A stability-indicating RP-HPLC-PDA method has been developed and subsequently validated for the simultaneous determination of Thiocolchicoside (THIO) and Aceclofenac (ACE) in commercial tablets. Formulations containing THIO with ACE are used as antispasmodic and anti-inflammatory agent. The proposed HPLC method utilizes Waters Qualisil BDS C8 column (250 mm × 4.6 mm, 5.0 µ) maintained at 60 °C using column oven. Isocratic elution with methanol: Acetonitrile: THF: acetate buffer (56:4:10:30 v/v) pH adjusted to 6.5 with acetic acid at a flow rate of 0.7 mL min⁻¹ was carried out. Quantitation was achieved with UV detection at 312 nm based on linear calibration curves at concentration ranges 0.4 - 24 µg mL⁻¹ for THIO and 10 - 600 µg mL⁻¹ for ACE (R² > 0.999 for both drugs). The method was validated according to the ICH guidelines with respect to accuracy, precision, linearity, limits of detection, limits of quantitation and robustness. This method has been successively applied to pharmaceutical formulation and no interference from the tablet excipients was found. THIO, ACE and their combination drug product were exposed to acid, base and neutral hydrolysis, oxidation, dry heat and photolytic stress conditions and the stressed samples were analyzed by the proposed method. As the proposed method could effectively separate the drug from its degradation products, it can be employed as stability-indicating method for the determination of these drugs in bulk and commercial products.

**Key words:** Column liquid chromatography, Stability-indicating method, Degradation products, Thiocolchicoside (THIO) and Aceclofenac (ACE)

**INTRODUCTION**

Thiocolchicoside (THIO); (S)-N-[3-(B-D-glucopyranosyloxy)-5, 6, 7, 9-tetrahydro-1, 2-dimethoxy-10-(methylthio)-9-oxobenzo[a]heptalen-7-yl] acetamide, it has a selective affinity for γ-amino-butyric acid (GABA) receptors and acts on the muscular contracture by activating the GABA inhibitory pathways thereby acting as a potent muscle relaxant. ¹¹

Chemically Aceclofenac (ACE) is, 2-[2-[(2, 6-dichlorophenyl) amino] phenyl] acetyl] oxacetic acid; which is structurally related to diclofenac, has anti-inflammatory and analgesic properties. ²² Although its mode of action is not well understood, it is known to suppress cyclo-oxygenase 2-dependent prostaglandin synthesis following long-term treatment. ³³

Literature survey reveals that an optimized reversed-phase HPLC method for the routine quality control analysis of THIO and ACE simultaneously from tablets are now reported. ⁴⁴ TLC method for THIO with other drugs, ⁴⁵ LC-MS method for 3-desmethyliicolchicine, ⁴⁶ have been reported. Several HPLC, ⁴⁷ UV, ⁴⁸ stability indicating (for ACE), ⁴⁹ have been cited in the literature for the estimation of ACE individually and also in combination with other drugs, ⁵⁰, ⁵¹ and few bioanalytical methods, ⁵², ⁵³, ⁵⁴ have been reported. Further, no stability-indicating method has been reported in literature for simultaneous determination of THIO and ACE in presence of their degradants. Therefore, the present study targets the development and subsequent validation of a stability-indicating RP-HPLC-PDA method for the simultaneous determination of THIO and ACE in presence of their degradants. To establish the stability indicating nature of the method, forced degradation of each API and drug product was performed under stress conditions and stressed samples were analyzed by the proposed method. The proposed LC method was able to separate both drugs from degradants generated during forced degradation studies and validated as per ICH guidelines.

**EXPERIMENTAL**

**Materials and Reagents**

Commercial formulation Tablets THIOSPAS A manufactured by Nicholas Piramal (Ahmadabad, India) were used for analysis containing THIO 4 mg and ACE 100 µg per tablet. Pure drug sample of THIO (99.92%) and ACE (99.8%) were obtained as a gift sample from Medley Pharmaceuticals Ltd., Baddi and Curex pharmaceuticals, Jalgaon, India, respectively. HPLC grade methanol, acetonitrile and tetrahydrofuran (THF) were procured from Merck and Qualigens fine Chemicals, respectively (Mumbai, India). Ammonium acetate and acetic acid were procured from Research Lab Fine Chem (Mumbai, India). Double distilled water and tablet placebo was made at lab scale only.

**HPLC Instrumentation and Conditions**

The HPLC system consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 717 plus Auto sampler), column heater, and PDA detector (Waters 2998). Data collection and analysis were performed using Empower- version 2 software. Separation was achieved on Qualisil BDS C8 column (250 mm × 4.6 mm, 5.0 µ) columns maintained at 60 °C using column oven. Isocratic elution with (methanol: acetonitrile: THF; acetate buffer (56:4:10:30 v/v) pH adjusted to 6.5 with acetic acid at a flow rate of 0.7 mL min⁻¹ was carried out. The detection was monitored at 312 nm and injection volume was 20 μL. The peak purity was checked with the photodiode array detector.

**Standard Solutions and Calibration Curve**

Standard stock solution of THIO and ACE (1000μg mL⁻¹) were prepared separately in methanol. To study the linearity range of each component, serial dilutions of THIO and ACE each were made from 0.4 - 24 μg mL⁻¹ and 10-600 µg mL⁻¹, respectively in mobile phase and injected on to column. Calibration curves were plotted as concentration of drugs versus peak area response. From the standard stock solutions, a mixed standard solution was prepared containing the analytes in the given ratio and injected on to column. The system suitability test was performed from six replicate injections of mixed standard solution.

**Analysis of Tablet Formulation**

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 4 mg of THIO and 100 mg of ACE was weighed and transferred to a 100 mL volumetric flask containing about 80 mL of methanol, ultrasonicated for 10 min and volume was made up to mark with the methanol, filtered and suitably diluted to get solutions of concentrations of 4 μg mL⁻¹ of THIO and 100 μg mL⁻¹ of ACE in mobile phase. The sample solution was then injected, chromatogram was recorded and the amounts of the drugs were calculated. A typical chromatogram obtained from a formulation is shown in Figure 1.

**Method Validation**

The stability indicating method was validated in terms of precision, accuracy and linearity as per ICH guidelines.

**Linearity**

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration...
Acid and Base Hydrolysis

Solutions for acid degradation studies were prepared in methanol and 3 M hydrochloric acid at 70°C. It was observed that acid hydrolysis was a fast reaction for both drugs and carried for 6 hrs of exposure time, therefore the samples were analyzed after this period of time. Solutions for base degradation studies were prepared in methanol and 1 M sodium hydroxide at 80°C and the resultant solutions analyzed 3 hrs exposure time.

Oxidation and Dry heat Degradation Product studies

Solutions for use in oxidation studies were prepared in methanol and 30% w/v hydrogen peroxide at ambient temperature and the resultant solutions were analyzed after 8 hr exposure time.

UV Degradation Product

For UV degradation study, the solution of THIO and ACE separately and in mixture was exposed to short UV radiation (254 nm) and long UV radiation at 312 nm. The sample was then diluted to obtain solution containing 16 µg mL⁻¹ of THIO & 400 µg mL⁻¹ of ACE and then 20 µl of the solution was injected into the system.

Neutral Hydrolysis

For Neutral Hydrolysis study, the solution of THIO and ACE separately and in mixture prepared using distilled water were kept at 80°C for 8 hour exposure time. The sample was then diluted to obtain solution containing 16 µg mL⁻¹ of THIO & 400 µg mL⁻¹ of ACE and then 20 µl of the solution was injected into the system.

Stress Degradation Studies for Tablet Formulation

Tablet powder equivalent to 10 mg of ACE (0.4 mg of THIO) were left at same condition for Acid and Base hydrolysis, Oxidation and Dry heat degradation product, UV degradation product and neutral hydrolysis. The sample was then diluted to obtain solution containing 16 µg mL⁻¹ of THIO & 400 µg mL⁻¹ of ACE and injected on to column.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

The HPLC method was optimized with a view to develop a well-defined symmetrical peak which was obtained upon measuring the response of eluent under the optimized conditions after thorough experimental trials. Two columns were used for performance investigations, including Kromasil C₁₈ (5 micron 4.6x250 mm) and Qualsil C₁₈ (5 micron 4.6x250 mm), the second column was the most suitable one, since it produced symmetrical peaks with high resolution.

Method Validation

The newly developed method was validated according to the ICH guidelines with respect to specificity, accuracy, precision and robustness. System suitability was established by injecting standard solution and results are given in Table 1.

Formulation analysis and Accuracy Studies

The assay for the marketed tablets was established with present chromatographic conditions developed and it was found to be accurate and reliable. The average drug content was found to be 99.98 % for THIO and 100.12 % for ACE of the labelled claim. No interfering peaks were found in chromatogram.
Table 1: System suitability parameters and results of precision study

<table>
<thead>
<tr>
<th>Compound</th>
<th>System Suitability Parameter</th>
<th>Precision of the Method (n=5)</th>
<th>Measured conc. (µg/mL)</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value</td>
<td>Actual Conc. (µg/mL)</td>
<td>Intra-day</td>
<td>Inter-day</td>
</tr>
<tr>
<td>THIO</td>
<td>3908</td>
<td>2</td>
<td>2.08, 1.04</td>
<td>1.99, 0.20</td>
</tr>
<tr>
<td>USP resolution*</td>
<td>-</td>
<td>4</td>
<td>4.05, 0.19</td>
<td>4.01, 0.68</td>
</tr>
<tr>
<td>Peak Tailing*</td>
<td>1.37</td>
<td>6</td>
<td>5.95, 0.67</td>
<td>6.19, 0.95</td>
</tr>
<tr>
<td>% R.S.D.</td>
<td>0.96</td>
<td>3433</td>
<td>50.08, 1.50</td>
<td>49.01, 0.20</td>
</tr>
<tr>
<td>ACE</td>
<td>4.56</td>
<td>100</td>
<td>100.07, 0.23</td>
<td>100.01, 0.19</td>
</tr>
<tr>
<td>USP resolution*</td>
<td>1.17</td>
<td>150</td>
<td>149.90, 0.79</td>
<td>150.09, 0.88</td>
</tr>
<tr>
<td>% R.S.D.</td>
<td>0.78</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*USP-NF 29 section 621, pp. 2135. *Data expressed as mean for “measure concentration” values indicating that the estimation of drug free from inference of excipients. The results for formulation analysis and accuracy studies are given in Table 2.

**Precision**
The result obtained for Intraday and Inter day variations are shown for THIO and ACE in Table 1.

**Robustness**
The robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The parameters considered (± values) for the study were, proportion of methanol in mobile phase (± 1%), flow rate (±0.2 mL min⁻¹), column temp. (± 2°C), measurement wavelength (± 2 nm), injection volume (± 2 µl), buffer strength (± 5 mM), pH (± 0.2) and effect of column from different supplier were studied. Results of robustness study are presented in Table 3.

**Stress Degradation**
The results of stress degradation are shown in Figure 2 and Table 3.

**Acid and Base induced – Degradation Product**
The chromatogram of the THIO acid degraded sample showed one additional peak. The degradation peak was observed at t = 5.17 min and the chromatogram of the THIO base degraded samples showed one additional peak at t = 5.05 minutes. The chromatogram of the ACE acid degraded sample showed two additional peaks at t = 6.1 & 7.9 min and the chromatogram of the ACE base degraded samples showed one additional peak at t = 7.9 min. The t = 2.9 min, which was the major degradation peak of ACE and was identified as Diclofenac free acid.

**Hydrogen Peroxide induced – Degradation Product**

<table>
<thead>
<tr>
<th>Compound (Label Claim)</th>
<th>Formation Study (n=5)</th>
<th>Recovery (accuracy) Study</th>
<th>% Assay Found.</th>
<th>Recovery Level</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>THIO (4mg)</td>
<td>Formulation I</td>
<td>99.65, 0.77</td>
<td>100.04, 0.67</td>
<td>100.19, 0.59</td>
<td>100.16, 0.92</td>
</tr>
<tr>
<td>Formulation II</td>
<td>99.09, 0.83</td>
<td>100.04, 0.94</td>
<td>100.00, 0.54</td>
<td>100.12, 1.29</td>
<td>100.13, 0.87</td>
</tr>
<tr>
<td>ACE (100 mg)</td>
<td>Formulation I</td>
<td>99.98, 0.56</td>
<td>98.40, 0.42</td>
<td>98.17, 0.87</td>
<td>99.80, 0.31</td>
</tr>
<tr>
<td>Formulation II</td>
<td>100.08, 0.54</td>
<td>98.40, 0.42</td>
<td>98.17, 0.87</td>
<td>99.80, 0.31</td>
<td>99.98, 0.56</td>
</tr>
</tbody>
</table>

**UV Degradation Product & Neutral Hydrolysis**
In HPLC, sample showed no degradation for short and long UV radiation and also for neutral hydrolysis conditions.

**CONCLUSIONS**
The developed methods was found to be simple, sensitive, accurate, precise and reproducible and can be used for the routine quality control analysis of THIO and ACE in bulk drug and marketed formulation. As the method could effectively separate the drugs from their degradation products it can be employed as a stability indicating one. Method uses MS compatible volatile buffer and can be used for LC-MS/MS bio analytical study and to determine titled drugs in biological fluids.

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