Simultaneous Estimation of Aceclofenac, Paracetamol and Tizanidine in Their Combined Dosage Forms by Spectrophotometric and RP-HPLC Method

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Abstract

A rapid and sensitive RP-HPLC method with UV detection and UV spectrophotometric method for routine Pharmaceutical quality control of aceclofenac, paracetamol and tizanidine in tablets formulation was developed. Chromatography was performed by using Phenomenex-Luna C₁₈ (250 x 4.6 mm, 5µ) with a mobile phase containing Methanol: Water (90:10v/v), flow rate 1mL/min, wavelength at 256 nm. Linearity observed over the concentration range between 5-30µg/mL, 10-60µg/mL and 2-12µg/mL for aceclofenac, paracetamol and tizanidine respectively. The UV spectrophotometric method was performed at 277, 248 and 323 nm by derivative spectrophotometry with simultaneous equation method. The entire three drugs obey Beer’s law in the concentration range between 5-30 µg/mL, 2-16 µg/mL and 2-30 µg/mL respectively. The proposed methods were simple, rapid, precise, accurate and sensitive and can be used for routine quality control in pharmaceuticals.

Keywords: Derivative spectrophotometry; RP-HPLC; Aceclofenac; Paracetamol; Tizanidine

Introduction

Aceclofenac (ACO) is 2-[2-[(2,6-dichlorophenyl) amino]phenyl] acetyl] oxacyclic acid and non-steroidal anti-inflammatory drug [1]. Aceclofenac is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis. Paracetamol (PCM) is N-(4 hydroxyphenyl) acetamide [2] is a potent analgesic and anti-pyretic drug.Tizanidine (TZN) is 5-chloro N(4,5-dihydro-1H-imidazol-2-yl)-2,1,3-benzothiadiazol-4-amine,used as a muscle relaxant [3]. It used to treat the spasms, cramping and tightness of muscles etc. (Figure 1).

A tablet dosage form containing all the three (ACO 100 mg, PCM 500 mg and TZN 2 mg) is commercially available and used as non-steroidal anti-inflammatory, analgesic, muscle relaxant. ACO, PCM and TZN are official in Indian pharmacopoeia. ACO has been analyzed separately and in combination with other drug by UV [4-7] and HPLC [8-12]. PCM has been analysed separately and combination with other drug by UV [13-15], HPLC [16], RP-HPLC [17]. TZN has been analysed separately and combination with other drug by UV [18-20], RP-HPLC [21-22], HPTLC [23-24] and spectrophotometric method has been reported for ACO and TZN [25]. Spectrophotometric method has been reported for estimation of PCM, NIMS (Nimesulide) and TZN [26]. However no spectrophotometric method for simultaneous analysis of ACO, PCM and TZN in combination dosage form has been reported yet, hence the present study is essential to develop simultaneous estimation method for all three drugs in tablet formulation.

Keywords: Derivative spectrophotometry; RP-HPLC; Aceclofenac; Paracetamol; Tizanidine

Experimental

Reagents and chemicals

The ACO, PCM and TZN reference standards were obtained from Micro labs Pvt. Ltd. Bangalore, Karnataka and Excel Parma Pvt. Ltd, Mhesana, Gujarat. Pharmaceutical dosage forms (Zerodol MRR) containing 500 mg PCM, 100 mg ACO and 2 mg TZN were obtained commercially (Manufactured by IPCA Laboratories Ltd. Mumbai). Acetonitrile and Methanol for chromatography were purchased from Merck and S.D. fine chemicals. HPLC Grade water from Milli-Q Gradient A10 (Milli-pore) were used to prepare the solutions for the UV spectrophotometric experiments.

Apparatus / Instrumentation and analytical conditions

Chromatographic analysis was performed using Shi-madzu LC-20AT HPLC system equipped with LC-20AT pump and SPD-M20A detector. Chromatography was performed on Phenomenex Luna C₁₈ column (250 x 4.6 mm i.d, 5µ) maintained at 40°C. The mobile phase composition was Methanol: Water 90:10 v/v (Binary flow) which was pumped at flow rate of 1 mL/min without splitter. The mobile phase was prepared freshly before use and filtered through 0.45 µm membrane filter (Milli-pore) and degassed by ultra sonication bath before use .The auto sampler temperature was set at 10°C. The wavelength of the

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UV detector was set to 256 nm. The UV spectrophotometric method was performed on a UV/Visible double beam spectrophotometer (Shimadzu model-1700) at 277 nm, 248 nm and 323 nm using 1 cm Quartz cells.

Preparation of the standard solution

For the HPLC method, ACO, PCM and TZN (10 mg each) were weighed accurately and transferred to separate 100 mL volumetric flasks. All drugs were dissolved in 100 mL methanol to prepare standard stock solution of 100 μg/mL. For the UV spectrophotometric method, ACO, PCM and TZN (10 mg each) were weighed and transferred to 100 mL volumetric flask, dissolved and made up to the volume using methanol. Accurately pipette out 10 mL of above solution separately into 100 mL standard flask and the volume was made up to 100 mL using methanol to get a concentration of 100 μg/mL of ACO, PCM and TZN respectively.

Preparation of the sample solutions

For both UV and HPLC analysis of the tablet dosage form, twenty tablets of Zerodol MRR were weighed individually and their average weight was determined. The tablets were then crushed to a fine powder and powder equivalent to the weight of one tablet was transferred to a 100 mL volumetric flask and dissolved in 50 mL methanol. The solution was shaken vigorously for 15 min and filtered through Whatman #41 filter paper.

Construction of calibration plots

Solutions of ACO, PCM and TZN drugs having different concentrations were prepared by dilution of the standard solutions. These solutions (20 μL) were chromatographed and the peak areas were measured. Peak areas were then plotted against the respective concentrations for ACO, PCM and TZN. From the plots it was found that the linear range for ACO, PCM and TZN was between 5-30, 10-60 and 2-12 μg/mL for HPLC method whereas for UV method was between 5-30, 2-16 and 2-30 μg/mL.

Method Validation

The methods were validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures [27]. The System suitability test was carried through for both the methods evaluating theoretical plates and asymmetry.

Linearity

The calibration curve was obtained with five concentrations of the standard solution in the range 5 to 30, 10 to 60 and 2 to 12 μg/mL for HPLC method and for UV spectrophotometric method the linearity range was optimized with 5 to 30, 2 to 16 and 2 to 30 μg/mL for ACO, PCM and TZN respectively (Figure 2-4).

Table 1: Result of assay by HPLC and UV method.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug</th>
<th>Label claim mg/tablet</th>
<th>HPLC method</th>
<th>UV method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zerodol-MRR</td>
<td>ACO</td>
<td>100</td>
<td>101.68±0.99</td>
<td>99.78±0.98</td>
</tr>
<tr>
<td></td>
<td>PCM</td>
<td>500</td>
<td>100.55±0.64</td>
<td>99.84±0.48</td>
</tr>
<tr>
<td></td>
<td>TZN</td>
<td>2</td>
<td>100.32±0.34</td>
<td>99.70±0.63</td>
</tr>
</tbody>
</table>

*Average of five readings
Precision

The assay precision was carried out by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying sample solutions of three different concentrations of ACO, PCM and TZN (5, 10 and 15µg/mL, 10, 20 and 30µg/mL, 6, 8 and 10µg/mL for HPLC method and 5, 10 and 15µg/mL, 6, 8 and 10µg/mL, 5, 10 and 15 µg/mL for UV spectrophotometric method) were prepared and assayed at same concentration and during the same day. The Percentage relative standard deviation (% RSD) values of peak area for repeated injections of ACO, PCM and TZN were found to be 0.08 %, 0.17%, 0.023 % and 0.023%, 0.014 %, 0.027% respectively. Intermediate precision of the method was checked by repeating studies on three different days. Percentage relative standard deviation (%RSD) was found to be less than acceptable limit of 2% for within a day and day to day variations, which proves that method is precise.

Accuracy

The accuracy of an analytical method is the closeness of the test results obtained by the method to the true value. For the spectrophotometric method, the accuracy was determined by recovery of known amounts of ACO, PCM and TZN reference standard added to the samples at the beginning of the process. The accuracy was assessed from five replicate determinations of three concentration levels and the absolute means obtained were shown in Table 3, and it is evident that the method is accurate within the desired range. For the HPLC method, the accuracy was determined by the assay of three concentrations of the sample solution in triplicate and the absolute means obtained were shown in Table 3.

Specificity

The specificity of the HPLC and UV spectrophotometric methods was determined. This parameter was performed to assess and ensure that the impurities, degraded products and diluents do not affect the samples analyzed. ACO, PCM and TZN were injected into the system and chromatograms are recorded. For the UV spectrophotometric method, the specificity was obtained by the absorption spectra of the

### Table 2: Validation parameter of HPLC and UV Method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HPLC method</th>
<th>UV method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACO</td>
<td>PCM</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>1.89</td>
<td>2.87</td>
</tr>
<tr>
<td>Working λmax (nm)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Linearity (µg/mL)</td>
<td>5-30</td>
<td>10-60</td>
</tr>
<tr>
<td>LOD (µg/mL)</td>
<td>0.019</td>
<td>0.224</td>
</tr>
<tr>
<td>LOQ (µg/mL)</td>
<td>0.588</td>
<td>0.678</td>
</tr>
<tr>
<td>Precision(%RSD)</td>
<td>Intra-day precision</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Inter-day precision</td>
<td>0.35</td>
</tr>
<tr>
<td>Theoretical plate/meter</td>
<td>20186.48</td>
<td>37466.62</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>0.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Resolution</td>
<td>6.914</td>
<td>3.175</td>
</tr>
<tr>
<td>Sandell’s sensitivity</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Slope</td>
<td>18802</td>
<td>99382</td>
</tr>
<tr>
<td>Intercept</td>
<td>8890</td>
<td>48412</td>
</tr>
<tr>
<td>Regression coefficient $\left(r^2\right)$</td>
<td>0.99</td>
<td>0.99</td>
</tr>
</tbody>
</table>

*Average of three readings

### Table 3: Result of recovery study.

<table>
<thead>
<tr>
<th>Drug</th>
<th>HPLC METHOD</th>
<th>UV METHOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount of sample (µg)</td>
<td>Amount of standard (µg)</td>
</tr>
<tr>
<td>ACO</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>PCM</td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>TZN</td>
<td>0.16</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>3</td>
</tr>
</tbody>
</table>

*Average of three readings
ACO, PCM and TZN reference standard 10µg/mL and by the use of the simulated sample of excipients.

Robustness

The effect of small, deliberate variation of the analytical conditions on the peaks area and retention time of the ACO, PCM and TZN were examined, such as small changes in the percentage (±5%) in the mobile phase and flow rate (±0.1mL/min) [28]. The robustness of the UV spectrophotometric method was determined by small changes in the solvent lot.

Limit of detection (LOD) and quantification (LOQ)

The LOD and LOQ of ACO, PCM and TZN by proposed methods were determined using calibration standards. LOD and LOQ of the drugs were calculated as 3.3σ/S and 10σ/S, respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot and showed in Table 2.

Results and Discussion

HPLC method

The mobile phase selection was based on peak parameters (symmetry, tailing), run time, ease of preparation and cost. Figure 5 shows a typical chromatogram which was obtained from the analysis of a standard and sample of ACO, PCM and TZN using the proposed method (Table 1) and were eluted forming a symmetrical peak. The retention time observed (1.87, 2.85 and 3.38 min) allowed a rapid determination of the drug, which is important for routine analysis. The calibration curves for ACO, PCM and TZN were constructed by plotting peak area verses concentration and showed good linearity within 5-30, 0.35, 0.54 and 0.047% respectively. The accuracy of the method was verified with a mean recovery of 99.78, 99.84 and 99.70% (RSD of 0.023, 0.014 and 0.027%) and inter-day variability (R.S.D. of 0.047, 0.023 and 0.029%). A good accuracy of the method was verified with a mean recovery of 99.78, 99.84 and 99.70% (Table 3). Finally, the method showed to be specific for the determination of ACO, PCM and TZN in tablets.

UV Spectrophotometric method

The proposed first derivative spectrophotometric method allowed a rapid and accessible quantitation of ACO, PCM and TZN in tablets without any time consuming sample preparation. Moreover, the spectrophotometric method involved simple instrumentation compared with other instrumental techniques. The absorption spectra of ACO, PCM and TZN showed Amax = 277 nm, 248 nm and 332 nm, which was the wavelength used (Figure 3). The calibration curves were constructed in the range of expected concentrations (5-30, 2-16 and 2-30µg/mL). The representative equation analysis was y = 0.033x + 0.006, y = 0.092x - 0.005 and y = 0.0189x + 0.0296 with a correlation coefficient of 0.9999 (Table 2). LOD and LOQ were found to be 0.81, 0.21, 0.29µg/mL and 1.4, 0.71, 0.58µg/mL respectively, showing that the experimental values obtained for the determination of ACO, PCM and TZN in the samples indicated a satisfactory intra-day variability (RSD of 0.023, 0.014 and 0.027%) and inter-day variability (R.S.D. of 0.047, 0.023 and 0.029%). A good accuracy of the method was verified with a mean recovery of 99.78, 99.84 and 99.70% (Table 3). Finally, the method showed to be specific for the determination of ACO, PCM and TZN in tablets.

Comparison between HPLC, UV Spectrophotometric and the official method

The HPLC method and the UV method developed and validated for the analysis of ACO, PCM and TZN in tablets were found to be reliable, simple, fast, precise, accurate and sensitive. Results of UV spectrophotometric method showed no significant difference from those obtained with the method of HPLC and the official method (P > 0.01) but when LOD, LOQ and assay parameter of both the methods were compared and HPLC method was found to be more sensitive than the UV method. Hence these HPLC methods can be used for simultaneous estimation of ACO, PCM and TZN routinely in tablet. In summary, the proposed method can be used for the routine of quality control in pharmaceuticals.

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