ABSTRACT: A rapid and sensitive high performance liquid chromatography with UV detection method was developed and validated for the quantification of paracetamol, chlorzoxazone and aceclofenac in dosage forms. The chromatographic separation was carried out on a Phenomenex Luna C18 column using a mixture of acetonitrile-0.05M disodium hydrogen orthophosphate (65:35) (pH adjusted to 3.0 using 10% orthophosphoric acid) as mobile phase with UV detection at 271 nm. The calibration curve was linear in the concentration range of 20-100 µg/ml for paracetamol, 20-100 µg/ml for chlorzoxazone and 4-20 µg/ml for aceclofenac. The lower limit of detection was found to be 0.9 µg, 1.81 µg, 0.9 µg for paracetamol, chlorzoxazone, aceclofenac respectively. The method was successfully used for quantitative determination of Hifenac-MR tablets.

KEYWORDS: Paracetamol; chlorzoxazone; aceclofenac; HPLC; analysis.

INTRODUCTION

Aceclofenac {2[(2,6-dichlorophenyl)amino]benzoic acid carboxymethyl ester} is an analgesic and non-steroidal anti-inflammatory drug. Paracetamol (p-hydroxy acetanilide) is a compound with analgesic and antipyretic properties. It is much safer than aspirin in terms of gastric irritation, ulceration and bleeding. Chlorzoxazone (5-chloro-2(3H)-benzoxazolone) is a compound with skeletal muscle relaxant property. It is used to decrease muscle tone and tension and used to relieve spasm and pain associated with musculoskeletal disorders. Aceclofenac is official in B.P\textsuperscript{1}, paracetamol in B.P & I.P\textsuperscript{2,3} and chlorzoxazone in U.S.P\textsuperscript{4}. B.P. suggests a potentiometric assay method for aceclofenac in bulk drugs. The I.P. & B.P. both suggest titrimetric and UV spectrophotometric assay method for paracetamol in bulk and tablet formulations. Literature survey revealed that high performance liquid chromatography spectrofluorimetric\textsuperscript{5}, calorimetric\textsuperscript{6}, densitometric\textsuperscript{7} and (HPLC)\textsuperscript{8,9} methods have been reported for the estimation of aceclofenac in pharmaceutical dosage forms. A spectrophotometric method\textsuperscript{10} have been reported for the simultaneous estimation of the three drugs in formulation. This prompted us to develop and validate HPLC method for the simultaneous estimation of paracetamol, chlorzoxazone and aceclofenac.

EXPERIMENTAL

MATERIALS AND REAGENTS:

All materials and reagents were analytical grade.

1. Pure drugs of paracetamol, chlorzoxazone and aceclofenac were obtained from Paris- Dakner Pharmaceuticals, Chennai, India.
2. Hifenac-MR (labeled to contain 500 mg of paracetamol, 500 mg of chlorzoxazone, and 100 mg of aceclofenac,) is obtained from Intas, India.
3. Acetonitrile, methanol and water of HPLC grade were
obtained from Qualigens Fine Chemicals, India.
4. Disodium hydrogen orthophosphate was obtained from S.D. Fine Chemicals, India.
5. Nylon syringe membrane filters (0.2µm) were purchased from Satoris, Germany.
6. Phosphate buffer pH 3 (dissolved 7.1 g of disodium hydrogen orthophosphate in water and made upto 1000 ml with water. The pH is adjusted with 10% phosphoric acid)
7. The mobile phase consisted of acetonitrile-0.05 M disodium hydrogen ortho phosphate (65:35) (pH adjusted to 3.0 using 10% ortho phosphoric acid). The solution was filtered through a 0.2 µm membrane filter.

INSTRUMENTATION
The HPLC system consisted of a Shimadzu LC-10AT-VP instrument with a reversed phase Phenomenex Luna C18 column, a Rheodyne sample injector with a 20 µl loop volume and a variable wavelength UV-Vis detector (Shimadzu SPD-10AVP).

CHROMATOGRAPHIC CONDITIONS
Separation was achieved using a Phenomenex Luna C18 5 µm 250 x 4.6 mm). The isocratic mobile phase pumped at a flow-rate of 1.5 ml/min consisted of acetonitrile-disodium hydrogen ortho phosphate (pH 3; 0.05 M) (65:35) prepared daily and degassed by passing through a 0.2 µm membrane filter and ultrasonication for 10 min. All separations were performed at room temperature with detection at 271 nm.

STANDARD SOLUTION
A standard solution of paracetamol (5 mg/ml), chlorzoxazone (5 mg/ml) and aceclofenac (1 mg/ml) were prepared in methanol. Subsequent dilutions were made in mobile phase to give the concentrations 20, 40, 60, 80 and 100 µg/ml for paracetamol; 20, 40, 60, 80, 100 µg/ml for chlorzoxazone and 4, 8, 10,16, 20 µg/ml for aceclofenac.

APPLICATION OF THE METHOD
Twenty tablets of Hifenac-MR were weighed accurately and finely powdered. The powder equivalent to 250 mg of paracetamol, 250 mg of chlorzoxazone and 50 mg of aceclofenac was weighed accurately and dissolved in 250 ml methanol (HPLC). The solution was ultrasonicated for 15 minutes and filtered through a 0.2 µm membrane filter. Four ml of the resulting solution was further diluted to 50 ml to get a solution having a concentration of 80 µg/ml of paracetamol, 80 µg/ml of chlorzoxazone and 16 µg/ml of aceclofenac. Twenty µl of this solution was injected in triplicate under the specified conditions. The concentrations of the drugs were determined by HPLC using the calibration curve.

RESULTS AND DISCUSSION

CHROMATOGRAPHIC CONDITIONS
Optimization of chromatographic conditions was achieved by monitoring varying mobile systems. After trying different ratios of mixtures acetonitrile-water buffer and methanol-water buffer, the best results were achieved by using a mixture of acetonitrile-disodium hydrogen phosphate (pH 3; 0.05 M) (65:35) as mobile phase. Excellent chromatographic specificity with no interference from the dosage form excipients was observed. Moreover, suitable retention times for paracetamol, chlorzoxazone and aceclofenac were achieved. Typical chromatograms obtained from the standard solution of paracetamol, chlorzoxazone and aceclofenac is presented in Fig.1. Under the chromatographic conditions described, paracetamol, chlorzoxazone and aceclofenac were well resolved and eluted at about 1.8, 2.6 and 4.2 min, respectively and the total run time was within 5 min. Good baseline resolution and peak shape can be observed. Also, the influences of small changes in the mobile phase composition (±10%) and buffer pH (±0.3) were studied to determine the robustness of the method, such as the changes in peak area and retention time. Peak area values were not influenced by changing the composition of mobile phase or pH of the phosphate buffer, whereas it was slightly influenced by changing the mobile phase composition.

LINEARITY
Calibration curves were constructed using five series of standard solutions of paracetamol, chlorzoxazone and aceclofenac in the range of 20-100 µg/ml, 20-100 µg/ml and 4-20 µg/ml respectively. A linear relationship was obtained between the peak area of the drug and the corresponding concentration, as shown by the equation presented in Table 1. Statistical data are presented in Table 1.

ACCURACY AND PRECISION
The accuracy and precision were determined by analyzing three samples of paracetamol, chlorzoxazone and aceclofenac on three separate days. Within-day and between-day data for the corresponding concentrations are given in Table 2. Good accuracy and repeatability were observed over the entire concentration range. The within-day and between-day variability showed CV values less than 1.2% in all the concentrations.

SPECIFICITY
The specificity test of the proposed method demonstrated
that the excipients from the tablet did not interfere in the drugs peak. Furthermore, well-resolved peaks indicated the specificity of the method. Thus the proposed HPLC method was useful to quantify paracetamol, chlorzoxazone and aceclofenac in dosage forms.

SENSITIVITY
The limit of quantification was found to be 3.0 µg/ml for aceclofenac, 3.06 µg/ml for paracetamol and 6 µg/ml for chlorzoxazone. The limit of detection with S/N ratio of 3 was found to be 0.9 µg/ml for aceclofenac, 0.9 µg/ml for paracetamol and 1.81 µg/ml for chlorzoxazone.

SOLUTION STABILITY:
The stability of the stock solution was determined by analyzing the standard solution solution in comparison to freshly prepared standard solution. No significant changes (<1%) were observed in stock solution after 7 day in comparison to freshly prepared standard.

ASSAY OF THE HIFENAC-MR TABLETS
The developed method was applied to quantitative determinatin of paracetamol, chlorzoxazone and aceclofenac in Hifenac-MR tablets. The results were shown to be in good agreement with the labelled amount (509.49mg ± 0.64 mg for paracetamol; 496.75 ± 0.65 mg for chlorzoxazone and 100.9182 ± 0.52 mg for aceclofenac) and the relative standard deviation did not exceed 0.6. The results are given in Table 3.

CONCLUSION
In conclusion, the proposed HPLC method provided a simple, accurate and reproducible method for routine in vitro tests of paracetamol, chlorzoxazone and aceclofenac in dosage forms. Only one method is now available for the simultaneous estimation of the drugs using spectrophotometry. This is the first report for the simultaneous estimation of paracetamol, chlorzoxazone and aceclofenac using HPLC. The short chromatographic time makes this method suitable for the processing of multiple samples in a limited amount of time.

ACKNOWLEDGMENTS
This work was supported by the grant from the Tamilnadu Government, India.

Figure 1. Standard Chromatogram of Paracetamol, Chlorzoxazone and Aceclofenac

Table 1. Statistical Data of Calibration Curves of Paracetamol, Chlorzoxazone and Aceclofenacin Standard Solutions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Aceclofenac</th>
<th>Paracetamol</th>
<th>Chlorzoxazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation</td>
<td>8.651 x + 0.0232</td>
<td>10.569 x – 0.7147</td>
<td>9.446 x – 0.1236</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9986</td>
<td>0.9999</td>
<td>0.9995</td>
</tr>
<tr>
<td>Standard deviation of residuals</td>
<td>2.303</td>
<td>8.221</td>
<td>3.723</td>
</tr>
</tbody>
</table>
Table 2. Precision and Accuracy of Method for Determination of Paracetamol, Chlorzoxazone and Aceclofenac in Standard Solutions (n=8)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration added (µg/ml)</th>
<th>Concentration calculated (mean ± S.D.) (µg/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within-day (n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>500</td>
<td>499.9751 ± 0.8084</td>
<td>0.1619</td>
</tr>
<tr>
<td>Chlorzoxazone</td>
<td>500</td>
<td>499.9849 ± 0.7813</td>
<td>0.1362</td>
</tr>
<tr>
<td>Aceclofenac</td>
<td>100</td>
<td>99.9969 ± 0.6747</td>
<td>0.6406</td>
</tr>
<tr>
<td></td>
<td>Between-day (n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>500</td>
<td>499.9751 ± 0.8056</td>
<td>0.1615</td>
</tr>
<tr>
<td>Chlorzoxazone</td>
<td>500</td>
<td>499.9849 ± 0.7235</td>
<td>0.1362</td>
</tr>
<tr>
<td>Aceclofenac</td>
<td>100</td>
<td>99.9857 ± 0.6726</td>
<td>0.6432</td>
</tr>
</tbody>
</table>

Table 3. Estimation of Paracetamol, Chlorzoxazone and Aceclofenac in Tablets

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim (mg/tablet)</th>
<th>Estimated amount (mg/tablet)</th>
<th>Standard deviation</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>500</td>
<td>501.4907</td>
<td>0.6489</td>
<td>0.1294</td>
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<tr>
<td>Chlorzoxazone</td>
<td>500</td>
<td>496.7597</td>
<td>0.6564</td>
<td>0.1321</td>
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<tr>
<td>Aceclofenac</td>
<td>100</td>
<td>100.9182</td>
<td>0.5287</td>
<td>0.5239</td>
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</table>

REFERENCES


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