A New Isocratic HPLC Method for Simultaneous Determination of Paracetamol and Flurbiprofen in Pharmaceutical Preparations

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Abstract
An isocratic HPLC method has been developed for simultaneous determination of paracetamol-acetaminophen (PAR) and flurbiprofen (FLU) in pharmaceutical preparations. The method was based on separation by using a mobile phase acetonitrile–0.1M sodium acetate solution (pH:5.00) (60:40,v/v) and reversed-phase C18 column. The mobile phase flow rate was 0.8mL/min with UV detection at 247nm. The linearity ranges were 0.1–1.5 μg/mL for PAR and FLU. The limits of detection and quantification were found to be 16.33 ng/mL and 54.45 ng/mL for PAR and 15.25 ng/mL and 50.82 ng/mL for FLU, respectively. The combination of these two drugs has not commercially available but patented as combined preparation. The proposed method was successfully validated and applied to the determination in pharmaceutical preparations. The developed and validated method is simple, sensitive and reproducible and can be used safely routine simultaneous analysis of PAR and FLU in pharmaceutical preparations in the future.

Keywords: Paracetamol; Acetaminophe; Flurbiprofen; HPLC; Pharmaceutical preparations.

1. Introduction
Paracetamol (PAR), N-acetyl-p-aminophenol , acetominophen (Figure 1) is an analgesic and antipyretic drug for relief of pain and fever reduction [1].

![Figure 1. Chemical structure of PAR](image)

Flurbiprofen, (±)-2-(2-fluoro-4-biphenylyl) propionic acid (Figure 2) is an important non-steroidal, anti-inflammatory drug (NSAID). It is usually recommended to the patients with rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and gout [2]. Various chromatographic methods were described for determination of PAR by HPLC with UV [3-12], and LC-MS/MS analysis [13-15], spectrophotometric [16]. Some electrochemical methods were also carried out by voltammetry [17, 18].
Several LC methods using UV detection [19-26], LC-MS/MS [27, 28] and fluorescence detection [29] techniques have been reported for the estimation of FLU either alone or together with their metabolites in plasma and tablet form. Although there is no any commercial preparation of PAR and FLU simultaneously, a patent has been developed for some preparations containing each other [30]. For this purpose, an isocratic LC method for simultaneous determination of PAR and FLU in tablet form has been developed and validated in this study.

2. Experimental
2.1. Chemicals and Reagents
PAR and FLU was obtained from (Atabay Kimya Sanayi ve Ticaret A.Ş.and FDC Limited ). Methanol which is HPLC grade was purchased from Sigma-Aldrich. Acetonitrile which is HPLC grade purchased from VWR. All other chemicals were analytical grade. HPLC grade solvents were used. Purified water for the HPLC was prepared through a Milli-Q® Integral Ultrapure Water Purification System (Merc Millipore ).

2.2. Pharmaceutical Formulation
Fixed-dose combination tablets containing 150 mg PAR, 100 mg FLU for active ingredient and 75 mg lactose, 50 mg corn starch, 4 mg magnesium stearate in each tablet were prepared. Ten tablets of the preparation were weighted and finely powdered.

2.3. Chromatographic Conditions
The HPLC system of Agilent Infinity 1260 Series HPLC-UV were used for analysis of PAR and FLU. The data acquisition and analysis were performed using the Chem32 software (Agilent Technologies). Chromatographic separation was carried out using a reversed phase Intersil ODS, 5 µm, 250x4.6 mm column at 25°C temperature. The mobile phase was consist of (acetonitrile: 0.1M sodium acetate pH:5) (60:40) (v/v). Flow rate of mobile phase was 0.8 mL/min. The detector was set 247 nm. The injection volume was 20 µL.

2.4. Preparation of Standard Solutions
Stock solutions of PAR (1.0 mg/mL) and FLU (1.0 mg/mL) were prepared and diluted to 10µg/mL with acetonitrile. All solutions were stored at 4°C. The five calibration standards were prepared with acetonitrile between the concentration ranges of 0.1–1.5 µg/mL. The quality control (QC) samples were prepared at concentrations of 100, 500, 1000, and 1500 ng/mL. All calibration standards and QC samples were store at 4°C.

2.5. Method Validation
The method was validated according to ICH guidelines for linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ), robustness, and system suitability. The linearity was evaluated by analyzing different concentrations of the standard solutions of PAR and FLU. The calibration curves were constructed over the concentration range of 0.1 – 0.3 – 0.6 – 0.75 - 1.5 µg mL⁻¹ for PAR and 0.1 - 0.2 - 0.4- 0.75- 1.5µg mL⁻¹ for FLU, and the linearity was evaluated by plotting the peak area against the concentration. The linear regression equation was computed. LOD and LOQ values of the method were evaluated using the following equations:
LOD = 3 x $\sigma$/S  
LOQ = 10 x $\sigma$/S

where $\sigma$ is the standard deviation of the response and “S” is the slope of the calibration curve.

2.6. Analysis of Dosage Form
Ten tablets were powdered and equal to one tablet was weighed. Then it was diluted to volume with acetonitrile in a 100 mL flask (6 replicates). Further dilution was carried out by transferring appropriate amounts for final concentrations of 0.6µg/mL PAR and 0.4µg/mL FLU.

3. Results and Discussion
Chromatographic separation of analytes were optimized to provide acceptable resolution, good peak shape and intensity of the response. Mobile phase composition was changed systematically to establish chromatographic conditions giving an acceptable resolution. A typical LC chromatogram of standard mixture containing PAR and FLU is given in Figure 3.

![Figure 3. LC chromatogram of standard mixture containing PAR (0.3 µg/mL) and FLU (0.2 µg/mL)](image)

The selectivity of method was assessed by analyzing inactive ingredients of pharmaceutical preparations. Chromatograms were compared for any interference from the matrix or any of the assay reagents. The linearity ranges were 0.1-1.5 µg/mL for both of PAR and FLU. The calibration curves were prepared from five calibration samples within the range of 0.1-1.5 µg/mL. The acceptance criteria of back calculated standard concentration was 15% deviation from nominal value except the LOQ (for LOQ 20 % deviation was applied). The standart calibration curves were linear over the concentration range from 1.5 µg/mL with mean $r^2=0.9994$ for PAR and $r^2=1.0000$ for FLU. LOD and LOQ values were found to be 16.33 ng/mL and 54.45 ng/mL for PAR and 15.25 ng/mL and 50.82 ng/mL for FLU, respectively (Table 1).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration Range (ngmL⁻¹)</th>
<th>Regression Equation</th>
<th>Correlation Coefficient ($r^2$)</th>
<th>LOD (ngmL⁻¹)</th>
<th>LOQ (ngmL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAR</td>
<td>100 - 1500</td>
<td>$y=0.110118x - 1.58599$</td>
<td>0.9994</td>
<td>16.33</td>
<td>54.45</td>
</tr>
<tr>
<td>FLU</td>
<td>100 - 1500</td>
<td>$y=0.116354x - 1.07823$</td>
<td>1.0000</td>
<td>15.25</td>
<td>50.82</td>
</tr>
</tbody>
</table>

Table 1. Linearity values of PAR and FLU

Intra batch accuracy and precision were determined by analysis of six replicates of 5 concentrations including low, medium and high concentration QC samples. Inter-batch accuracy and precision were
determined by the analysis of these QC samples on three separate states. The overall precision of the method was expressed as percentage of coefficient of variation and the accuracy of the method was expressed in terms of relative errors. Table 2 gives a summary of the precision. Recovery values were found 101.13 % ± 0.41 for PAR and 98.17% ± 0.77 for FLU in pharmaceutical preparation (Table 3).

Table 2. Precision studies of PAR and FLU

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (ngmL⁻¹)</th>
<th>Interday Determination [n=6]</th>
<th>%RSD</th>
<th>Interday Determination (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak Area (mAU*5)</td>
<td>Concentration Measured (ngmL⁻¹)</td>
<td></td>
<td>Concentration Measured (ngmL⁻¹)</td>
</tr>
<tr>
<td>PAR</td>
<td>500</td>
<td>53,9496</td>
<td>0.9351</td>
<td>53,5519</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>109,3039</td>
<td>0.5681</td>
<td>108,2281</td>
</tr>
<tr>
<td>FLU</td>
<td>500</td>
<td>59,02584</td>
<td>0.9814</td>
<td>57,4288</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>116,0660</td>
<td>0.4954</td>
<td>115,4786</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>174,6009</td>
<td>0.50245</td>
<td>173,9653</td>
</tr>
</tbody>
</table>

Table 3. Accuracy studies of PAR and FLU

<table>
<thead>
<tr>
<th>Drug</th>
<th>Percent of Nominal (level)</th>
<th>Amount Added (ngmL⁻¹)</th>
<th>Amount Recovered (ngmL⁻¹ ± RSD%)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAR</td>
<td>50%</td>
<td>300</td>
<td>300,7982 ± 1,3389</td>
<td>100,26</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>600</td>
<td>604,9395 ± 0,7354</td>
<td>100,82</td>
</tr>
<tr>
<td></td>
<td>125%</td>
<td>750</td>
<td>745,5867 ± 0,8864</td>
<td>99,41</td>
</tr>
<tr>
<td>FLU</td>
<td>50%</td>
<td>300</td>
<td>301,1910 ± 0,3643</td>
<td>100,39</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>600</td>
<td>611,8420 ± 0,9112</td>
<td>101,97</td>
</tr>
<tr>
<td></td>
<td>125%</td>
<td>750</td>
<td>755,8400 ± 0,6784</td>
<td>100,77</td>
</tr>
</tbody>
</table>

4. Conclusion

A new isocratic HPLC method has been developed and validated for simultaneous determination of PAR and FLU in pharmaceutical preparations in the first time. This method; simple, accurate, precise and selective can be used for the quality control analysis of PAR and FLU in pharmaceutical preparations in the future.

Acknowledgement

The present work was supported by the Research Fund of Istanbul University. Project No:40085

References


