Using RP-HPLC Method for Determination of Some Anti-Inflammatory (NSAIDs) in Pharmaceutical Drugs

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Abstract

Determination of some Anti-Inflammatory (Ketoprofen (KP), Flurbiprofen (FP), and Diclofenac Sodium (DS)) in pharmaceutical drugs were carried out employing Reverse Phase of High Performance Liquid Chromatographic using isocratic separation. Separation was performed on an Enable C18 column (250 mm x 4.6 mm, 5.0 µm) using ((1%) Triethylamine aqueous buffer adjust pH=2 by H₃PO₄ (85%): Methanol: Acetonitrile); (35: 20: 45 v/v%) as the mobile phase at a flow rate of 2.0 ml/min. The wavelength detection was set at 220 nm. The linearity was observed over a concentration range of (0.05–200) µg/ml for RP-HPLC method (correlation coefficient=0.999). The developed method was validated according to ICH guidelines. The relative standard deviation values for the method precision studies were < 2% and the accuracy was > 99%. The developed method was used successfully for the determination of Ketoprofen (KP), Flurbiprofen (FP) and Diclofenac Sodium (DS) in Tablets and Dry Powder for inject pharmaceutical formulations.

Keywords: Ketoprofen (KP), Flurbirprofen (FP), Diclofenac Sodium (DS), Anti-Inflammatory, RP-HPLC.

1. Introduction

Ketoprofen, (KP): C₁₆H₁₄O₃, M. W= 254.3 gr/mole, (2RS)-2-(3-Benzoylphenyl) Propanoic acid. (Fig. 1) [1].
Flurbiprofen, (FP): C₁₅H₁₃FO₂, M. W= 244.3 gr/mole, (2RS)-2-(2-Fluorobiphenyl-4-yl) Propanoic acid. (Fig. 2) [1].
Diclofenac Sodium, (DS): C₁₄H₁₀Cl₂NNaO₂, M. W= 318.1 gr/mole, Sodium 2-[(2.6-dichlorophenyl) amino] phenyl] acetate. (Fig. 3) [1].

Anti-Inflammatory Painkillers are used to treat arthritis, sprains, painful periods, tendinitis and other painful conditions. Anti-Inflammatory painkillers are sometimes called non-steroidal anti-Inflammatory drugs (NSAIDs) or just Anti-Inflammatory such as: Ketoprofen (KP), Flurbiprofen (FP), and Diclofenac sodium (DS) [2].

Numerous NSAIDs analysis of ways because of the many types and its indications currently, there are various methods available to identify NSAIDs in the world such as: colorimetric [3], spectrophotometry [4] capillary electrophoresis [5], Gas Chromatography [6]. The determination of DS in pharmaceutical formulation including colorimetric [7], spectrophotometry [8], capillary electrophoresis [9], Flow injection [10] Spectrofluorsence [11-12] Voltammetry [13], RP-HPLC [14]. But no analytical methods are reported for the determination of Anti-Inflammatory in Tablets and Dry powder for inject pharmaceutical formulations using the mobile phase ((1%) Triethylamine aqueous buffer adjust pH=2 by H₃PO₄ (85%); Methanol: Acetonitrile); (35: 20: 45 v/v%) by Reverse Phase of High Performance liquid chromatography. So a successful attempt was made to develop and validate a fast, simple, precise, and accurate RP-HPLC method for the determination of KP, FP, and DS in Tablets and Dry powder for inject pharmaceutical formulations. Specificity and stability parameters for the drug were assessed according to ICH [15].

2. Experimental and Chemical Solvents

Ketorprofen (KP), Flurbiprofen (FP), and Diclofenac Sodium (DS); (purity of all > 99.90%) was obtained as a gift sample from Merck Ltd, Germany. Methanol, Acetonitrile and Triethylamine was chemical solvents from Merck Ltd, Germany for Analytical HPLC grade. The Ultra-pure water for RP-HPLC was obtained by using the TKA Water Purification System, Germany. The Tablets formulation containing 100 mg/tab for KP, FP, and DS, and dry powder for inject formulations containing 75 mg/3ml for DS were bought from the local market.
3. Instrumentation
Quantitative RP-HPLC was performed on Lab Alliance machine Prominence LC pumps with a 20 µl sample injection loop and UV-Vis detector. The signal was recorded and integrated using Clarity Software. An Enable C18 column, (250 mm × 4.6 mm, particle size 5µm) was used for separation. Chromatographic analysis was carried out at ambient temperature on the column using the ((1%) Triethylamine aqueous buffer adjust pH=2 by H₃PO₄ (85%): Methanol: Acetonitrile); (35: 20: 45 v/v%) as the mobile phase at a flow rate of 2.0 ml/min in Isocratic mode. The wavelength detection was carried out at 220 nm.

4. Preparation of Standard and Sample Solution
Standard stock solutions of KP, FP, and DS were prepared by transferring 100 mg of the drug into two separate 100 ml volumetric flasks having 10 ml of diluents and were ultra-sonicated for 5 minutes. Finally, the volume was made up with suitable diluents, which gave 1000 µg/ml solutions. Powder (Tablets and Dry Powder for Inject Forms) equivalent to 100 mg of KP, FP, and DS was accurately measured and transferred into two separate 100 ml volumetric flasks, containing 10 ml of diluents and ultra-sonicated for 15 minutes; the volume was made up and mixed well. Solutions were filtered by a 0.2 µm filter to remove particulate matter, if any found. The filtered solutions were properly diluted for analysis as already described. The drug present in the sample solutions was calculated by using the calibration curves. All the solutions were stored at (2-8) ºC for future use.

5. Method Validation
5.1 Linearity
A twenty-two-point (0.05, 0.1, 0.4, 0.8, 1, 4, 8, 12, 18, 22, 28, 34, 40, 48, 50, 60, 70, 100, 150 and 200) µg/ml calibration curves were prepared for the RP-HPLC method. The peak area for the RP-HPLC was obtained by injecting 20 µl of the drug solution into the column. Calibration curves were plotted by taking the peak area curve on the y-axis and the concentration (µg/ml) on the x-axis.

5.2 Precision and Accuracy
The intraday precision study was carried out to check the reproducibility of the results. A concentration of (8, 14, 22) µg/ml and (34) µg/ml of KP, FP, and DS (n=6) were analyzed to find out relative standard deviation (RSD) for RP-HPLC methods. The recovery study was performed six times at each level. The amount of KP, FP, and DS present in the sample was calculated using the calibration curves.

5.3 Robustness
The robustness of the RP-HPLC method was studied by deliberately changing the method parameters like flow rate of the mobile phase, detection wavelength, and organic phase composition. A series of system suitability parameters like retention time, theoretical plates, and tailing factor were determined for each changed condition according to ICH [15].

5.4 Limit of Detection and Limit of Quantitation
The LOD and LOQ were determined separately according to the ICH guidelines [15]. For the RP-HPLC method, concentrations providing a signal-to-noise ratio 3:1, and 10:1 were considered as the LOD and LOQ, respectively.

5.5 Results and Discussion
Optimization of the mobile phase was carried out based on the tailing factor and theoretical plates obtained for KP, FP and DS. During the trial runs, the drug was tested with different mobile phase compositions ((1%) Triethylamine aqueous buffer adjust pH=2 by H₃PO₄ (85%): Methanol: Acetonitrile) at various compositions (10: 5: 85 v/v%), (20: 10: 70 v/v%), and (30: 20: 50 v/v%), and flow rates (0.5, 1.0, 1.5, and 2ml/min). The mobile phase consisting of ((1%) Triethylamine aqueous buffer adjust pH=2 by H₃PO₄ (85%): Methanol: Acetonitrile); (35: 20: 45 v/v%) at a flow rate of 2.0 ml/min was selected which gave a sharp, symmetric peak for KP, FP, and DS. The retention time for KP, FP, and DS was found to be (3.20, 4.49, and 5.07) min, respectively. The run time was 6 min. The tailing factor for KP, FP, and DS was found to be (1.21, 1.08, and 1.02). The wavelength detection was carried out at 220 nm. The separation of KP, FP, and DS chromatograms was carried out at room temperature. (Fig. 4).
Fig. 4. Overlay chromatograms of KP, FP, and DS for RP-HPLC Proposed Method

The tested the proposed method in the process of chromatographic separation, separated the three vehicles at a concentration of 50 µg/ml. The results of the chromatographic lobe in the following form, as in the (Table. 1) and (Fig. 5).

Fig. 5. Chromatograms of KP, FP, and DS at 50 µg/ml concentration

Tab. 1. Analysis of method Chromatographic parameters for KP, FP, and DS at 50 µg/ml concentration

<table>
<thead>
<tr>
<th>Name Compound</th>
<th>Area (mV. s)</th>
<th>Number of Theoretical Plate</th>
<th>Tailing Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ketoprofen (KP)</strong></td>
<td>789.759</td>
<td>8462</td>
<td>0.983</td>
</tr>
<tr>
<td><strong>Flurbiprofen (FP)</strong></td>
<td>423.406</td>
<td>9355</td>
<td>0.939</td>
</tr>
<tr>
<td><strong>Diclofenac Sodium (DS)</strong></td>
<td>694.055</td>
<td>9672</td>
<td>0.879</td>
</tr>
</tbody>
</table>

4.1.1 Linearity

The calibration curves were found to be linear over a concentration range of (0.05–200) µg/ml for methods (correlation coefficient 0.999 for all the methods). The method parameters and regression data are shown in (Fig. 6, 7, and 8) and (Table. 2).
Tab. 2. Analysis of method parameters and regression data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ketoprofen (KP)</th>
<th>Flurbiprofen (FP)</th>
<th>Diclofenac Sodium (DS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>5.409</td>
<td>9.7593</td>
<td>4.3034</td>
</tr>
<tr>
<td>Intercept</td>
<td>15.053</td>
<td>8.1986</td>
<td>8.2426</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9998</td>
<td>0.9997</td>
<td>0.9998</td>
</tr>
<tr>
<td>Detection Wavelength, nm</td>
<td></td>
<td></td>
<td>220</td>
</tr>
<tr>
<td>Linear rang, µg/mL</td>
<td>0.05-200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.2.1 Precision and Accuracy
The methods were found to be precise as the RSD (%) values for the precision studies were well below 2% (n=6). The results are shown in Table. 3. The accuracy of the developed methods was found out by the standard addition method. High recovery values suggest that all three methods are accurate. The results are shown in (Table. 3).

4.3.1 Limit of Detection and Limit of Quantitation
The LOD and LOQ values shown in (Table. 3). Suggest that the developed methods are sensitive to determine Anti-Inflammatory (NSAIDs).
### Tab. 3. Summary of validation parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Accuracy(recovery),%</th>
<th>*Precision(RSD),%</th>
<th>LOD, µg/ml</th>
<th>LOQ, µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoprofen (KP)</td>
<td>99.78</td>
<td>1.49</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>Flurbiprofen (FP)</td>
<td>100.02</td>
<td>1.70</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>Diclofenac Sodium (DS)</td>
<td>100.79</td>
<td>1.56</td>
<td>0.02</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Average of six determinations at each level.

#### 4.4.1 Robustness

The RP-HPLC method was found to be robust under deliberate changes in the mobile phase flow rate (±0.1 mL/min), detection wavelength (±5 nm), and organic phase composition (± 2%). The results of system suitability for the robustness study are shown in (Table. 3). No significant changes were obtained in the content of KP, FP, and DS during the solution stability studies by the developed methods. The recoveries for the solution stability by Method was found to be 99.78%, 100.02%, and 100.79%, respectively.

### 4.6 Analysis of Commercial Dry Syrup Formulation

The developed methods were successfully applied for the determination of KP, FP, and DS in the dry syrup formulation. The result for the assay of KP, FP, and DS is shown in (Table. 4). The assay results obtained for KP, FP, and DS in Tablets and Dry Powder for Inject pharmaceutical formulations using the RP-HPLC method.

#### Tab. 4. Assay of pharmaceutical formulations

<table>
<thead>
<tr>
<th>Name-Company (Formulation Label Claim)</th>
<th>*RSD%</th>
<th>Recovery(%)±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketophen-Medico Labs (100 mg/Tab)</td>
<td>1.63</td>
<td>99.80±1.03</td>
</tr>
<tr>
<td>Voltamed-Medico Labs (100 mg/Tab)</td>
<td>1.57</td>
<td>101.25±1.05</td>
</tr>
<tr>
<td>Diclon-Ibn Hayan (75 mg/3mL)</td>
<td>1.62</td>
<td>101.20±1.04</td>
</tr>
<tr>
<td>Diclon-Ibn Hayan (100 mg/Tab)</td>
<td>1.54</td>
<td>99.90±1.03</td>
</tr>
<tr>
<td>Flurbiprofen-Ultramedica (100 mg/Tab)</td>
<td>1.31</td>
<td>100.65±1.14</td>
</tr>
</tbody>
</table>

* Average of six determinations at each level.

#### 4.7 Conclusion

The novel RP-HPLC analytical method was developed for the determination of (KP, FP, and DS). The validation study shows the methods are specific, linear, precise, accurate, and sensitive in the proposed working range. The method was found to be fast, simple, accurate, precise, and sensitive. The excipients present in the commercial formulation were found to be non-interfering in the assay results. The method was successfully applied for the determination of the drug in Tablets and Dry powder for inject pharmaceutical formulations. Furthermore, the developed method may be applied for the routine analysis of the drug in API, formulations, and dissolution medium.

**5.0 Acknowledgement**

The authors are thankful to Medico Labs Pharmaceutical Company, Homs, Syria for providing the gift sample of Ketoprofen (KP), Flurbiprofen (KP), and Diclofenac Sodium (DS).

**5.1 References**


