DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF THIOCOLCHICOSIDE AND DEXKETOPROFEN TROMETAMOL IN PHARMACEUTICAL DOSAGE FORM


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ABSTRACT
Rapid, economical accurate and precise, methods have been developed for estimation of Thiocolchicoside and Dexketoprofen trometamol in tablet dosage form. In first order derivative spectroscopy, wavelengths selected for quantitation were 233.7 nm for Thiocolchicoside (zero cross for Dexketoprofen trometamol) and 243.0 nm for Dexketoprofen trometamol (zero cross for Thiocolchicoside). Linearity for detector response was observed in the concentration range of 4-12 μg/ml for Thiocolchicoside and 25-75 μg/ml for Dexketoprofen trometamol. The proposed method was successfully applied for the simultaneous determination of both drugs in commercial tablet preparation. The results of the analysis have been validated statistically.

Keywords: Thiocolchicoside, Dexketoprofen trometamol, First Order derivative spectroscopy.

INTRODUCTION
Thiocolchicoside (THIO) chemically, N-[3-(B-D-glucopyrano xyloxy)-5, 6, 7, 9-tetrahydro-1,2-imethoxy-10-(methylthio)-9-oxobenzo[a] heptalen-7yl] acetamide. It has selective affinity for g-amino- butyric acid (GABA) receptors and acts on the muscular contracture by activating the GABA- inhibitory pathways thereby acting as a potent muscle relaxant [1].

Dexketoprofen trometamol (DEX) Chemically, (2S)-2-[3-(benzoyl) phenyl] propanoic acid. Dexketoprofen trometamol belongs to a class of medicines called non-steroidal anti-inflammatory drugs (NSAIDs). It works by blocking the action of a cyclo-oxygenase (COX) [2].
Both the drugs are available in combined dosage form (4: 25 mg) \[^{[3]}\]. Literature survey reveals that Thiocolchicoside can be estimated by spectrophotometry \[^{[4-12]}\], HPLC \[^{[13-23]}\] and by HPTLC \[^{[24-25]}\] methods individually or in combination with other drugs. Dexketoprofen trometamol is reported to be estimated by spectrophotometry \[^{[26]}\], HPLC \[^{[27-28]}\] and HPTLC \[^{[29]}\] individually or in combination with other drugs. However, there is no analytical method reported for the estimation of THIO and DEX in a combined dosage formulation. Present work describes First order derivative spectroscopic method for simultaneous estimation of THIO and DEX in tablet formulation.

**MATERIALS AND METHODS**

**Instruments:**
Shimadzu UV-1800, UV-Visible double beam Spectrophotometer with matching pair of 1 cm quartz cuvettes (Shimadzu Corporation, Kyoto, Japan). The spectral bandwidth is 0.5 nm. Electronic analytical balance (Shimadzu AUX-220) was used in study.

**Reagents and Chemicals:**
Standard Thiocolchicoside (THIO) and Dexketoprofen trometamol (DEX) were kindly gifted by Lincoln pharma Ltd., Khatraj., distilled water was used as solvent. Combined dose tablet formulation containing THIO and DEX (4:25 mg) were purchased from local market.

**Methodology (First order derivative method):**
In this method solutions of THIO (4-12 g/ml) and DEX (25-75 g/ml) were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra thus obtained were derivatized for first order. From the overlain spectra of both drugs (Fig. 1), wavelength selected for
quantitation were 233.7 nm for THIO (zero cross for DEX) and 243.0 nm for DEX (zero cross for THIO). The calibration curves for THIO (4-12 g/ml) and DEX (25-75 g/ml) were plotted at wavelength 233.7 nm and 243.0 nm, respectively. The concentration of the individual drug present in the mixture was determined against the calibration curve in quantitation mode.

Method validation:

Linearity and Range:
The linearity of measurement was evaluated by analyzing different concentration of the standard solution of THIO and DEX. Beer-Lambert’s was followed in the concentration range of 4-12 g/ml and 25-75 g/ml for THIO and DEX, respectively (Table no.1.2.).

Accuracy:
To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels. A known amount of drug was added to preanalyzed tablet powder and percentage recoveries were calculated (Table no.4.).

Precision:
The Repeatability study of THIO (4 g/ml) and DEX (25 g/ml) were determined by estimating the corresponding response 6 times. The intraday and interday precisions of the proposed spectroscopic method were determined by estimating the corresponding response 3 times on the same day and on 3 different days for 3 different concentrations of THIO (4, 8 and 12 g/ml) and DEX (25, 52 and 75 g/ml) and the results are reported in terms of % C.V (Table no.4.)

LOD and LOQ:
The LOD and LOQ were determined based on the standard deviation of the y-intercept and slope of the calibration curves. LOD and LOQ, 0.12 g/ml and 0.36 g/ml for THIO, 1.44 g/ml and 4.36 g/ml for DEX (Table no.4.).

Estimation of Thiocolchicoside and Dexketoprofen trometamol in the marketed formulation:
Twenty tablets were weighed and finely powdered. The powder equivalent to 4 mg of THIO and 25 mg of DEX was weighed accurately, mixed with distilled water (50 ml) in 100 ml volumetric flask, kept in ultrasonic water bath for 10 min and diluted up to mark
with distilled water. The solution was filtered through Whatman No.41 filter paper. 1 ml of aliquot was transferred in 10 ml volumetric flask and make up the volume up to 10 ml with distilled water. An Absorbance of diluted solution was measured at 233.7 nm and 243 nm. Amounts of THIO and DEX in the tablets were calculated by referring equation of straight line (Table no.5.).

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of THIO and DEX. In first order derivative spectroscopy, wavelengths selected for quantitation were 233.7 nm for THIO (zero cross for DEX) and 243.0 nm for DEX (Zero cross for THIO). Linearity was observed in the concentration range of 4-12 μg/ml for THIO and 25-75 μg/ml for DEX. In method Percent label claim for THIO and DEX in tablet analysis, was found 99.65% and 99.81%, respectively. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Percent recovery for THIO and DEX was found in the range of 98.99% to 101.66% and 99.34% to 100.31%, respectively. Values of standard deviation and coefficient of variation was satisfactorily low, indicating the accuracy of both the methods. Intra-day and inter-day precision studies were carried out by analyzing the tablet powder at different time interval on same day and on three different days, respectively. Standard deviation and coefficient of variance for intra-day and inter-day precision studies was found to be less than 2 indicating precision of proposed method. Based on the result obtained, it is found that the proposed method are accurate, precise, reproducible & economical and can be employed for routine quality control of Thiocolchicoside and Dexketoprofen trometamol in combined dose tablet formulation.
Figure 1
Overlain 1st order derivative spectra of THIO and DEX

Table 1: Lambert-Beer’s Curve Data for THIO at 233.7 NM

<table>
<thead>
<tr>
<th>Conc. of THIO (µg/ml)</th>
<th>Response Mean ± S.D</th>
<th>% C.V</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.004 ± 0.000002</td>
<td>0.51</td>
</tr>
<tr>
<td>6</td>
<td>0.007 ± 0.00005</td>
<td>0.82</td>
</tr>
<tr>
<td>8</td>
<td>0.010 ± 0.00007</td>
<td>0.70</td>
</tr>
<tr>
<td>10</td>
<td>0.012 ± 0.00007</td>
<td>0.56</td>
</tr>
<tr>
<td>12</td>
<td>0.016 ± 0.00007</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Figure 2
Calibration curve for THIO (4 – 12 g/ml) at 233.7 nm
**TABLE 2: LAMBERT-BEER’S CURVE DATA FOR DEX AT 243 NM**

<table>
<thead>
<tr>
<th>Conc. of DEX (µg/ml)</th>
<th>Response Mean ± S.D</th>
<th>% C.V</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.017 ± 0.00008</td>
<td>0.49</td>
</tr>
<tr>
<td>37.5</td>
<td>0.034 ± 0.00011</td>
<td>0.33</td>
</tr>
<tr>
<td>50</td>
<td>0.052 ± 0.00013</td>
<td>0.24</td>
</tr>
<tr>
<td>62.5</td>
<td>0.069 ± 0.00005</td>
<td>0.70</td>
</tr>
<tr>
<td>75</td>
<td>0.083 ± 0.00007</td>
<td>0.80</td>
</tr>
</tbody>
</table>

*Figure 3*
Calibration curve for DEX (25 - 75 µg/ml) at 243 nm

**TABLE 3: DATA OF REGRESSION ANALYSIS OF THIO AND DEX**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Straight line equation of calibration curve</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>THIO</td>
<td>$y = 0.0015x - 0.0019$</td>
<td>0.9987</td>
</tr>
<tr>
<td>DEX</td>
<td>$y = 0.0013x - 0.0159$</td>
<td>0.9986</td>
</tr>
</tbody>
</table>

**TABLE 4: SUMMARY OF VALIDATION PARAMETERS**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>THIO</th>
<th>DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>4 – 12  µg/ml</td>
<td>25 – 75  µg/ml</td>
</tr>
<tr>
<td>Accuracy (% Recovery) (n=3)</td>
<td>98.99-101.66%</td>
<td>99.34-100.31%</td>
</tr>
<tr>
<td>Precision (% CV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability (n=6)</td>
<td>0.30</td>
<td>0.48</td>
</tr>
<tr>
<td>Intraday (n=3)</td>
<td>0.61–1.30</td>
<td>0.58–1.11</td>
</tr>
<tr>
<td>Interday (n=3)</td>
<td>0.93–1.48</td>
<td>0.70–1.45</td>
</tr>
<tr>
<td>LOD</td>
<td>0.12</td>
<td>1.44</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.36</td>
<td>4.36</td>
</tr>
</tbody>
</table>
TABLE 5: ANALYSIS OF MARKETED FORMULATION (N = 5)

<table>
<thead>
<tr>
<th>DRUG</th>
<th>ACTUAL CONC. (mg)</th>
<th>CONC. FOUND (mg)</th>
<th>% C.V</th>
<th>% PURITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>THIO</td>
<td>4</td>
<td>3.97</td>
<td>0.76</td>
<td>99.65%</td>
</tr>
<tr>
<td>DEX</td>
<td>25</td>
<td>24.86</td>
<td>0.48</td>
<td>99.81%</td>
</tr>
</tbody>
</table>

CONCLUSION

First order derivative method for simultaneous estimation of Thiocolchicoside and Dexketoprofen trometamol in combined dosage form was developed and validated. The method was found to be accurate as it is more sensitive to the smallest changes in the concentration. It has advantage that it eliminates the spectral interference from one of the two drugs while estimating the other drug by selecting zero crossing point in the derivative spectra of each drug at selected wavelength. The % assay results of 99.65% for THIO and 99.81% for DEX indicate that the developed method was successfully utilized for the estimation of THIO and DEX in their tablet formulation.

ACKNOWLEDGEMENT:

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