Validated RP-HPLC Method for Estimation of Aceclofenac, Paracetamol and Chlorzoxazone in Dosage Form


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Abstract

A simple, reproducible and efficient reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for estimation of Aceclofenac, Paracetamol and Chlorzoxazone in its tablet dosage forms. Separation was done by using mobile phase consists of pH 2.8 buffer solution. The mobile phase was a 85:15 (v/v) mixture of methanol and phosphate buffer solution (ph 0.04±0.05, adjusted by addition of glacial acetic acid) the flow rate was 1.5ml min⁻¹ and the analyte was monitored at 232 nm. The slope, intercept and correlation coefficient were found to be 0.998 for paracetamol, 0.999 for chlorzoxazone and 0.997 for aceclofenac respectively. The percentage recovery was found to be 99.76% for paracetamol, 99.99% for chlorzoxazone and 99.56% for aceclofenac respectively. Proposed methods were found to be simple, accurate, precise, and rapid and could be used for routine analysis. This condition is applied for tablet dosage form. The statistical parameters and recovery studies were carried out and reported.

Key words: Aceclofenac, Chlorzoxazone, Paracetamol, Estimation, Tablets, RP-HPLC.
INTRODUCTION

Aceclofenac[1-4] (2-[2-(6-Dichloro phenylamino)phenyl]-acetoxy acetic acid) Fig.1, Paracetamol[5-12] (N-(4-Hydroxyphenyl)acetamide, Fig.2 and Chlorzoxazone[13-15,16] (5-Chloro Benzoxazol-2-ol Fig.3.) Aceclofenac has been shown to exert effect on many mediators of inflammation it inhibits synthesis of many inflammatory release, Paracetamol has analgesic and antipyretic action with weak anti inflammatory activity, chlorzoxazone it primarily act at the level of the spinal cord and sub cortical area of brain where it inhibits multisyanaptic area involved in producing and maintaining skeletal muscle spasm of varied etiology.

Literature survey reveals that as such no HPLC method[17-22] has yet been reported for simultaneous estimation of these three drugs. The nonavailability of Spectrophotometric and HPLC method till date for the analysis of the combination made it worthwhile to persue the present research work.

In the proposed work, a successful attempt has been made to develop simple, accurate and economic method to estimate Paracetamol[23-25], Chlorzoxazone and Aceclofenac in tablet dosage form and also to validate it.

RESULTS AND DISCUSSION

Method Development and Optimization

Column chemistry ,solvent selectivity (solvent type) ,solvent strength (volume fraction of organic solvent(s) in the mobile phase ),additive strength, detection wavelength ,and flow rate were varied to determine the chromatographic condition giving the best separation .the mobile phase condition were optimized so there was no interference with the Paracetamol, Chlorzoxazone and Aceclofenac peak from solvent or axcipint peaks .other criteria ,for example the time required for analysis ,assay sensitivity ,solvent noise ,and use of same solvent system for extraction of drug from formulation matrices during drug analysis ,were also considered .after each change of mobile phase the column was equilibrated by passage of at least twenty column volumes of the new mobile phase(10).

To investigate the appropriate wavelength for determination of paracetamol, aceclofenac and chlorzoxazone, UV-visible spectra in the range 200-400nm were acquired from a solution of the
drug in the mobile phase (Elico, India, model SL-164 spectrophotometer). From the UV spectra obtained the wavelength selected for monitoring the drug was 232nm. Solution of the drug in the mobile phase were injected directly for HPLC analysis and the response (peak area) were recorded at 232nm. It was observed there was no interference from the mobile phase or baseline distribution at 232nm. It was, therefore, concluded that 232nm was the most appropriate wavelength for analysis of the substance with suitable sensitivity.

**Chromatography**
Symmetrical peaks were obtained for paracetamol chlorzoxazone and aceclofenac. Typical chromatograms obtained a blank and from a solution of drug are illustrated in Fig 3, Fig 4, Fig 5, Fig 6 and Fig 7. The retention time for paracetamol was 1.9min, for chlorzoxazone was 2.3 min and for aceclofenac was 2.7min. and the overall chromatographic run time was 9.0 min.

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**Fig 3.** 2D Chromatogram of Paracetamol, Chlorzoxazone, Aceclofenac
Fig 4  2D Chromatogram of Paracetamol

Fig 5  2D Chromatogram of Chlorzoxazone
Fig 6 2D Chromatogram of Aceclofenac

Fig 7 3D Chromatogram of Paracetamol, Chlorzoxazone and Aceclofenac
Method validation

Linearity
The linearity of the method was tested using the calibrations describe above. Plots of concentration against response were linear in the range 32.5-162.5µg/mL (Fig 8, paracetamol), 20-100µg/mL (Fig 9, chlorzoxazone), 10-50 µg/mL (Fig 10, aceclofenac). The correlation coefficient for paracetamol was 0.998 and correlation coefficient for chlorzoxazone was 0.9990. Correlation coefficient for aceclofenac was 0.997(Table 1)

![Fig 8 Linearity graph for Paracetamol](image1)

![Fig 9 Linearity graph for Chlozoxazone](image2)
The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be readily detected but not necessarily quantified. It is usually regarded as the amount for which the signal-to-noise ratio (SNR) is 1:3. The limit of quantification (LOQ) is defined as the lowest of an analyte that can be quantified with acceptable precision and accuracy. It is usually regarded as the amount for which the SNR is 10:1. Two types of solution, solution blank and solution containing known, progressively decreasing, concentration of the analyte, were prepared and analyzed. LOD and LOQ were 514.26 and 41.21 µg/mL, respectively.

### Table 2 Statistical data for accuracy

<table>
<thead>
<tr>
<th>Statistical data</th>
<th>Paracetamol</th>
<th>Chloroxazone</th>
<th>Aceclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Mean</td>
<td>97.81</td>
<td>92.51</td>
<td>98.63</td>
</tr>
<tr>
<td>SD</td>
<td>1.941</td>
<td>1.83</td>
<td>1.78</td>
</tr>
<tr>
<td>% R.S.D.</td>
<td>1.984</td>
<td>1.98</td>
<td>1.80</td>
</tr>
</tbody>
</table>

**Accuracy**

Drug assay was performed in triplet after spiking raw material in volumetric flask with amount of paracetamol, chloroxazone and aceclofenac 32.5, 20, 10 µg/ml of the standard concentration of drugs (100 µg/ml) as in the analyte method. The result obtained (Table 2) indicate the...
recovery was were excellent, not less than 99%, and that relative standard deviation and relative percentage error was less than 2%.

**Precision**

Intra-day precision was calculated from results obtained from fivefold replicate analysis of sample at three different concentration on the same day. Inter day precision was calculated from results from the same sample analyzed on five consecutive days. The result obtained are listed in table 3.

### Table 3 Statistical data for precision

<table>
<thead>
<tr>
<th>Statistical parameter</th>
<th>Paracetamol</th>
<th>Chlorzoxazone</th>
<th>Aceclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>%RSD</td>
<td>SD</td>
</tr>
<tr>
<td>Repeatability</td>
<td>1.24</td>
<td>1.23</td>
<td>1.08</td>
</tr>
<tr>
<td>Intermediate Precision</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Day to day</td>
<td>1.18</td>
<td>1.18</td>
<td>0.09</td>
</tr>
<tr>
<td>b. Analyst to Analyst</td>
<td>0.73</td>
<td>0.73</td>
<td>0.92</td>
</tr>
</tbody>
</table>

**Robustness**

The ratio of mobile phase was methanol: phosphate buffer (0.04% ortho phosphoric acid and adjusted PH 4 with glacial acetic acid) 58:15 v/v. Change in ratio of mobile phase from 85:15 and 92:8 for robustness (Table 4).

### Table 4 Statistical data for Robustness

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Paracetamol</th>
<th>Chlorzoxazone</th>
<th>Aceclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in ratio of mobile phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.39</td>
<td>1.52</td>
<td>0.47</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.39</td>
<td>1.45</td>
<td>0.45</td>
</tr>
</tbody>
</table>

**Application of the method to tablet**

Simultaneous estimation of the marketed formulation of Paracetamol, Chlorzoxazone and Aceclofenac. The results of tablet analysis are given in the table 5.

### Table 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Paracetamol</th>
<th>Chlorzoxazone</th>
<th>Aceclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>%mean</td>
<td>99.76</td>
<td>99.99</td>
<td>99.56</td>
</tr>
<tr>
<td>SD</td>
<td>0.55</td>
<td>0.33</td>
<td>1.60</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.55</td>
<td>0.33</td>
<td>1.61</td>
</tr>
</tbody>
</table>
MATERIALS AND METHODS

**Chemical and Reagents**

A reference standard of Aceclofenac, Paracetamol and Chlorzoxazone was obtained from Cadilla pharmaceutical ltd. (Ahmedabad, India). A pharmaceutical product containing the same drug of (200 mg, 100 mg and 325 mg per tablet respectively), obtained from the mankind laboratories was used in the experiments. Disodium hydrogen phosphate, potassium dihydrogen phosphate, methanol (HPLC grade), orthophosphoric acid and triple distilled water purchased from Merck ltd. India. Mobile phase was filter through a 0.45-µ Millipore nylon 66 membrane filter. Whatman no. 41 filter papers (obtained commercially) were used for the preparation of sample solutions.

**Chromatographic system and conditions**

Analysis was performed with a Shimadzu (Japan) chromatograph comprising an LC-10 AT VP solvent delivery module, an SPD-M10 UV-visible detector and a array detector , and a rheodyne model 7125 injection valve with 20µl sample loop. Chromatographed on 250mm×4.60mmi.d, 5µ particle phenomnex OSD inertsil analytical column under reversed –phase partition conditions. The mobile phase was a 85:15 (v/v) mixture of methanol and phosphate buffer solution (ph 0.04±0.05, adjusted by addition of glacial acetic acid) the flow rate was 1.5ml min-1 and the analyte was monitored at 232 nm. The equipment was controlled by pc workstation with class LC-10/M10A chromatography software installed .The system was used in an air conditioned HPLC laboratory (20±2º).before analysis the mobile phase was degassed by use of Frontline ultra sonic cleaner Fs-10 sonicator and filter through a 0.45µm injection filter the column was equilibrated each injection

**Calibration**

Calibration plot were constructed by analysis of appropriate working solutions ranging from 32.5-162.5µg/ml for Paracetamol, 20-100 µg/ml for Chlorzoxazone and 10-50 µg/ml for Aceclofenac in the mobile phase and plotting concentration against peak –area response for each injection .unknown samples were quantified by reference to these calibration plots

**Sample preparation**

Twenty tablet of each brand were weighed and powered separately. Weigh equivalent to 325mg Paracetamol 200mg Chlorzoxazone and 100 mg Aceclofenac was dissolve in 100ml diluents and then sonicated for 15 min. and filtrated through whatman filter paper no 41 then different concentration of solution were prepared by serial dilution technique, as per standard

**Statistical Calculations**

Standard regration curve analysis was performed by use of Microsoft (USA) office excel 2003 software ,without forcing through zero .means and standard derivations were calculated by use of SPSS software version 9.5(SPPS, Cary, NC, USA) homoscedasticity for the calibration plots was tested by using Graphpad prism software, demo version.
CONCLUSION

This RP-HPLC method for estimation of paracetamol, chlorzoxazone and aceclofenac is very simple, sensitive and accurate. The run time is 9 min. only so many samples can also be processed and analyzed in a short period of time. The procedure describe is suitable for the routine estimation of paracetamol, chlorzoxazone and aceclofenac in pharmaceutical formulation.

Acknowledgement
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REFERENCES

