SIMULTANEOUS DETERMINATION OF DICLOFENAC SODIUM AND THIOCOLCHICOSIDE IN FIXED DOSE COMBINATION BY SPECTROPHOTOMETRY

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Abstract: Three accurate, precise, sensitive and economical procedures for simultaneous determination of diclofenac sodium and thiocolchicoside in tablet dosage form have been developed. The methods employed were absorbance correction method (I), first order derivative spectrophotometric method (II) and dual wavelength method (III). In the first method diclofenac sodium concentration was determined directly from calibration plot by measuring absorbance at 276.6 nm and thiocolchicoside was determined after correction for absorbance of diclofenac sodium at 372.8 nm. The second method is based on first order derivative spectroscopy to overcome spectral interference from other drug. Wavelengths 278.6 nm and 243.2 nm were selected for the determination of the diclofenac sodium and thiocolchicoside, respectively. In the third method, diclofenac sodium was determined by plotting the difference in absorbance in 244 and 269 nm (difference is zero for thiocolchicoside) against the concentration of diclofenac sodium. Similarly for the determination of thiocolchicoside, the difference in absorbance at 266.8 and 290 nm (difference is zero for diclofenac sodium) was plotted against the concentration of diclofenac sodium. The Beer’s law obeyed in the concentration range 5-30 μg/ml for diclofenac sodium and 10-60 μg/ml for thiocolchicoside. The results of analysis have been validated statistically and by recovery studies.

Keywords:Diclofenac sodium, Thiocolchicoside, Absorbance correction method, Dual wavelength method.

INTRODUCTION

Diclofenac sodium (DIC) [Sodium (o-[(2, 6-dichlorophenyl) amino phenyl] acetate) is a synthetic non steroidal anti-inflammatory drug (NSAID), has been proved to be safe and efficacious drug in the treatment of a variety of inflammatory and rheumatoid disorders [1]. Thiocolchicoside (THIO) chemically, C₂₃H₂₇Cl₂N₂O₂S₂ is a muscle relaxant which has been claimed to possess GABA mimetic and glycnergic actions. It is used in the symptomatic treatment of painful muscle spasm [2].

Literature survey reveals spectrophotometric [3, 4] and HPTLC [5, 6] determination of DIC in combination with other drugs. HPLC [7] and bioanalytical chromatographic methods [8] for quantification of DIC are also reported. For simultaneous determination of THIO with other drugs spectrophotometric [9], HPTLC [10] and HPLC methods [11, 12] are reported. No reports were found for determination of DIC and THIO by HPTLC method in fixed dose combination. Aim of present work was to develop simple, economical, rapid, accurate and precise spectrophotometric methods for determination of these drugs in fixed dose combination. The proposed methods were optimized and validated as per the International Conference on Harmonization (ICH) guidelines [13].

MATERIALS AND METHODS

Instrumentation

The instrument used in the present study was JASCO double beam UV/Visible spectrophotometer (Model UV-550) with slit width fixed at 2 nm, equipped with spectra manager software (Version 1.5-A).

Reagents and chemicals

Analytically pure samples of DIC and THIO were kindly supplied by Cipla Pvt. Ltd., Mumbai, India and Aventis Pharma Pvt. Ltd., Goa, India respectively and were used as such without further purification. The pharmaceutical dosage form used in this study was THIOACT-D4 capsules (Sun Pharmaceuticals Industries Ltd, Mumbai, India) labeled to contain 4 mg of thiocolchicoside and 50 mg of diclofenac sodium as enteric coated tablet, per capsule.

Methods

Absorbance correction method (Method I)

This method involves absorbance correction for DIC determination by subtracting absorbance of THIO from total absorbance of sample at 276.6 nm (λmax of DIC). THIO concentration was determined directly from calibration plot by measuring absorbance at 372.8 nm with ICH guidelines [13].

\[ C_{THIO} = \frac{A_{372.8} \times ax_1}{\lambda_1} \] ........................ (1)

\[ C_{DIC} = A_{276.6} - \frac{(23.335 \times C_{THIO})}{10.2346} \] ........................ (2)

First order derivative spectroscopic method (Method II)

This method is based on first order derivative spectroscopy to overcome spectral interference from other drug. Zero order spectrums of both the drugs were converted to first order derivative spectra with the help of spectra manager software (Figure 1 and Figure 2).

Absorbance correction method (Method I)

This method involves absorbance correction for DIC determination by subtracting absorbance of THIO from total absorbance of sample at 276.6 nm (λmax of DIC). THIO concentration was determined directly from calibration plot by measuring absorbance at 372.8 nm

\[ C_{THIO} = \frac{(dA/d\lambda)_{278.6} + 0.00005}{‐0.0006} \] ........................ (3)

\[ C_{DIC} \] = \[ \frac{(dA/d\lambda)_{244} + 0.00005}{0.00006} \] ........................ (4)

Dual wavelength method (Method III)

In this method difference in absorbance at two selected wavelengths

\[ C_{THIO} = (dA/d\lambda)_{266.8} \] ........................ (5)

\[ C_{DIC} = (dA/d\lambda)_{290} \] ........................ (6)

It was observed that DIC showed dA/d\λ zero at 278.6 nm in contrast to THIO that has considerable dA/d\λ at this wavelength. Further, THIO has zero dA/d\λ at 243.2 nm while at this wavelength DIC has significant dA/d\λ. Therefore wavelengths 278.6 nm and 243.2 nm were employed for the determination of THIO and DIC respectively without interference of other drug. The calibration curves were plotted at these two wavelengths of concentrations against dA/d\λ. The equations of line obtained to determine concentrations of DIC and THIO were as follows

\[ C_{DIC} = (dA/d\lambda)_{278.6} + 0.00003)/0.0009 \] ........................ (7)

\[ C_{THIO} = (dA/d\lambda)_{243.2} + 0.00005)/0.00006 \] ........................ (8)
is calculated. The difference in absorbance at 266.8 and 290 nm was found to be zero for DIC. Hence these two wavelengths were selected for the determination of THIO. Similarly, 244 and 269 nm was selected for the determination of DIC, where the difference in absorbance was found to be zero for THIO. Zero order spectra were recorded for solutions of different concentration of DIC and THIO between 200-400 nm. The difference in absorbance at 244 and 269 nm were plotted against the concentration of DIC and that 266.8 and 290 nm were plotted against the concentration of THIO to construct two separate calibration curves for DIC and THIO. The equations of line obtained to determine concentrations of DIC and THIO are as follows

\[ C_{THIO} = A_{266.8,290} - 0.0863/0.0206 \quad \ldots \ldots \ (5) \]

\[ C_{DIC} = A_{244,269} - 0.0036/0.0067 \quad \ldots \ldots \ (6) \]

Preparation of standard stock solutions

Standard stock solution of DIC was prepared by dissolving 5 mg of pure DIC in 10 ml methanol to get concentration of 0.5 mg/ml. Standard stock solution of THIO was prepared by dissolving 10 mg of pure THIO in 10 ml of methanol to get concentration of 1 mg/ml. Aliquots of the stock solution were further diluted to 10 ml with distilled water and scanned in the wavelength range of 200-400 nm to determine linearity.

Analysis of capsule formulation

Contents of twenty capsules were weighed accurately and powdered. Powder equivalent to 10 mg of DIC was weighed and dissolved in 5 ml of methanol with the aid of sonication for 5 min in 10 ml volumetric flask. Volume was made up to the mark with methanol to get final concentration of 10 μg/ml of DIC. Similarly powder equivalent to 2 mg of THIO was weighed and dissolved in 5 ml of methanol with the aid of sonication for 5 min in 10 ml volumetric flask. Volume was made up to the mark with methanol to get final stock solution. Solution was filtered through whatman filter paper no. 41. From the filtrate 0.5 ml was taken and diluted to 10 ml with methanol to get final concentration of 10 μg/ml of THIO. Absorbance of these solutions was recorded in the wavelength range of 200-400 nm. Spectra were processed separately as mentioned in theory section to determine the concentration of each drug by mentioned methods. Analysis was repeated six times to study the precision of the method.

Recovery studies

The accuracy of the proposed method was checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels within the range of linearity for both the drugs.

RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve, assay of tablets, precision and recovery studies were performed. The drugs obey beer’s law in the concentration range 5-30 μg/ml for DIC and 10-60 μg/ml for THIO for all the methods with good correlation coefficient > 0.998. The results of commercial formulation analysis are presented in Table 1. Results of recovery studies are shown in Table 2. The accuracy and reproducibility is evident from the data as results are close to 100 % and low standard deviation. The proposed methods are simple, economical, rapid, precise and accurate. Hence these can be used for routine analysis of DIC and THIO in tablet formulation. Of the three methods developed dual wavelength method found to be more accurate and precise as standard deviation is less.

Table 1. Results of commercial formulation analysis

<table>
<thead>
<tr>
<th>Method</th>
<th>Label claim (mg/tab)</th>
<th>% Label claim estimated * (Mean ± SD)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance Correction Method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIC-50</td>
<td>10.09 ± 0.888</td>
<td>100.49 ± 0.806</td>
<td>0.849</td>
</tr>
<tr>
<td>THIO-4</td>
<td>10.10 ± 0.535</td>
<td>100.34 ± 0.546</td>
<td>0.534</td>
</tr>
<tr>
<td>First Derivative Method</td>
<td></td>
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</tr>
<tr>
<td>DIC-50</td>
<td>10.18 ± 0.589</td>
<td>100.34 ± 0.546</td>
<td>0.578</td>
</tr>
<tr>
<td>THIO-4</td>
<td>10.91 ± 0.468</td>
<td>100.34 ± 0.546</td>
<td>0.459</td>
</tr>
<tr>
<td>Dual Wavelength Method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIC-50</td>
<td>100.56 ± 0.346</td>
<td>100.94 ± 0.351</td>
<td>0.344</td>
</tr>
<tr>
<td>THIO-4</td>
<td>101.13 ± 0.345</td>
<td>100.94 ± 0.351</td>
<td>0.340</td>
</tr>
</tbody>
</table>

* Mean of six determinations, RSD is relative standard deviation

Table 2. Recovery studies of DIC and THIO

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. of drug added</th>
<th>% Level</th>
<th>% Recovery * (Mean ± S.D)</th>
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<tbody>
<tr>
<td>DIC</td>
<td>5</td>
<td>50</td>
<td>100.78 ± 0.507</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>100</td>
<td>101.35 ± 0.679</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>150</td>
<td>100.12 ± 0.688</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>50</td>
<td>101.96 ± 0.827</td>
</tr>
<tr>
<td>THIO</td>
<td>20</td>
<td>100</td>
<td>100.59 ± 0.427</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>150</td>
<td>102.38 ± 0.446</td>
</tr>
</tbody>
</table>

*Average of three determinations

CONCLUSION

The validated spectrophotometric methods employed here proved to be simple, economical, rapid, precise and accurate. Thus these can be used for routine simultaneous determination of DIC and THIO in tablet dosage form.

ACKNOWLEDGEMENTS

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