Simultaneous HPTLC Determination of Paracetamol and Dexketoprofen trometamol in pharmaceutical dosage form

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ABSTRACT

A fast, simple, precise, accurate and robust HPTLC method was developed for simultaneous determination of paracetamol and dexketoprofen trometamol in tablet dosage form. Silica gel G 60F$_{254}$ plates were used as stationary phase and toluene: ethyl acetate: acetic acid (6: 4: 0.2 v/v/v) as mobile phase. Wavelength selected for analysis was 256 nm. The two drugs were satisfactorily resolved with $R_f$ 0.20 ± 0.02 and 0.55 ± 0.02 for paracetamol and dexketoprofen trometamol respectively. Linearity was found to be in the range of 25-150 ng/spot for paracetamol, 100-600 ng/spot for dexketoprofen trometamol. Intra-day and inter-day precision were found to be (%RSD) 0.94–1.58 % and 1.14–1.83 % for paracetamol and (%RSD) 0.67–1.03 % and 0.49–1.18 for dexketoprofen trometamol. Accuracy of paracetamol and dexketoprofen trometamol was found to be 99.91 % and 99.56 %, respectively. The proposed method can be used for routine simultaneous analysis of paracetamol and dexketoprofen trometamol.

Keywords: Paracetamol and dexketoprofen trometamol, HPTLC.

INTRODUCTION

Paracetamol, $N$-(4-hydroxyphenyl) acetamide (Figure 1a). Paracetamol has negligible anti-inflammatory action. It is a poor inhibitor of of prostaglandin synthesis in peripheral tissues. It inhibits the action of cyclooxygenase (COX), it is highly selective for COX-2. It has analgesic and antipyretic properties[1]. Dexketoprofen trometamol, (2S)-2-[3-(benzoyl) phenyl] propanoic acid (Figure 1b). Dexketoprofen trometamol is a new, quick acting analgesic for the treatment of painful musculoskeletal conditions such as osteo-arthritis and low back pain. It is also used as a treatment for post-operative pain, toothache and dysmenorrhoea. It is the active optical isomer of ketoprofen[2].

Hence, the combination of paracetamol and dexketoprofen trometamol provides antiinflammatory as well as analgesic effects in painful musculoskeletal conditions. Today TLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs,
high sample throughput and the need for minimum sample preparation. The major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC thus reducing the analysis time and cost per analysis.

Literature review reveals that methods have been reported for paracetamol and dexketoprofen trometamol, spectrophotometric estimation of paracetamol and lornoxicam[3], HPTLC method for determination of paracetamol in combination with other drugs [4-6].

To date there have been no published reports on simultaneous quantitation of paracetamol and dexketoprofen trometamol by HPTLC in bulk drug and in tablet dosage form. This present study reports for the first time the simultaneous quantitation of paracetamol and dexketoprofen trometamol by HPTLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH Guidelines [7-9].

**Figure 1 a Structure of Paracetamol**

**Figure 1 b Structure of Dexketoprofen trometamol**

**MATERIALS AND METHODS**

Working standards of pharmaceutical grade paracetamol and dexketoprofen trometamol were obtained as generous gifts from Emcure Pharmaceuticals Ltd. Pune, India. They were used without further purification and certified to contain 99.92 %, and 99.85 % (w/w) on dry weight basis for paracetamol and dexketoprofen trometamol respectively. Fixed dose combination tablets (Brand Name: Infen-P) containing 500 mg of paracetamol and 25 mg of dexketoprofen trometamol were procured from Emcure Pharmaceutical Ltd. India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

**Instrumentation**

Camag HPTLC System (with TLC Scanner), WinCATS Software V 4.0 and Linomat 5 (as application device) used for the analysis. Precoated silica gel 60F254 on aluminium sheets (200µm thick) of E-Merck, Germany were used as stationary phase. Pre-washing of plate was done with methanol and then it was activated by keeping in an oven at 115°C for 10 minutes. The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonded, Switzerland) syringe. A constant application rate of 0.1 µL/s was used and the space between two bands was 5 mm. The slit dimension was kept at 5 mm × 0.45
mm and the scanning speed was 10 mm/s. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. Each chromatogram was developed over a distance of 8 cm. The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm.

**Preparation of Standard Stock Solutions**
Standard stock solutions with a concentration of 1000 µg/mL were prepared in methanol for paracetamol and dexketoprofen trometamol, respectively. From the standard stock solutions, diluted mixed standard solutions were prepared containing 100 µg/mL of paracetamol and dexketoprofen trometamol respectively.

**Optimization of the HPTLC method**
The TLC procedure was optimized with a view to develop a simultaneous assay method for paracetamol and dexketoprofen trometamol. Both the drug solutions were spotted on to TLC plates and run in different solvent systems. Finally the mobile phase consisting of toluene: ethyl acetate: acetic acid (6: 4: 0.2 v/v/v) was found to be optimum, resulting in an Rf 0.20 ± 0.02 and 0.55 ± 0.02 for paracetamol and dexketoprofen trometamol respectively (Figure 2). The mobile phase was run up to a distance of 8 cm; which takes approximately 30 min for complete development of the TLC plate.

**Validation of the method**
Validation of the optimized TLC method was carried out with respect to the following parameters.

**Linearity and range**
A stock solution containing 25 µg/mL for paracetamol and 100 µg/mL for dexketoprofen trometamol were prepared in methanol. Different volumes of this solution were applied to the plate resulting in application of 25-150 ng/spot for paracetamol and 100-600 ng/spot for dexketoprofen trometamol to the plate. Each concentration was applied six times to the plate and the plate was developed as described above. Peak areas were plotted against corresponding concentrations to furnish the calibration plot.

**Precision**
The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (25 ng/spot, 50 ng/spot and 75 ng/spot for paracetamol and 100 ng/spot, 200 ng/spot and 300 ng/spot for dexketoprofen trometamol) of the drugs six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

**Limit of detection and limit of quantitaition**
To determine the limits of detection (LOD) and quantitation (LOQ), solutions of concentration in the lower part of the linear range of the calibration plot were used. LOD and LOQ were calculated using the equations LOD = 3.3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration plot.

**Robustness of the method**
Robustness was assessed by deliberately changing the chromatographic conditions and studying the effects on the results obtained.
Specificity
The specificity of the method was determined by analyzing standard drug and test samples. The spot for paracetamol and dexketoprofen trometamol in the samples was confirmed by comparing the R_F and spectrum of the spot with that of a standard. The peak purity of paracetamol and dexketoprofen trometamol 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).

Accuracy
Accuracy of the method was carried out by applying the method to drug sample (Paracetamol and Dexketoprofen trometamol combination tablet) to which known amount of Paracetamol and Dexketoprofen trometamol standard powder corresponding to 80, 100 and 120% of label claim had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

Analysis of a marketed formulation
Twenty tablets were weighed (each containing 500 and 25 mg each of paracetamol and dexketoprofen trometamol and their average weight was calculated. The tablets were finely powdered and powder equivalent to 500 mg of paracetamol and 25 mg of and dexketoprofen trometamol was accurately weighed and dissolved in 30 ml of methanol and sonicated for 30 min and the resulting solution was centrifuged at 3000 rpm for 5 min. The solution was filtered through Whatman filter paper no. 41 and the residue was washed with methanol and volume was adjusted to 50 ml with the same solvent. Both the solutions were further diluted with methanol to obtain the final concentration 1000 ng/µl of paracetamol and 50 ng/µl dexketoprofen trometamol, respectively. The amount of paracetamol and dexketoprofen trometamol present per tablet was calculated by comparing peak area of sample with that of standard.

RESULTS AND DISCUSSION
The results of validation studies on simultaneous estimation of paracetamol and dexketoprofen trometamol was carried out by using toluene: ethyl acetate: acetic acid (6: 4: 0.2 v/v/v) as mobile phase are given below.

Linearity
The drug response was linear (r^2 = 0.9970 for paracetamol and 0.9980 for dexketoprofen trometamol) over the concentration range between 25-150 ng/spot for paracetamol and 100-600 ng/spot for dexketoprofen trometamol.

Precision
The results of the repeatability and intermediate precision experiments are shown in Table 1. The developed method was found to be precise as the % RSD values for repeatability and intermediate precision studies were < 2%, as recommended by ICH guidelines.

LOD and LOQ
Signal-to-noise ratios of 3:1 and 10:1 were obtained for LOD and LOQ respectively. The LOD and LOQ were found to be 15 ng/spot and 25 ng/spot for paracetamol and 80 ng/spot and 100 ng/spot for dexketoprofen trometamol.
Robustness of the method
The standard deviation of peak areas was calculated for each parameter and the % RSD was found to be less than 2. The low values of the % RSD, as shown in Table 2 indicated the robustness of the method.

Specificity
The peak purity of paracetamol and dexketoprofen trometamol was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e., \( r^2 \) (S, M) = 0.9973 and \( r^2 \) (M, E) = 0.9981. A good correlation (\( r^2 = 0.9994 \)) was also obtained between the standard and sample spectra of paracetamol and dexketoprofen trometamol.

Table 1 Precision Studies

<table>
<thead>
<tr>
<th>Concentration (ng/spot)</th>
<th>Repeatability (n=6)</th>
<th>Intermediate precision (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured conc. ± SD</td>
<td>(%) RSD</td>
</tr>
<tr>
<td><strong>Paracetamol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>24.68 ± 2.4</td>
<td>1.28</td>
</tr>
<tr>
<td>50</td>
<td>49.79 ± 1.6</td>
<td>0.94</td>
</tr>
<tr>
<td>75</td>
<td>74.31 ± 8.8</td>
<td>1.58</td>
</tr>
<tr>
<td><strong>Dexketoprofen trometamol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>99.48 ± 10.7</td>
<td>1.03</td>
</tr>
<tr>
<td>200</td>
<td>199.46 ± 16.0</td>
<td>0.67</td>
</tr>
<tr>
<td>300</td>
<td>299.98 ±17.3</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Table 2 Robustness testing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Paracetamol ± SD</th>
<th>% RSD</th>
<th>Dexketoprofen trometamol ± SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase composition (±0.1 ml)</td>
<td>4.07</td>
<td>0.73</td>
<td>3.74</td>
<td>0.48</td>
</tr>
<tr>
<td>Amount of mobile phase (±5%)</td>
<td>21.09</td>
<td>0.95</td>
<td>8.72</td>
<td>0.15</td>
</tr>
<tr>
<td>Time from spotting to chromatography (± 10 min.)</td>
<td>5.22</td>
<td>0.89</td>
<td>3.31</td>
<td>0.85</td>
</tr>
<tr>
<td>Time from chromatography to scanning (± 10 min.)</td>
<td>6.02</td>
<td>0.49</td>
<td>2.63</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Table 3 Recovery studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim (mg per tablet)</th>
<th>Amount Added (%)</th>
<th>Total amount (mg/mL)</th>
<th>Amount recovered (mg) ± % RSD</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>500</td>
<td>80</td>
<td>900</td>
<td>900.44 ± 1.21</td>
<td>100.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>1000</td>
<td>999.98 ± 0.78</td>
<td>99.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>1100</td>
<td>1100.19 ± 1.56</td>
<td>100.01</td>
</tr>
<tr>
<td>Dexketoprofen trometamol</td>
<td>25</td>
<td>80</td>
<td>45</td>
<td>44.92 ± 1.13</td>
<td>99.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>50</td>
<td>50.09 ± 1.43</td>
<td>100.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>55</td>
<td>54.88 ± 1.12</td>
<td>99.78</td>
</tr>
</tbody>
</table>

Recovery studies
Recovery studies were performed. From the data obtained, recoveries of standard drugs were found to be accurate (Table 3).
Analysis of a formulation

Experimental results of the amount of paracetamol and dexketoprofen trometamol in tablets, expressed as a percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets. Two different lots of paracetamol and dexketoprofen trometamol combination tablets were analyzed using the proposed procedures (Table 4).

Table 4 Analysis of commercial formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim (mg/tablet)</th>
<th>Amount found* (mg)</th>
<th>% of drug content*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>500</td>
<td>499.58</td>
<td>99.91</td>
</tr>
<tr>
<td>Dexketoprofen</td>
<td>25</td>
<td>24.89</td>
<td>99.56</td>
</tr>
<tr>
<td>trometamol</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Each value is a mean of six determinations.

![Figure 2 Chromatogram of paracetamol and dexketoprofen trometamol. Peak 1 and 2 are paracetamol and dexketoprofen trometamol, respectively.](image)

CONCLUSION

Introducing TLC into pharmaceutical analysis represents a major step in terms of quality assurance. The developed TLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of paracetamol and dexketoprofen trometamol as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of paracetamol and dexketoprofen trometamol, also for its estimation in plasma and other biological fluids. The proposed TLC method is less expensive, simpler, rapid, and more flexible than HPLC.
Acknowledgement
The authors would like to thank, Emcure Pharmaceutical Ltd. Pune, India for providing a gift sample of standard Paracetamol and Dexketoprofen trometamol. The authors would like to thank, Dr. K. R. Mahadik, Principal, Poona College of Pharmacy, Pune, India for providing necessary facilities to carry out the work.

REFERENCES