SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF THIOCOLCHICOSIDE AND DEXKETOPROFEN TROMETAMOL IN PHARMACEUTICAL DOSAGE FORM

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Abstract
The present manuscript describes simple, sensitive, rapid, accurate, precise and economic dual wavelength spectrophotometric method for simultaneous determination of Thiocolchicoside and Dexketoprofen trometamol in combined tablet dosage form. The utility of dual wavelength data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. The method was based on property of additivity of absorbances. The two wavelengths on UV spectrum of Thiocolchicoside were found out where it showed same absorbance, which were 368 nm and 284.60 nm. At 368 nm Thiocolchicoside showed some absorbance whereas Dexketoprofen trometemol showed zero absorbance. Both the drugs gave absorbance at 284.60 nm. The method involved solving of an equation based on measurement of absorbances at two wavelengths 284.6 and 368 nm. The two drugs follow Beer-Lambert’s law over the concentration range of 2-24 µg/ml. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found. The results of analysis have been validated statistically and by recovery studies.

Keywords: Dual wavelength spectrophotometric method, Validation, Thiocolchicoside, Dexketoprofen Trometamol

1. Introduction
Dexketoprofen trometamol (DKP) chemically, 2-amino-2-(hydroxymethyl) propane-1,3-diol; 2-(3-benzoylphenyl propanoic acid is a water-soluble salt of the dextrorotatory enantiomer or (S)+(+)-enantiomer of the nonsteroidal anti-inflammatory drug (NSAID) ketoprofen[1, 2]. The enantiomer is a relatively new oral NSAID with analgesic, anti-inflammatory and anti-pyretic properties and is one of the most potent in vitro inhibitors of prostaglandin synthesis. Thiocolchicoside (THC) chemically, N-[(7S)-3-(beta-D-glucopyranosyloxy)-1, 2-dimethoxy-10-(methylsulfanyl)-9-oxo-5, 6, 7, 9-tetrahydrobenzo[a]heptalen-7-yl] acetamide, a semi-synthetic derivative of the naturally occurring compound colchicoside with a relaxant effect on skeletal muscle, has been found to displace both [3H]gamma-amino butyric acid ([3H]GABA) and [3H]strychnine binding, suggesting an interaction with both GABA and strychnine-sensitive glycine receptors[3, 4, 5], potent competitive antagonist of GABA function. THC is also shows muscular relaxant and displays anti-inflammatory and analgesic properties. The combination of 4mg of Thiocolchicoside and 25mg of dexketoprofen trometamol is available in tablet dosage form. Thiocolchicoside is official in IP [6] and dexketoprofen trometamol is not official in any pharmacopoeia. A deep Literature survey shows that combination of these two drugs is not official in any pharmacopoeia and no official or reported method is available for simultaneous estimation of THC and DKP in combined dosage form. Various reported methods are available for estimation of THC such as U.V [7, 8, 9], HPTLC [10], HPLC [11, 12], and U.V [13], HPLC [13, 14, 15, 16], HPTLC [17] for DKP. In the present investigation an attempt has been made to develop simple, rapid, economic and accurate spectrophotometric method for simultaneous estimation of THC and DKP from the pharmaceutical formulation. The method was based on dual wavelength data processing programe (dual wavelength spectrophotometry).

2. Materials and Methods
2.1 Apparatus: A double beam UV/Visible spectrophotometer (shimadzu model 1600,
Japan), attached to the computer software UV-Probe system software (UV Probe version 2.10), with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was employed. Spectra were automatically obtained by a Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

2.2 Reagents and Chemicals: Kindly gifted reference standard of THC (Astron Research Centre, Ahmadabad) and DKP (Troika Pharmaceuticals, Ahmadabad) were used without further purification for the study. The commercial fixed dose combination of tablet dosage form (each tablet contains THC, 4 mg and DKP, 25 mg) was procured from the local market. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

2.3 Preparation of Standard and sample Solution: Accurately weighed 5 mg of THC and DKP standard powder were transferred in two separate 50 ml volumetric flasks, dissolved in methanol and volumes were made up to mark with the same solvent to obtained the final solution having concentration of 100 µg/ml.

Twenty tablets were weighed and the average weight was calculated. The tablet powder equivalent to 4 mg of THC and 25 mg of DKP were weighed and transferred to 100 ml volumetric flask. Methanol (30 ml) was added and sonicated for 20 min, and volume was made up to the mark with methanol. The solution was filtered through Whatman filter paper no. 41 and filtrate was suitably diluted with methanol to achieve a final concentration of 2 µg/ml of THC and 12.5 µg/ml of DKP. The absorbance of final solution was recorded at selected wavelengths for determination of THC and DKP. The analysis procedure was repeated three times with tablet formulation.

2.4 Selection of wavelength for estimation of THC and DKP: Absorbance spectrum of pure THC was scanned in the spectrum basic mode. DKP shows absorbance value at 284.60 nm (\(\lambda_2\)) while it doesn’t shows any absorbance at 368 nm (\(\lambda_1\)). The absorbance at 368 nm was due to THC only in combined dosage form. The absorbance of various dilutions of THC and DKP in methanol was measured at \(\lambda_1\) and \(\lambda_2\) as depicted in Fig. 1. At these two wavelengths, the absorbance difference for THC at any concentration was found to be zero while for DKP, absorbance difference was found to increase as the concentration was increased.

2.5 Validation of the proposed method: The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [18].

2.5.1 Linearity (Calibration curve): Aliquots from the standard stock solutions (100 µg/ml) of THC and DKP were used to prepare different sets of dilutions. Mixed series consisted of different concentration of THC and DKP in the range of 2-24 µg/ml. The solutions were prepared by pipetting out 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 ml of the stock solutions of both drugs in to a series of 10 ml volumetric flasks and the volume was made up to the mark with methanol.

![Fig. 1: Overlaid Spectra of Thiocochicoside and Dexketoprofen trometemol (12 µg/ml)](image-url)
2.5.2 Method precision (repeatability): The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for THC and DKP (12 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method.

2.5.3 Intermediate precision (reproducibility): The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of THC and DKP (4, 8, 12 µg/ml for THC and DKP). The result was reported in terms of relative standard deviation (% RSD).

2.5.4 Accuracy (recovery study): The accuracy of the method was determined by calculating the recoveries of THC and DKP by the standard addition method. Known amounts of standard solutions of THC and DKP were added at 50%, 100% and 150 % level to prequantified sample solutions of THC and DKP (2 µg/ml for THC and 12.5 µg/ml DKP). The amounts of THC and DKP were estimated by applying obtained values to the respective regression line equations.

2.5.5 Limit of detection and Limit of quantification: The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S} \\
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where, \(\sigma\) = the standard deviation of the response
And, \(S\) = slope of the calibration curve.

2.6 Analysis of THC and DKP in combined tablet dosage form: The absorbance of the final sample solution was measured against methanol as blank at 284.60 nm and 368 nm. The amount of THC and DKP was computed using equation of the straight line.

3. Result and Discussion
The utility of dual wavelength data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. Two specific wavelengths are chosen: (I) First wavelength, \(\lambda_1\) at which minimum absorbance of THC and there was zero absorbance of DKP was observed. (II) Second wavelength \(\lambda_2\) was the wavelength at which the absorbance of THC was same as at \(\lambda_1\). In the proposed procedure the absorbance of DKP alone in the mixture of THC and DKP was determined using dual wavelength data processing program. To remove the interference of THC to the absorbance at 284.60 nm \(\lambda_2\), the wavelength of the reasonable absorbance for DKP, another wavelength 368 nm \(\lambda_1\) was found out at which the absorbance of DKP was zero. This was confirmed by various dilution of THC in methanol at 284.60 and 368 nm, respectively. The absorbance at these two wavelengths was found to be equal for THC. These two selected wavelengths were employed to determine the concentration of DKP from the mixture of THC and DKP. The difference in absorbance at these two wavelengths \((A_{284.60} - A_{368})\) cancels out the contribution of absorbance of THC in measurement of DKP at 284.60 nm and the difference in the absorbance was proportional to the concentration of DKP in the mixture.

The proposed method was found to be simple, sensitive, rapid, accurate, precise and economic for the routine simultaneous estimation of two drugs. The linearity range for Thicocolchicoside and Dexketoprofen Trometemol was found to be 2-24 µg/ml. Regression analysis data and summary of all validation parameters are given in Table 1. Precision was calculated as repeatability (% RSD) and intra and inter day variation (% RSD) for both the drugs. The LOD and LOQ were found to be 0.15 and 0.48 µg/ml, respectively for THC and 0.14 and 0.42 µg/ml, respectively for DKP indicates sensitivity of the proposed method.

Accuracy was determined by calculating the recovery study at 3 different concentration levels. Known amounts of standard solutions of THC and DKP were added at 50%, 100% and 150 % level to prequantified sample solutions of THC and DKP (2 µg/ml for THC and 12.5 µg/ml DKP), and the mixture were analyzed by the proposed method and the result was shown in the Table-2. The method was successfully used to determine the amounts of THC and DKP present in tablet formulation containing 4mg of THC and 25mg of DKP. The result was shown in the Table-3.
Table 1: Regression analysis data and summary of validation parameters for the proposed method

| Parameters                      | THC            | DKP            |
|--------------------------------|----------------|----------------|
| Wavelength (nm)                | 368            | 284.60         |
| Beer’s Law Limit (µg/ml)       | 2-24 µg/ml     | 2-24 µg/ml     |
| Regression equation (y = mx + c)| y = 0.022x + 0.032 | y = 0.024x - 0.017 |
| Slope (m)                      | 0.022          | 0.024          |
| Intercept (c)                  | 0.032          | 0.017          |
| Correlation Coefficient (r²)   | 0.997          | 0.997          |
| Sandell’s sensitivity (µg/cm²/0.001AU) | 0.037 | 0.044 |
| Repeatability (%RSD, n=6)      | 0.25           | 0.47           |
| Interday (n=3) (% RSD)         | 0.73-1.40%     | 0.90-1.57%     |
| Intraday (n=3) (% RSD)         | 0.17-0.22%     | 0.37-0.52%     |
| LOD b                          | 0.159          | 0.14           |
| LOQ c                          | 0.48           | 0.42           |

RSD a = Relative standard deviation. LOD b = Limit of detection. LOQ c = Limit of quantification

Table 2: Recovery data of proposed method

| Drug | Level | Amount taken (µg/ml) | Amount added (%) | %Recovery ± RSD. (n = 5) |
|------|-------|----------------------|-------------------|--------------------------|
| THC  | I     | 4                    | 50                | 102±1.75                 |
|      | II    | 4                    | 100               | 102.4±1.69               |
|      | III   | 4                    | 150               | 103.3±1.17               |
| DKP  | I     | 25                   | 50                | 98.22±1.28               |
|      | II    | 25                   | 100               | 102.2±0.29               |
|      | III   | 25                   | 150               | 100.22±0.79              |

RSD is relative standard deviation and n is number of replicate

Table 3: Analysis of THC and DKP by Proposed method

| Label claim (mg/tab) | Amount found (mg/tab) | %Label claim± RSD. (n=6) |
|----------------------|-----------------------|--------------------------|
| THC                  | DKP                   | THC                      | DKP                      |
| 4                    | 25                    | 3.9±0.25                 | 25.32±0.039              | 97.50±0.64 | 101.28±0.07 |

RSD is relative standard deviation and n is number of replicate

**Conclusion**

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of THC and DKP in tablet dosage form. The method utilizes easily available and cheap solvent for analysis of THC and DKP hence the method was also economic for estimation of THC and DKP from tablet dosage form. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of THC and DKP in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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