STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
The Aim of present work is to develop a simple, selective and precise, stability indicating RP-HPLC method for the simultaneous estimation of Amlodipine and Hydrochlorothiazide. The chromatographic separation of the two drugs was achieved on a reverse phase Hypersil Gold, C18, 250×4.6 mm, 5µm column using mobile as Potassium dihydrogen buffer – Acetonitrile in ratio of 600:400 v/v (pH adjusted to 3.2±0.05 using orthophosphoric acid) with flow rate of 1.0 ml/min with injection volume 20 µl and the detection was carried out at 237 nm using UV detector. The retention time of amlodipine (Amlo) and hydrochlorothiazide (HCT) were found to be 3.80 and 6.48 min respectively. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range of 0.84-1.98 μg/ml for hydrochlorothiazide and 4.2-9.8 μg/ml for amlodipine. The method was validated for precision, linearity, LOD and LOQ, specificity, accuracy, system suitability and ruggedness as per ICH guidelines and the results were found to be within the limits. The developed method was used for the stability studies. The validated method can be used for routine quality control testing for HCT and Amlo combine dosage form.

Keywords: Amlodipine, Hydrochlorothiazide, RP-HPLC, Validation, Stability.

INTRODUCTION
Hydrochlorothiazide (HCT), 6 - chloro - 3, 4 dihydro - 7 - sulfamoyl - 2H - 1, 2, 4 - benzothiadiazine - 1, 1 – dioxide, is a thiazide diuretic. Inhibition of the initial influx of calcium decreases the contractile activity of arterial smooth muscle cells and results in vasodilation. The vasodilatory effects of amlodipine result in an overall decrease in blood pressure. All the two drugs are official in IP and BP. Literature survey revealed that there are several methods reported for the estimation of HCT and Amlo individually as well as in combination with some other drugs. As no method is available for their simultaneous determination indicating stability over long period, however, because HPLC methods have been widely used for routine quality control assessment of drugs, because of their accuracy, repeatability, selectivity, sensitivity and specificity. We have developed a simple, precise and specific stability indicating RP-HPLC method for the simultaneous determination of HCT and Amlo in pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals and Reagents
The standard amlodipine and hydrochlorothiazide, marketed preparation and other required chemicals used for the present investigation are procured from Shreya Life science Pvt. Ltd. Aurangabad (India). The entire chemical were methanol, Acetonitrile form (Rankem grade), buffer and HPLC water (HPLC grade) were used for study.

Instruments
Stability Indicating RP-HPLC method development and validation was done on [Shimadzu ALD-02-013] HPLC instruments UV-detector and column Hypersil gold C18, 250x4.6 mm, 5µm particle size. HPLC system was equipped with LC solution software. Also the following instruments were used UV- spectrophotometer (waters), ultrasonic cleaning bath (spectralab model USB), pH analyser (Labinda), weighing balance (shimadzu), Fuming chamber (Labexel), hot air oven (Thermo lab to905), magnetic stirrer (Whilmatic) used in study.
day and inter-day variation studies, in the intra and inter-day studies, six repeated injections of standard solutions were made and the response factor of drug peak and % RSD were calculated. Repeatability was determined by carrying out six times analysis for same sample and at same condition. Intra and inter-day precision was determined by analysing both the drugs at three different concentrations of 1.12 μg/ml, 1.4 μg/ml, 1.68 μg/ml of HCT and 5.6 μg/ml, 7 μg/ml, 8.4 μg/ml for Amlo respectively twice, on same day. Linearity was determined at 5 levels over the range of 60% to 140% with respect to the test concentration. A standard stock solution was prepared and further diluted to attain concentration of about 60%, 80%, 100%, 120% and 140% of sample concentration. Accurate precision and accuracy with a signal to noise ratio, was determined by calculating LOD and LOQ using equation 1 and 2. The specificity of the method was performed by injecting blank solution and then a drug solution of 10μl injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both HCT and Amlo from any of the impurities, if present. The accuracy of the method was carried out at three levels 80%, 100% and 120% of the working concentration of sample. Each level was prepared in triplicate manner and each preparation was injected in duplicate. From the final stock solution, sample solution of HCT and Amlo was prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured by same concentrations solutions, six times. The ruggedness was found to be well within specific limit % RSD NMT 2.0 %. Suitability values were calculated from the first injection of six replicates of standard and % RSD is calculated from six replicate injections of standard.

\[
\text{LOD} = \frac{3.3 \times SD}{S} \quad \text{--- (1)}
\]

\[
\text{LOQ} = \frac{10 \times SD}{S} \quad \text{--- (2)}
\]

Where, SD is standard deviation of response and S is slop of calibration curve.

**Stability study**[8-10]

The stability study of tablet preparation containing HCT and Amlo was carried out as per the ICH and WHO guidelines. The marketed preparations were subjected to different stability condition for one month and three month periods.

**RESULT AND DISCUSSION**

Optimum solubility of both the drugs was obtained in the methanol. Spectral study showed that the \( \lambda_{\text{max}} \) for the HCT at 238.5 nm and Amlo at 271 nm. The solution of mixture exhibited maxima at about 237 nm. To achieve resolution between all two drugs and its degradation product by RP-HPLC, stationary phase C-18 was used. A mobile phase consisting of Buffer: Acetonitrile ratio was selected at proportion of 600: 400. This shows good resolution chromatogram with symmetrical peaks. The final RP-HPLC method was successfully developed for estimation of HCT and Amlo. The method was developed in consideration of optimized chromatographic parameters. The chromatograms of developed method for standard and sample are mentioned in figure 1. The percent assay by developed method also mentioned in table no.1. The chromatograms of standard and sample of HCT and Amlo indicating the retention time for HCT 3.80 and 3.80 for standard and sample respectively. Similarly the retention time for Amlo 6.48 and 6.49 for standard and sample respectively. The figure 2 shows system precision and method precision in which the % RSD was found to be for HCT 1.8 & 1.6 and for Amlo 2.0 & 1.9. In intra-day and inter-day precision the % RSD for HCT and Amlo found to within acceptable limit of ≤2. Hence method is reproducible (Table 2 and figure 3). The linearity curve of HCT was found to be linear over the range of 0.84-1.98 μg/ml and Amlo over the range of 4.2-9.8 μg/ml (figure 4). These were represented by a linear regression equation as follows, HCT \((r^2=0.998), \text{Amlo} = (r^2=0.999)\), which indicates that has good linearity. LOD and LOQ for HCT and Amlo were 1.4376 and 1.9410 μg/ml respectively and for HCT And Amlo were 1.84962 μg/ml and 1.65190 μg/ml and, respectively. The accuracy of developed method was determined and the data is shown in table no.3. The specificity of the method was determined by checking the interference of the components against placebo. No interference was observed for any of the excipients of both drugs (Figure 5). The % Mean recoveries for HCT are 98.8-100.1% and for Amlo are 98-100% respectively and % RSD for HCT and Amlo is within limit of s2. Hence the proposed method is accurate. The method is rugged by different analyst, different time intervals and the method did not significantly affect the recoveries, peak area and retention time of all the above drugs indicating that the proposed method is rugged. The % RSD was found to be for HCT analyst-1 1.9 and Amlo-1.4 and for analyst-2 2.0 and 1.4. System suitability parameters such as number of peak tailing (1.3, 1.2), retention time (3.80, 6.49) and resolution factor (7.678) were found. The total run time required for the method is only 15 minutes for eluting both HCT and Amlo. In stability study the % RSD limit are ≤2. The solution stability in terms of percent assay at 25°C/60%RH and 40°C/75%RH was found in the range of 99.26 % to 100.94 % for the both drugs, indicating good solution stability[7-9] (figure 6).

![Standard Chromatogram](image1.png)

![Sample Chromatogram](image2.png)

**Fig. 1: Standard and sample chromatogram**

<table>
<thead>
<tr>
<th>Component</th>
<th>Label Claim [μg]</th>
<th>% Amount found</th>
<th>Mean</th>
<th>SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT</td>
<td>12.5</td>
<td>100.1%</td>
<td>100.6</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Amlo</td>
<td>5</td>
<td>101.1%</td>
<td>101.2</td>
<td>0.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 1: % Assay of tablet formulation.
Table 2: Data showing Intra-day and inter-day precision

<table>
<thead>
<tr>
<th>Concentration level</th>
<th>Area</th>
<th>Amount added</th>
<th>Amount Found</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>26.4290</td>
<td>1.12</td>
<td>1.10</td>
<td>99.8%</td>
</tr>
<tr>
<td>100%</td>
<td>27.8298</td>
<td>1.4</td>
<td>1.42</td>
<td>100.2%</td>
</tr>
<tr>
<td>120%</td>
<td>33.2563</td>
<td>1.68</td>
<td>1.68</td>
<td>100.1%</td>
</tr>
<tr>
<td>Amlo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80%</td>
<td>23.7512</td>
<td>5.6</td>
<td>5.1</td>
<td>98%</td>
</tr>
<tr>
<td>100%</td>
<td>24.7855</td>
<td>7</td>
<td>7.1</td>
<td>97%</td>
</tr>
<tr>
<td>120%</td>
<td>25.6265</td>
<td>8.4</td>
<td>8.9</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 3: Data showing accuracy of HCT and Amlo

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Validation parameter</th>
<th>% RSD (Acceptance criteria &lt;2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HCT</td>
</tr>
<tr>
<td>1</td>
<td>Intra-day precision</td>
<td>1.7</td>
</tr>
<tr>
<td>2</td>
<td>Inter-day precision</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Fig. 2: Chromatogram of system precision and method precision

Fig. 4: Chromatogram of linearity

Fig. 5: Chromatogram of specificity

Fig. 6: Chromatogram of solution stability of HCT and Amlo
CONCLUSION
Statistical analysis result showed that the proposed procedure has showed good precision and accuracy. The method completely validated and shows satisfactory result for all method parameter. The developed method was found with good stability over long period. Result of the study indicate that the develop method was found to be simple, reliable, accurate, linear, sensitive, economical and reproducible and have a short run time which makes the method rapid. Hence it is concluded that the proposed method is precise, simple, sensitive, accurate, rugged and rapid and can be applied successfully for the estimation of hydrochlorothiazide and amlodipine in pharmaceutical dosage form.

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