Development and Validation of an Analytical Method for Ketoprofen Determination in Matrix Tablets by High Performance Liquid Chromatography

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ABSTRACT SUMMARY
The present study describes the analytical parameters that are aimed at achieving a valid alternative for ketoprofen (KTP) determination in matrix tablets. The chromatographic separation was carried out isocratically at a flow rate of 2.0 ml/min and Ultraviolet – visible detection at 254 nm. A solution of fenoprofen (FNP) was used as internal standard, in a column of LiChroCART® RP18 Purospher Star® 250 mm length x 4.6 mm internal diameter 5μm particle size, coupled to a pre-column LiChro® RP-18 (4 mm x 4 mm, the average particle diameter of 5μm). A mixture of acetonitrile, 0.01 M phosphate buffer (40:60, v/v) adjusted to pH 3.5 with phosphoric acid (H3PO4) were used as mobile phase. It was prepared a stock solution into a 100 mL volumetric flask, dissolving in the mobile phase exactly 200 μg/ml of KTP. The validation parameters of the chromatographic method were analyzed: optimization of chromatographic conditions, selectivity, linearity, precision (repeatability and intermediate precision) and accuracy. The validation results showed that the time of separation, resolution and retention of the method was optimized by modifying the mobile phase at a flow rate to achieve a suitable resolution of drug and FNP in a short time for each analysis.

INTRODUCTION
The selection of a suitable analytical methodology is of fundamental interest for the quality control procedure of an active substance or dosage form. The tendency of the pharmaceutical industry has always been to produce medicines with quality, efficacy and safety, and such trend over the years has led to the development of recommendations and incorporation of requirements that have evolved to strict regulation, with the main objective to implementation of specific analytical techniques for identification and quantification of substances involved in the production of pharmaceutical dosage forms. Following control efforts developed under the quality control comes the concept of validation1. However, in general, the various existing concepts of validation reflect the same general direction, differing only as to how it had been previously observed by Chapman2. The prominence given to the validation of analytical methods varies depending on the application area, the concentration of the analyte, the purpose of the study and the nature of the method3.

EXPERIMENTAL METHODS
Materials: Drug - ketoprofen (KTP) (batch 043K0684), Sigma - Aldrich Chemie, Germany. Polymers - methylcellulose Methocel® MC25 (batch MC25MFCD00081763), Fluka, Switzerland; hydroxypropyl cellulose (HPC batch 8174), Klucel, HF, USA; hydroxypropyl methylcellulose Methocel® K15M (batch OG20012N31) and Methocel® K100M (batch OB12012N11), Colorcon, England. Binders - monohydrate lactose (LAC) Granulac® 200, Meggle, Wasserburg, Germany, β - cyclodextrin (β - CD), Kleptose®, Roquette, Lestrem, France. Lubricant - magnesium stearate and talc analytical grade. Fenoprofen (FNP) (batch 122K1268), Sigma - Aldrich Chemie GmbH, Steinheim, Germany, which was used as internal standard. Other reagents and solvents such as methanol and acetonitrile (ACN), analytical grade (Sigma-Aldrich and Merck KGaA, Darmstadt, Germany). Deionized water system (Millipore Elix 5) was used for all studies. Shimadzu UV-1603, Japan and chromatograph (Hewlett Packard Model HP 1050 Waldburg, Germany

Chromatographic conditions. The chromatographic conditions were based KTP by the proposed method by Roda et al4. The chromatographic separation was reverse phase on a column of LiChroCART® RP-18 Purospher Star® 250 mm length x 4.6 mm internal diameter 5μm particle size (Merck, Darmstadt, Germany) coupled to a pre-column LiChro® RP-18 (4 mm x 4 mm ID, average particle diameter of 5μm, Merck). The mobile phase consisted of a mixture of ACN, 0.01 M phosphate buffer (40:60, v/v) adjusted to pH 3.5 with phosphoric acid. The chromatographic separation was performed isocratically with a flow rate of 2.0 ml/min and UV detection at 254 nm. A solution of FNP was used as internal standard. The HPLC method was validated KTP for determining the parameters of selectivity, linearity, precision (repeatability and accuracy/intermediate) and accuracy.

RESULTS AND DISCUSSION
Optimization of chromatographic conditions. The separation time, resolution and retention of both methods is optimized by modifying the mobile phase and the flow rate to obtain adequate resolution of the drug (KTP) and internal standard (PNF) for each analysis in a short time. The KTP gave a retention time of 9 min and 18 min for FNP.
Selectivity

As can be seen in Figure 1, the selectivity of the analytical method was demonstrated by the fact of not having seen any other peak, corresponding to the addition of drug and internal standard, after injection of the solution where excipients were present.

Figure 1. Corresponding to assess the selectivity of the assay method of KTP chromatogram (A) solution containing 10 g/ml of FNP, (B) buffer solution containing 6 mg/ml of KTP and 10 g/ml of FNP, (C) solution sample.

Linearity

Linearity was also observed between the ratio of the peak area of the internal standard KPT depending on the concentration of the KPT in the concentration of 2-10μg/ml. The linear regression equation obtained for the ratio of the peak area (y) versus concentration (x) is as follows: y = 0.1829x - 0.0103, with a regression coefficient of 0.9999 (n = 5).

Precision (repeatability and intermediate precision)

Table 2 is represented corresponding to the study of precision (repeatability and intermediate precision) results.

Table 1. Repeatability (n = 10) and intermediate precision (n = 5) HPLC method for determination of KTP, expressed as coefficient of variation (CV).

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Repeatability CV (%)</th>
<th>Intermediate precision CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.56</td>
<td>0.46</td>
</tr>
<tr>
<td>6</td>
<td>0.41</td>
<td>0.15</td>
</tr>
<tr>
<td>8</td>
<td>0.17</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Accuracy

The percent recovery of the solutions prepared above in relation to the theoretical value (100%), as well as the percentage difference between the determined average concentration and the theoretical concentration (bias) are summarized in Table 2. The percentage recovery and bias varied only between 97.68 and 97.85% (n = 5) and between 0.32 and 0.47 (n = 5) for KTP, which indicated good accuracy of this HPLC.

Table 2. Accuracy of the HPLC method of KTP as a percentage of recovery and the presence of excipients bias.

<table>
<thead>
<tr>
<th>Excipients</th>
<th>Concentration (μg/ml)</th>
<th>Recovery (%)</th>
<th>Bias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC25</td>
<td>4</td>
<td>97.68 ± 0.44</td>
<td>-2.32</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>99.15 ± 0.15</td>
<td>-0.85</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>101.37 ± 0.48</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>98.39 ± 1.39</td>
<td>-1.61</td>
</tr>
<tr>
<td>HPC</td>
<td>6</td>
<td>97.90 ± 0.14</td>
<td>-2.10</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>100.71 ± 1.64</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>99.69 ± 2.40</td>
<td>-0.31</td>
</tr>
<tr>
<td>HPMC K15M</td>
<td>6</td>
<td>99.15 ± 0.15</td>
<td>-0.85</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>100.50 ± 1.70</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>98.38 ± 1.43</td>
<td>-1.62</td>
</tr>
<tr>
<td>HPMC K100M</td>
<td>6</td>
<td>100.47 ± 2.02</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>101.37 ± 0.48</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>101.06 ± 2.39</td>
<td>1.06</td>
</tr>
<tr>
<td>LAC</td>
<td>6</td>
<td>98.41 ± 0.86</td>
<td>-1.59</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>100.63 ± 1.52</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>101.56 ± 1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>β-CD</td>
<td>6</td>
<td>99.42 ± 0.56</td>
<td>-0.58</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>102.42 ± 1.61</td>
<td>2.42</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>99.53 ± 0.63</td>
<td>-0.47</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>100.76 ± 1.14</td>
<td>0.76</td>
</tr>
</tbody>
</table>

CONCLUSION

The results have demonstrated that the HPLC method developed for the quantification of KTP in the presence of MC25, HPC, HPMC K15M or K100M, LAC or β-CD, present parameters of selectivity, linearity, precision and acceptable accuracy for dosing of drugs, and have been widely used in various stages of research and development of tablets and other dosage forms.

REFERENCES


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