APPLICATION OF HPTLC IN THE SIMULTANEOUS ESTIMATION OF THIOCOLCHICOSIDE AND DICLOFENAC IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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A simple, precise and accurate HPTLC method was developed for the simultaneous estimation of thiocolchicase (THIO) and diclofenac potassium (DICLO) as the bulk drug and in capsule dosage form. Chromatographic separation was performed on silica gel 60 F254 as the stationary phase and the toluene: acetone: methanol: formic acid (5:2:2:0.01 v/v/v/v) as mobile phase. Densitometric evaluation of the separated zones was performed at 280 nm. The two drugs were satisfactorily resolved with Rf values of 0.29±0.02 and 0.71±0.02 for THIO and DICLO, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (160-800 ng spot-1 for THIO and 1000-5000 ng spot-1 for DICLO), precision ( repeatability RSD 0.658-0.788% and intermediate RSD 0.579-1.012% for THIO, and repeatability RSD 0.340-1.092% and intermediate RSD 0.429-1.007% for DICLO), accuracy (100.97±0.921% for THIO and 99.22±0.022% for DICLO) and specificity in accordance with ICH guidelines.

Key words: Thiocolchicase, Diclofenac potassium, HPTLC, Validation, ICH guidelines.

INTRODUCTION
Thiocolchicase, a thiocholine analog with chemical name N-[3-(β-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylthio)-9-oxo-5,6,7,9-tetrahydrobenzo[α]heptalen-7-yl]acetamide is a muscle relaxant drug with anti-inflammatory, analgesic action and used topically for the treatment of musculoskeletal disorders. Thiocolchicase (THIO) allosterically inhibits strychnine sensitive glycine receptor in brain stem and spinal cord, may provide a possible mechanism for myorelaxant activity (Gimino et al 1996; Balduini et al 1999). Diclofenac, as the potassium salt, is a benzene acetic acid derivative, designated chemically as 2-[(2,6-dichlorophenyl)amino] benzene acetic acid, monopotassium salt. The mechanism of action of diclofenac potassium (DICLO), like that of other NSAIDs, is not completely understood but may be related to prostaglandin synthetase inhibition (BP, 2003).

In literature, analytical methods are described for determination of THIO and other drugs in pharmaceuticals, including the UV spectrophotometry (Patil et al 2011; Shukla et al 2011; Shah et al 2011), HPLC (Rosso and Zuccaro, 1998; Vargas et al 2001; Prasanthi et al 2011), LC-MS methods for their quantitative estimation in human plasma [Ferrari et al 2001; Sutherland et al 2002] and HPTLC method for quantification (El-Ragehy et al 2003).

To date, there are no published reports about the simultaneous quantification of THIO and DICLO by TLC in bulk drug and in pharmaceutical dosage forms. The present study reports for the first time simultaneous quantification of THIO and DICLO by TLC in bulk drug and in pharmaceutical dosage forms. The
proposed method was validated as per ICH guidelines (ICH, 2005).

EXPERIMENTAL
Materials
Working standards of pharmaceutical grade THIO (Batch No. 077101805008) and DICLO (Batch No. 160-2008) were obtained as generous gifts from Alchem International Ltd. (Haryana, India) and Brihons Laboratories, Pune (Maharastra, India) respectively. THIO was polar whereas DICLO was non-polar in nature. The mixed standard stock solution containing THIO and DICLO was spotted on to HPTLC plates and run in different solvent systems. Out of various solvent systems tried, the mobile phase consisting of toluene: acetone: methanol: formic acid (5:2:2:0.01 v/v/v/v) was found to be optimum. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25°C±2) at relative humidity of 60%±5. The flow rate in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed at 280 nm using a Camag TLC scanner III in the reflectance-absorbance mode and operated by CATS software (V 3.15, Camag).

Chromatographic conditions
An attempt was made to develop simultaneous assay method for the combination of THIO and DICLO. THIO was used as standards whereas DICLO was used as working standards for developing the method. The mixed standard stock solution containing THIO and DICLO was spotted on to HPTLC plates and run in different solvent systems. Out of various solvent systems tried, the mobile phase consisting of toluene: acetone: methanol: formic acid (5:2:2:0.01 v/v/v/v) was found to be optimum. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25°C±2) at relative humidity of 60%±5. The flow rate in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed at 280 nm using a Camag TLC scanner III in the reflectance-absorbance mode and operated by CATS software (V 3.15, Camag).

Preparation of standard solution and construction of calibration plots
Standard solution was prepared by dissolving 10 mg of THIO and DICLO in 10 ml methanol (1000 μg ml⁻¹). The working standard solutions were prepared by dilution of the stock solution with methanol to obtain concentration range of 160-800 ng spot⁻¹ and 1000-5000 ng spot⁻¹ for THIO and DICLO respectively. Each concentration was applied six times to the TLC plate. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves. The drug response was linear over the concentration range between 160-800 ng spot⁻¹ and 1000-5000 ng spot⁻¹ for THIO and DICLO respectively.

Assay of capsule formation
To determine the contents of THIO and DICLO in conventional capsules [Brand name: Thioact D 8, label claim: 8 mg and 50 mg THIO and DICLO respectively per capsule], twenty capsules were emptied and content was weighed, their mean weight determined and finely powdered. The weight of the content equivalent to 8 and 50 mg of THIO and DICLO respectively were transferred into a 50 ml volumetric flask containing 25 ml methanol, sonicated for 30 min and diluted to 50 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45 micron filter (Millipore, Milford, MA). The above stock solution was further diluted to obtain sample solution at three different concentration levels of 240, 320, 400 ng spot⁻¹ and 1500, 2000, 2500 ng spot⁻¹ for THIO and DICLO respectively. The plate was developed in the previously described chromatographic conditions. The peak areas were measured at 280 nm, concentrations in the samples were determined using multilevel calibration developed on the same HPTLC system under the same conditions using linear regression equation.

RESULTS AND DISCUSSION
HPTLC method development and optimization
The mixed standard stock solution of THIO and DICLO was spotted on to HPTLC plates and run in different solvent systems. The mobile phase consisting of toluene: acetone: methanol: formic acid in the ratio of 5:2:2:0.01 v/v/v/v was found to be optimum. Densitometric scanning was done at 280 nm as both drugs showed maximum response at that wavelength. Under the optimum chromatographic conditions, the Rf values obtained for THIO and DICLO were 0.29 and 0.71 respectively (Figure 1).

Validation of the developed method
Validation of the optimized TLC method was carried out with respect to the following parameters:

Linearity
The linear regression data (n=6) showed a good linear relationship over a concentration range of 160-800 ng spot⁻¹ (r² ± S.D. = 0.9904±0.961) and
Fig. 1. Densitogram of THIO and DICLO

1000-5000 ng spot⁻¹ \((r^2 \pm \text{S.D.} = 0.9983 \pm 0.694)\) for THIO and DICLO, respectively. The drug response was linear over the concentration range between 160-800 ng spot⁻¹ and 1000-5000 ng spot⁻¹ for THIO and DICLO, respectively.

Accuracy
These studies were carried out at three levels i.e. multiple level recovery studies. Sample stock solution from capsule formulation of each drug was prepared. To the sample solutions, 50%, 100% and 150% of the standard drug solutions were added. Dilutions were made and recovery studies were performed. % recovery was found to be within the limit as listed in Table 1.

Precision
The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2%, respectively as recommended by ICH guidelines. Separation of the drug was found to be similar when analyses were performed using chromatographic system on different days. The results of the repeatability and intermediate precision experiments are shown in Table 2.

LOD and LOQ
The signal to noise ratios of 3:1 and 10:1 were considered as LOD and LOQ respectively. The limit of detection (LOD) and the limit of quantitation (LOQ) were found to be 10 ng spot⁻¹ and 33 ng spot⁻¹ for THIO and 76.77 ng spot⁻¹ and 255.90 ng spot⁻¹ for DICLO respectively.

Table 1. Recovery studies of THIO and DICLO by HPTLC

<table>
<thead>
<tr>
<th>Label claim per capsule</th>
<th>Amount added (%)</th>
<th>Total amount (mg)</th>
<th>Amount recovered (mg) ± % RSD</th>
<th>Recovery* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THIO (8 mg)</td>
<td>50</td>
<td>12</td>
<td>12.22 ± 0.028</td>
<td>101.83</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>16</td>
<td>15.96 ± 0.159</td>
<td>99.75</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>20</td>
<td>19.94 ± 0.102</td>
<td>99.70</td>
</tr>
<tr>
<td>DICLO (50 mg)</td>
<td>50</td>
<td>75</td>
<td>76.5 ± 0.091</td>
<td>102.00</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>100.55 ± 0.166</td>
<td>100.55</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>125</td>
<td>125.23 ± 0.864</td>
<td>100.18</td>
</tr>
</tbody>
</table>

*\(n = 6\)

Table 2. Precision studies of proposed HPTLC method

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Conc. (µg ml⁻¹)</th>
<th>Repeatability*</th>
<th>Intermediate precision*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found conc. ± SD</td>
<td>RSD (%)</td>
<td>Found conc. ± SD</td>
</tr>
<tr>
<td>THIO</td>
<td>160</td>
<td>164.07 ± 1.294</td>
<td>0.788</td>
</tr>
<tr>
<td></td>
<td>480</td>
<td>477.51 ± 3.146</td>
<td>0.658</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>809.37 ± 5.568</td>
<td>0.687</td>
</tr>
<tr>
<td>DICLO</td>
<td>1000</td>
<td>998.50 ± 7.408</td>
<td>0.741</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>3035.91 ± 33.180</td>
<td>1.092</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>4955.98 ± 16.890</td>
<td>0.340</td>
</tr>
</tbody>
</table>

*\(n = 6\)

Specificity
The peak purity of THIO and DICLO was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e. \(r (S, M) = 0.9975, r (M, E) = 0.9969\) and \(r (S, M) = 0.9984, r (M, E) = 0.9974\), respectively. A good correlation \((r^2 = 0.9904\) and \(r^2 = 9983)\) was also obtained between the
standard and sample spectra of THIO and DICLO.

**Assay of capsule formulation**

The content of THIO and DICLO were found to be 100.97±0.93% and 99.02±1.02% with %RSD of 0.921 and 0.022, respectively. The low %RSD value indicated the suitability of this method for routine analysis of THIO and DICLO in pharmaceutical dosage form. The result of analysis are reported in Table 3.

### Table 3. Determination of THIO and DICLO in capsule formulation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>THIO</th>
<th>DICLO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Label claim (mg) found</td>
<td>8</td>
<td>50</td>
</tr>
<tr>
<td>Drug content (%) ± SD</td>
<td>100.97 ± 0.930</td>
<td>99.22 ± 0.022</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.921</td>
<td>0.022</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Over the past decade, HPTLC has been successfully used in the analysis of pharmaceuticals, plant constituents and biomacromolecules. Review of literature reveals that no HPTLC method has been reported so far for simultaneous estimation of thiocolchicoside and diclofenac, therefore, densitometric method (HPTLC) was developed and validated according to ICH guidelines, for the combinations of simultaneous estimation of thiocolchicoside and diclofenac. The results meet with the requirements of ICH guidelines. The proposed method was found to be rapid, specific, precise and accurate which could be used as effective quality control tool for routine analysis of above compounds as bulk drugs and in dosage formulations.

**ACKNOWLEDGEMENT**

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**REFERENCES**


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