Stability Indicating HPTLC Method for Simultaneous Estimation of Eperisone Hydrochloride and Diclofenac Sodium in Bulk and Solid Dosage Form

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ABSTRACT
The objective of present work was to develop and validate a simple, accurate, rapid and precise stability indicating HPTLC method for simultaneous estimation of Eperisone hydrochloride (EPE) and Diclofenac sodium (DICLO) in bulk and in capsule dosage form. The method employed Merck TLC plates precoated with Silica F254. After several trials, ethyl acetate: methanol (8:2, v/v), was chosen as the mobile phase with saturation time 15 min, which showed good resolution and acceptable peak parameters. The densitometric analysis of both drugs was carried out at 266 nm. The linearity coefficient was found to be 0.999 for Eperisone hydrochloride (EPE) and 0.999 for Diclofenac sodium (DICLO). The Rf values were found to be 0.38 ± 0.02 and 0.73 ± 0.03 for EPE and DICLO. Stability study of EPE and DICLO was carried out by Forced degradation study. The developed method was successfully applied to estimate the amount of Eperisone hydrochloride and Diclofenac sodium in bulk and capsule dosage form.

Keywords: eperisone hydrochloride, diclofenac sodium, HPTLC, forced degradation study

INTRODUCTION
Eperisone hydrochloride (EPE) is chemically 1-(4-ethylphenyl)-2-methyl-3-(piperidin-1-yl) propan-1-one (Figure 1). It is an antispasmodic agent. It is official in Japanese Pharmacopoeia (JP) [1]. It exhibits both skeletal muscle relaxant and vasodilator properties due to its actions on the central nervous system and on vascular smooth muscles and a variety of pharmacological effects such as cervical spondylosis, headache and low back pain [2].

Diclofenac sodium (DICLO) is chemically 2-[2-[(2, 6-dichlorophenyl) amino] phenyl] acetic acid sodium salt (Figure 2). It is official in IP, BP and USP [3-5]. It is a non-steroidal anti-inflammatory agent (NSAID) with antipyretic and analgesic actions [6]. It is also used for the
acute and chronic treatment of signs and symptoms of osteoarthritis and rheumatoid arthritis [7].

For the development of stability-indicating assay method, the drug is subjected to various ICH (International Conference on Harmonization) stress conditions such as photolytic, hydrolytic, thermal and oxidative [8]. As per the ICH drug stability test guidelines Q1A (R2), validated stability-indicating assay method should be developed for the analysis of drug substance and drug product [9-11]. The ICH guidelines Q6A explains about specifications and tests criteria for drug substance and product in order to perform stability-indicating assays.

According to literature many methods have been described for the determination of EPE by Spectrophotometric [12-19], RP-HPLC [20-24], HPTLC [25], TLC [26] and DICLO by Spectrophotometric [27-31], HPLC [32] individually and in combinations with other drugs from bulk drugs and pharmaceutical formulations. However, there is no stability indicating HPTLC method reported for the simultaneous estimation of these drugs in combined dosage forms. Fixed dose combination containing Eperisone (150mg) and Diclofenac (100mg) is available in capsule form in the market.

The aim of this work was to develop a stability indicating HPTLC method for the simultaneous determination of Eperisone hydrochloride and Diclofenac sodium in pharmaceutical dosage form.
MATERIALS AND METHODS

Reagents and materials

The gift sample of Eperisone hydrochloride and Diclofenac sodium was provided by Sharon Bio-Medicine Ltd. Mumbai, and Themis Medicare, Mumbai respectively. Capsule dosage form Rapisone-DSR (containing Eperisone 150mg, Diclofenac Sodium 100mg) Mfg.by Abbott Healthcare was purchased from local market. All the chemicals and solvents used in study of analytical grade.

Instrumentation

1. Camag HPTLC system comprising:
   - Linomat - 5 sample applicator
   - Camag TLC Scanner 3 operated by Win CATS software V- 1.4.2
   - Merck TLC plates precoated with Silica F$_{254}^{}$
   - Hamilton syringe (100 μl capacity)
2. Shimadzu balance- Model AY-120
3. Ultrasonic Bath-HMG India: CD-4820
4. Digital pH Meter: Systronic

Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200 - 400 nm and the overlain spectra was obtained. It was observed that both the drug showed considerable absorbance at 266 nm (Figure 3).

Preparation of standard stock solution

Standard stock solution of EPE was prepared by dissolving 15 mg of drug in 10 ml of methanol to get concentration of 1500 μg ml$^{-1}$ from which 1 ml was further diluted with methanol to get the final concentration 150 ng μl$^{-1}$. For preparation of standard stock solution of DICLO, 10 mg of drug was accurately weighed and was dissolved in 10 ml of methanol to

### Table 1. Optimized chromatographic condition

<table>
<thead>
<tr>
<th>Stationary phase precoated silica plates</th>
<th>Silica gel 60G F$_{254}^{}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>Ethyl Acetate: Methanol</td>
</tr>
<tr>
<td>Mobile phase ratio (%v/v)</td>
<td>(8: 2 )</td>
</tr>
<tr>
<td>Saturation time</td>
<td>15 min</td>
</tr>
<tr>
<td>Solvent front</td>
<td>80 mm</td>
</tr>
<tr>
<td>Scan wavelength</td>
<td>266 nm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rf values</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Eperisone hydrochloride</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>0.73 ± 0.03</td>
</tr>
</tbody>
</table>
get concentration of 100 µg ml\(^{-1}\) from which 1 ml was further diluted with methanol to get the final concentration 100 ng µl\(^{-1}\).

**Analysis of marketed formulation**

Accurately weighed 20 capsules. A quantity of powder equivalent to EPE (15 mg) and DICLO (10 mg) was weighed and transferred to a 10 ml volumetric flask containing approximately 5 ml of methanol. The mixture was sonicated for 15 min and diluted to volume with methanol. The solution was filtered using Whatman paper no. 41. One milliliter of the above solution was further diluted with methanol to obtain the concentration 150 ng band\(^{-1}\) for EPE and 100 ng band\(^{-1}\) for DICLO. Two microliter volume of this solution was applied on TLC plate to furnish concentration 300 ng band\(^{-1}\) for EPE and 200 ng band\(^{-1}\) for DICLO and developed under optimized chromatographic condition. Six determinations were carried out.
from homogenous sample to determine % assay. The % drug content (Mean±R.S.D.) was found to be 100.02±1.57 for EPE and 99.92±1.22 for DICLO.

VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) Guidelines [33].

Calibration curve (Linearity)

The standard stock solutions of EPE (150 ng µl⁻¹) and DICLO (100 ng µl⁻¹) were applied by over spotting on HPTLC plate in range of 1, 2, 3, 4, 5 and 6 µl with the help of CAMAG 100 µl sample syringe, using Linomat 5 sample applicator to obtain final concentration 150-900 ng band⁻¹ for EPE and 100-600 ng band⁻¹ for DICLO. Calibration curves of EPE and DICLO were plotted separately of peak area vs respective concentration.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intraday studies, 3 replicates of 3 different concentrations (450, 600, 750 ng band⁻¹) of EPE and (300, 400, 500 ng band⁻¹) of DICLO were analyzed in a day and percentage % RSD was calculated. For the inter day variation studies, 3 replicates of different concentrations were analyzed on 3 consecutive days and percentage % RSD was calculated.

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 300 ng band⁻¹ for EPE and 200 ng band⁻¹ for DICLO. These solutions were applied on TLC plates in triplicate to obtain the densitogram.

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drugs was determined by using following equation

\[
\text{LOD} = 3.3 \times \sigma / S \quad \text{and} \quad \text{LOQ} = 10 \times \sigma / S
\]

where, \( \sigma \) is the standard deviation and \( S \) is the slope.

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**Table 2.** Analysis of formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount taken (ng band⁻¹)</th>
<th>Amount found (ng band⁻¹)</th>
<th>% Drug content</th>
<th>% R.S.D.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPE</td>
<td>300</td>
<td>300.07</td>
<td>100.02</td>
<td>1.57</td>
</tr>
<tr>
<td>DICLO</td>
<td>200</td>
<td>199.85</td>
<td>99.92</td>
<td>1.22</td>
</tr>
</tbody>
</table>

* Average of six determinations
Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio and chamber saturation time were altered and the effects on the area were noted. One factor at a time was changed at a concentration level of 900 ng band\(^{-1}\) for EPE and 600 ng band\(^{-1}\) for DICLO respectively, to study the effect on the peak area of the drugs.

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The spot for EPE and DICLO in the samples was confirmed by comparing the RF and spectrum of the spot to that of a standard. The peak purity was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

RESULTS AND DISCUSSION

Method development

It was observed that both the drug showed considerable absorbance at 266 nm. After several trials, Ethyl Acetate: Methanol (8:2 v/v), was chosen as the mobile phase with
saturation time 15 min, which gave good resolution and acceptable peak parameters. The densitometric analysis of both drugs was carried out at 266nm. The $R_f$ values were found to be 0.38 ± 0.02 and 0.73 ± 0.03 for EPE and DICLO respectively.

**Linearity**

Results were found to be linear in the concentration range over 150-900 ng band$^{-1}$ for EPE and 100-600 ng band$^{-1}$ for DICLO. The slope, intercept and correlation coefficient values of Eperisone hydrochloride were found to be 4.645, 1138 and 0.999 respectively and 6.955, 869 and 0.999 respectively for Diclofenac sodium. The results are shown in Table 3.

**Precision**

Precision was calculated as inter day and intra day variations. The %RSD should not be more than 2%. The results are shown in Table 4 and 5.
Accuracy

The % recovery for Eperisone hydrochloride was found to be 100.45 (at 80%), 100.1 (at 100%), 99.67 (at 120%) with %RSD values ranging from 0.7124, 0.8974, 0.8948 and 100.54 (at 80%), 100.81 (at 100%), 100.30 (at 120%) for Diclofenac sodium with %RSD values ranging from 1.4372, 1.1050, 0.9060.

LOD & LOQ

The LOD for Eperisone hydrochloride was found to be 44.69 ng band\(^{-1}\) and for Diclofenac sodium 19.39 ng band\(^{-1}\) respectively. The LOQ for Eperisone hydrochloride was found to be 135.44 ng band\(^{-1}\) and for Diclofenac sodium 58.76 ng band\(^{-1}\) respectively.

Specificity

The non-interference of any other peak of degradation product or impurity with active drugs indicated specificity of the developed method. The peak purity values were found to be 0.999 for both EPE and DICLO respectively. So that method is specific.

Forced degradation study

Forced degradation studies have been performed at various stress conditions and % degradation of the individual drug was determined along with the % assay of drug after

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**Table 5. Inter day precision**

<table>
<thead>
<tr>
<th>Concentration (ng band(^{-1}))</th>
<th>Peak Area</th>
<th>% R.S.D. *</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPE 450</td>
<td>DICLO 300</td>
<td>EPE 3255.33</td>
</tr>
<tr>
<td>EPE 450</td>
<td>DICLO 300</td>
<td>EPE 3212.16</td>
</tr>
<tr>
<td>EPE 600</td>
<td>DICLO 400</td>
<td>EPE 3936.58</td>
</tr>
<tr>
<td>EPE 600</td>
<td>DICLO 400</td>
<td>EPE 3909.33</td>
</tr>
<tr>
<td>EPE 600</td>
<td>DICLO 400</td>
<td>EPE 3966.18</td>
</tr>
<tr>
<td>EPE 750</td>
<td>DICLO 500</td>
<td>EPE 4622.31</td>
</tr>
<tr>
<td>EPE 750</td>
<td>DICLO 500</td>
<td>EPE 4648.91</td>
</tr>
<tr>
<td>EPE 750</td>
<td>DICLO 500</td>
<td>EPE 4603.20</td>
</tr>
</tbody>
</table>

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**Table 6. Recovery study**

<table>
<thead>
<tr>
<th>Level</th>
<th>% Recovery</th>
<th>% R.S.D. *</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPE</td>
<td>DICLO</td>
<td>EPE</td>
</tr>
<tr>
<td>80%</td>
<td>100.45</td>
<td>100.54</td>
</tr>
<tr>
<td>100%</td>
<td>100.10</td>
<td>100.81</td>
</tr>
<tr>
<td>120%</td>
<td>99.61</td>
<td>100.30</td>
</tr>
</tbody>
</table>

*Average of three determinations
exposure to the particular stress condition. The results of forced degradation studies were found in the range (0.01%-24.07% for EPE) and (8.41%-25.98% for DICLO) respectively.

**CONCLUSION**

A simple, precise, accurate and selective stability indicating HPTLC method has been developed for the simultaneous determination of EPE and DICLO in combined capsule dosage form. The degradation products formed under stress conditions were well separated from the peaks of EPE and DICLO indicating the specificity of the method. The developed method is validated in accordance with ICH guidelines. The low % RSD values indicates high degree of precision of the method. The results of the recovery studies indicated that the method is accurate for estimation of drugs in capsule dosage form. The method can be used to determine the purity of the drugs available from various sources by detecting the related impurities.

**Figure 7.** Densitogram of Sample (EPI of 300 ng band⁻¹ and DICLO of 200 ng band⁻¹)

**Table 7.** Data of forced degradation studies of EPE and DICLO

<table>
<thead>
<tr>
<th>Stress Conditions</th>
<th>EPE</th>
<th>DICLO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Assay of active substance</td>
<td>% Recovered</td>
</tr>
<tr>
<td>Acid/ 0.1 N HCl /Reflex at 60°C for 4hrs</td>
<td>20.77</td>
<td>79.23</td>
</tr>
<tr>
<td>Alkali/0.1N NaOH/ Reflux at 60°C for 4hrs</td>
<td>14.49</td>
<td>85.51</td>
</tr>
<tr>
<td>Oxidative /3 % H₂O₂ / Reflux at 60°C for 4hrs</td>
<td>11.37</td>
<td>88.63</td>
</tr>
<tr>
<td>Neutral/H₂O/ Reflux at 60°C for 4hrs</td>
<td>24.07</td>
<td>75.93</td>
</tr>
<tr>
<td>Dry heat/ 80°C/ 2hrs</td>
<td>0.11</td>
<td>99.89</td>
</tr>
<tr>
<td>Photolysis</td>
<td>12.84</td>
<td>87.16</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

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