoing referencing work, till date, we have come across to Simultaneous equation method, Q ratio method, Absorption correction method and RP-HPLC method for simultaneous estimation of Thiocolchicoside and Etodolac in their combined dosage form. Therefore, the objective is to develop simple Area under curve method for simultaneous estimation of THC and ETD in their formulation and to validate the developed method according to ICH guidelines.

## MATERIALS AND METHODS

### 1. Instruments:

Shimadzu UV-1800, UV-Visible double beam Spectrophotometer with matching pair of 1 cm quartz cuvettes (Software –UV Probe, Version 2.42). The spectral bandwidth is 0.5 nm.

### 2. Reagents and Chemicals:

Standard APIs were kindly gifted by Genera Hindustan Inc and Tablets Dolgen MR were procured from Genera Hindustan Inc.

### 3. Methodology

### Selection of wavelength range for estimation of THC and LNC

The suitable concentrations of THC and LNC were prepared separately in methanol to get the final conc. 10 µg/ml for both THC and LNC. Both drug solutions were scanned in range of 200-400 nm and overlain spectrum was studied. After repeated observations of response the wavelength ranges were selected for estimation of THC and LNC further area was integrated between the selected wavelength ranges and estimation was carried out.

### Preparation of standard solution

#### Preparation of standard stock solution of THC (1000 µg/ml):

Accurately weighed quantity 10 mg of THC was transferred into 10 ml volumetric flask, dissolved and diluted up to mark with methanol. This gave a stock solution having strength of 1000 µg/ml.

#### Preparation of working standard solution of THC (100 µg/ml):

100 µg/ml of THC solution was prepared by diluting 2.5 ml of stock solution up to the mark with methanol in 25 ml volumetric flask.

#### Preparation of standard stock solution of LNC (1000 µg/ml):

Accurately weighed quantity 10 mg of LNC was transferred into 10 ml volumetric flask, dissolved and diluted up to mark with methanol. This will give a stock solution having strength of 1000 µg/ml.

#### Preparation of working standard solution of LNC (100 µg/ml):

100 µg/ml of LNC solution was prepared by diluting 2.5 ml of stock solution up to the mark with methanol in 25 ml volumetric flask.

#### Preparation of combined working standard solution containing THC and LNC

Accurately weighed quantity of tablet powder equivalent to 40 mg of THC and 40 mg of LNC were transferred to 100 ml volumetric flask, dissolved in sufficient amount of methanol and diluted up to mark with methanol to give concentration of 4 µg/ml of THC and 4 µg/ml of LNC.

#### Preparation of calibration curve

Calibration curve for THC consisted of different concentrations of standard THC solution ranging from 4-20 µg/ml. The solutions were prepared by transferring 0.4, 0.8, 1.2, 1.6 and 2.0 ml aliquots of the working standard solution of THC (100

µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol to get the final conc. of 4, 8, 12, 26 and 20 µg/ml for THC. Similarly for LNC, Calibration curve were ranging from 4-20 µg/ml. The aliquots from LNC (100 µg/ml) 0.4, 0.8, 1.2, 1.6 and 2.0 ml were transferred into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol to get the final conc. of 4, 8, 12, 26 and 20 µg/ml for LNC.

**Analysis of marketed formulation**

Twenty tablets were weighed; average weight was calculated then powered. A quantity of powder equivalent to 8 mg of THC and 8 mg of LNC was transferred in 100 ml volumetric flask. Methanol (50 ml) was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and residue was washed with methanol. Washing and filtrate were combined in another 100 ml volumetric flask and the volume was adjusted up to the mark with methanol. The above solution was suitably diluted with methanol to get a final concentration of 4 µg/ml of THC and 4 µg/ml of LNC.

For the simultaneous determination using the area under curve (AUC) method, sample solution of drugs were scanned in the range of 200-400 nm. The area was measured at 365-375 nm (λ<sub>1</sub>-λ<sub>2</sub>) and 285-295 nm (λ<sub>3</sub>-λ<sub>4</sub>) for quantification of THC and LNC respectively. The amounts of THC and LNC present in sample solutions were determined by fitting the response into the regression equation obtained from calibration curves of THC and LNC.

The concentration of THC and LNC was obtained by following equations:

$$C_{THC} = \frac{A_2aY_1 - A_1aY_2}{aX_2aY_1 - aX_1aY_2} \dots\dots\dots(1)$$

$$C_{LNC} = \frac{A_1aX_2 - A_2aX_1}{aX_2aY_1 - aX_1aY_2} \dots\dots\dots(2)$$

Where,

C<sub>THC</sub>= Concentrations of THC,

C<sub>LNC</sub>= Concentrations of LNC,

A<sub>1</sub>= Area of mixture at 365-375 nm,

A<sub>2</sub>= Area of mixture at 285-295 nm,

aX<sub>1</sub> = Absorptivity value of THC at 365-375 nm,

aX<sub>2</sub>= Absorptivity value of THC at 285-295 nm,

aY<sub>1</sub>= Absorptivity value of LNC at 365-375 nm,

aY<sub>2</sub>= Absorptivity value of LNC at 285-295 nm.

**VALIDATION OF AUC METHOD**

**Linearity and range**

Linearity is expressed in terms of correlation co-efficient of linear regression analysis. The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 4-20 µg/ml for THC and LNC. A lot of calibration curve of area vs. concentration and correlation coefficient and regression line equations for THC and LNC determined.

**Accuracy**

**Preparation of Sample solution:**

It was carried out to determine the suitability and reliability of the proposed method. Accuracy was determined by calculating the % Recovery. The amount of THC and LNC was calculated at each of three level and % recoveries were computed at three different levels (80%, 100% and 120%). For both the drugs THC and LNC the accuracy study performed on 4 µg/ml.

**Precision**

**Repeatability**

From the Sample solution aliquot was transferred to a separate 10 ml volumetric flask and diluted up to mark with methanol such that it gives the concentration of 4 µg/ml for both THC and LNC. Each concentration was prepared Six times. The area of the each solution was measured at selected wavelength rangess and % CV was calculated.

**Intraday precision**

Sample solutions containing 4, 12, 20 µg/ml of THC and LNC were separately analyzed three times on the same day and % CV was calculated.

**Interday precision**

Sample solutions containing 4, 12, 20 µg/ml of THC and LNC were analyzed on three different days and % CV was calculated.

**Limit of detection**

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. Then LOD was measured by using mathematical expressions given in section. The limit of detection (LOD) of the drug was calculated by using the following equations designated by ICH guideline:

$$LOD = 3.3 \times \sigma/S,$$

Where, σ = the standard deviation of the Intercept

S = Mean slope of the calibration curve

**Limit of quantification**

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. Then LOQ was measured by using mathematical expressions given in section. The limit of quantification (LOQ) of the drug was calculated by using the following equations designated by ICH guideline:

$$LOQ = 10 \times \sigma / S$$

Where,  $\sigma$  = the standard deviation of the Intercept  
 S = Mean slope of the calibration curve

**RESULTS**

The wavelength ranges selected for estimation of THC were 365 - 375 nm ( $\lambda_1$ - $\lambda_2$ ) and for LNC wavelength ranges selected were 285 - 295 nm ( $\lambda_3$ - $\lambda_4$ ). Linearity were found to be in range of 4-20  $\mu\text{g/ml}$  ( $r^2 = 0.9998$ ) for THC and 4-20  $\mu\text{g/ml}$  ( $r^2 = 0.9994$ ) for LNC. The results in terms of % CV for repeatability study were found to be 0.16 % and 0.28 % for THC and LNC respectively. The % CV for intraday precision was found to be 0.11 - 0.27 % for THC and 0.16 - 0.44 % for LNC. Similarly % CV from the interday precision data were found to be 0.16 - 0.54 % for THC and 0.28 - 0.62 % for LNC. The % recoveries for THC and LNC obtained in the accuracy study were 99.25-100.41% and 99.43-100.27% respectively. The LOD for THC and LNC was found to be 0.0330  $\mu\text{g/ml}$  and 0.3588  $\mu\text{g/ml}$  respectively while LOQ for THC and LNC was found to be 0.1002  $\mu\text{g/ml}$  and 1.0875  $\mu\text{g/ml}$  respectively. The % assay results by AUC method were found to be 99.20% for THC and 99.42% for LNC.

**CONCLUSION**

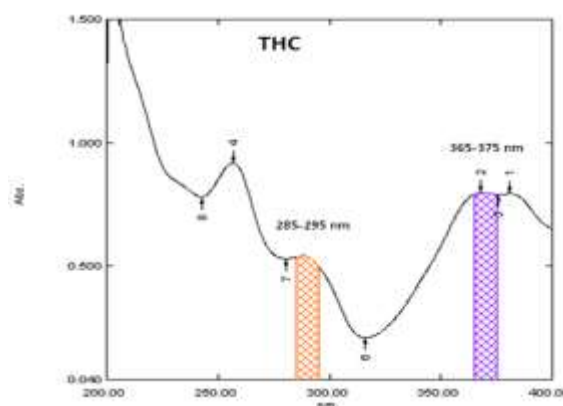
The simple Area under curve method for simultaneous estimation of Thiocolchicoside and Lornoxicam in combined dosage form was developed and validated. The method was found to be accurate and precise and it was sensitive to the smallest changes in the concentration. The % assay results of 99.20% for THC and 99.42% for LNC indicate that the developed method can be successfully utilized for routine analysis for simultaneous estimation of THC and LNC in their tablet formulation.

**ACKNOWLEDGEMENT**

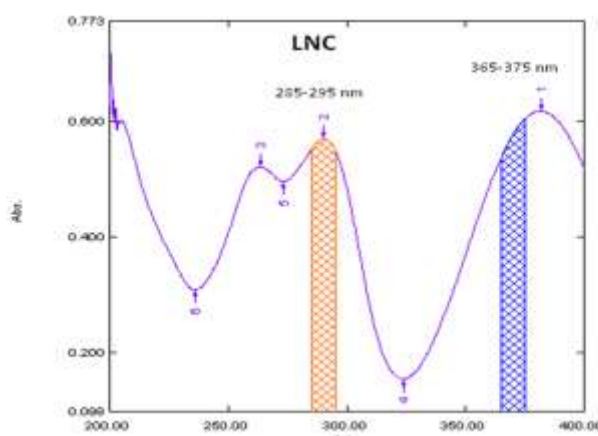
The authors are thankful to Genera Hindustan Inc, Hyderabad, India for providing standard sample of drugs and also to the ROFEL Shri G. M. Bilakhia College of Pharmacy, India for providing facilities to carry out research work.

**Table 1: Summary of Validation Parameters**

PARAMETERS	THC	LNC
<b>Linearity (n=5)</b>	0.9998	0.9994
<b>Linear Range</b>	4-20 $\mu\text{g/ml}$	4-20 $\mu\text{g/ml}$
<b>Accuracy (n=3)</b>	99.25-100.41 %	99.43-100.27 %
<b>Precision (% CV)</b>		
<b>Repeatability (n=6)</b>	0.1647	0.2874
<b>Intraday (n=3)</b>	0.11 - 0.27	0.16 - 0.44
<b>Interday (n=3)</b>	0.16 - 0.54	0.28 - 0.62
<b>LOD (<math>\mu\text{g/ml}</math>)</b>	0.0330 $\mu\text{g/ml}$	0.3588 $\mu\text{g/ml}$
<b>LOQ (<math>\mu\text{g/ml}</math>)</b>	0.1002 $\mu\text{g/ml}$	1.0875 $\mu\text{g/ml}$



**Figure 1: Spectrum of THC (365-375 nm)**



**Figure 2: Spectrum of LNC (285-295 nm)**

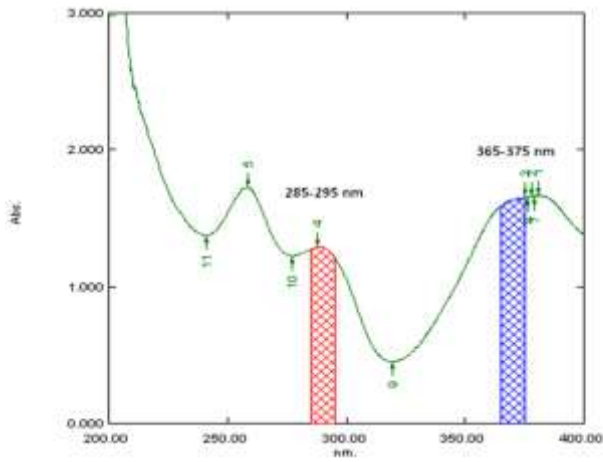


Figure 3: Spectrum of Sample solution

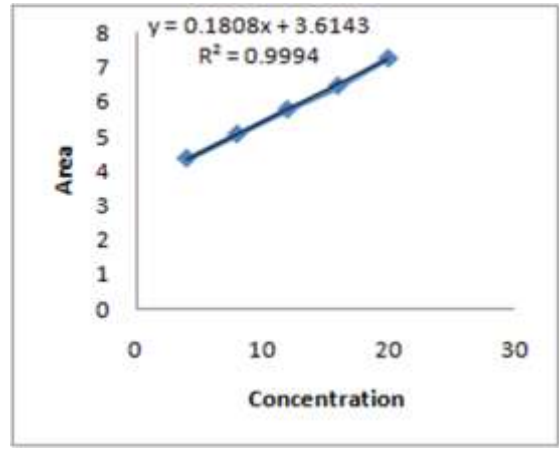


Figure 6: Calibration curve of LNC for area at 285-295 nm

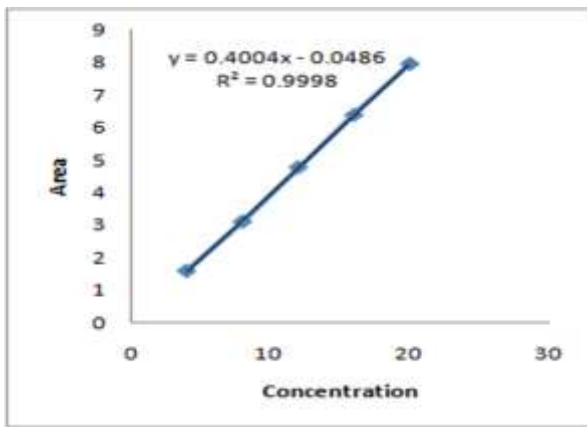


Figure 4: Calibration curve of THC for area at 365-375 nm

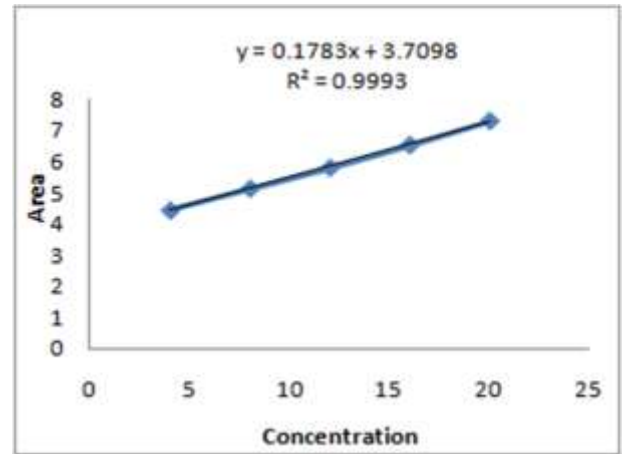


Figure 7: Calibration curve of LNC for area at 365-375 nm

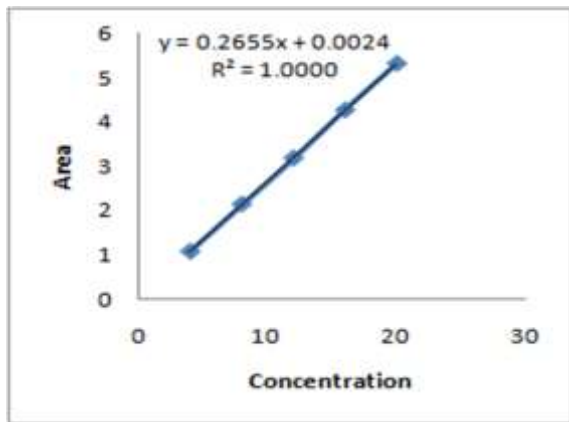


Figure 5: Calibration curve of THC for area at 285-295 nm

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