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Original Article

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

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ABSTRACT

Objective: 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.

Methods: 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.

Results: 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.

Conclusion: 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 - benzothia diazine - 1, 1–dioxide, is a thiazide diuretic. Inhibits water re absorption in nephorn by inhibiting the sodium chloride symporter in distal convoluted tubules, which responsible for 5 % of total sodium reabsorption [1]. Amlodipine is: 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5dicarboxylate benzene sulphate [2]. 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 USP. Amlo and HCT are official in IP and BP. Literature survey revealed that there are several methods were reported for the estimation of Amlo and HCT individually as well as in combination with some other drugs. As no method is available for their simultaneous determination indicating stability over the 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, accurate and specific stability indicating RP-HPLC method for the simultaneous determination of HCT and Amlo in pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals and reagent

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 was 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, 250×4.6 mm, 5 µm particle size. HPLC system was equipped with LC solution software. Also the following instruments were used UV-spectrophtometer (waters), ultra sonic cleaning bath (spectralabe model USB), pH analyser (Labindia), weighing balance (shimadzu), Fuming chamber (Labexel), hot air oven (Thrmo lab to905), magnetic stirrer (Whilmatic) used in study.

Chromatographic conditions

Chromatographic analysis was carried out at C18 (250×4.6 mm,5 µm) and mobile phase consisting of Buffer: Acetonitrile (600:400 v/v, pH adjusted to 3.2±0.05 with ortho phosphoric acid) and was filtered through Millipore HPLV 0.45 µm, and flow rate was adjust at 1.0 ml/min, and injection volume was 20 µm. all the drug shows good absorbance at 237 nm. The retention time of HCT and Amlo was observed to be at 3.89 and 6.48 min respectively.

Preparation of mobile phase

A mixture in a ratio of 600:400 ratio of buffer solution (600 ml)**:** Acetonitrile (400 ml) was prepared. This mixture was degassed in an ultrasonic water bath for 10 min and was filtered through 0.45µ filter under vacuum. This mobile phase was also used as diluents.

RP-HPLC method development [3, 4]

Preparation of standard solution of Hydrochlorothiazide and Amodipine

The standard solution of hydrochlorothiazide (HCT) and amlodipine (Amlo) was prepared in concentration of 14 ppm and 7 ppm respectively using methanol solvent.

Final standard solution

The standard solution was prepared by transferring 2 ml standard solutions of HCT and 1 ml standard solution Amlo stock solution in a 50 ml volumetric flasks and volume was made up with the mobile phase.

Preparation of sample solution of hydrochlorothiazide and amlodipine

Accurately 20 tablets were weighed to determine an average weight of each tablet. Tablets were finely crushed and tablet powder equivalent to 119.91 mg was transferred into100 ml volumetric flask. The final concentration 14 ppm and 7 ppm were made and filtered through no.42 filter paper.

Method validation [5-7]

The following parameters were validated according to International Conference on Harmonization guidelines for validation of analytical procedures. The precision of the method was demonstrated by intra-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 was calculated. Repeatability was determined by carring out six times analysis for the same sample and at same condition. Intra and inert-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 the 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 the 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.

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

$$LOD = \frac{10 \times SD}{S} \quad \text{---------- (2)}$$

Where, SD is standard deviation of response and S is the slope of the 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.

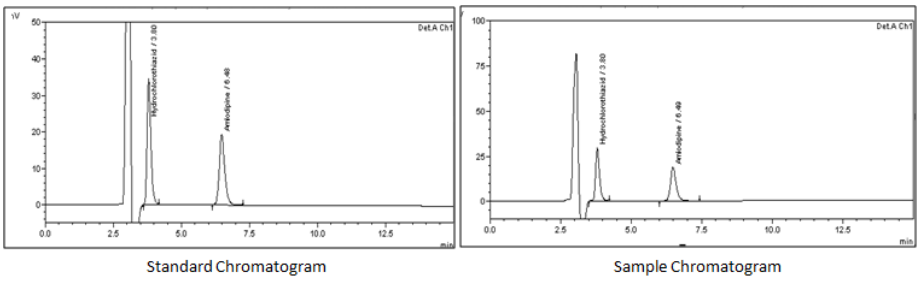

Fig. 1: Standard and sample chromatogram

Table 1: % Assay of tablet formulation

Component	Label claim [mg]	% Amount found	Mean	SD	% RSD
HCT	12.5	100.1 % 101.1 %	100.6	0.7	0.6
Amlo	5	101.1 % 101.2 %	101.2	0.1	0.01

Table 2: Data showing Intra-day and inter-day precision

HCT				
Concentration level	**Area**	**Amount added**	**Amount found**	**% Recovery**
80 %	26.4290	1.12	1.10	99.8 %
100 %	27.8298	1.4	1.42	100.2 %
120 %	33.2563	1.68	1.68	100.1 %
Amlo				
80 %	23.7512	5.6	5.1	98 %
100 %	24.7855	7	7.1	97 %
120 %	25.6265	8.4	8.9	100 %

Table 3: Data showing accuracy of HCT and Amlo

S. No.	Validation parameter	% RSD (Acceptance criteria <2)	
		HCT	Amlo
1	Intra-day precision	1.7	0.5
2	Inter-day precision	0.9	1.4

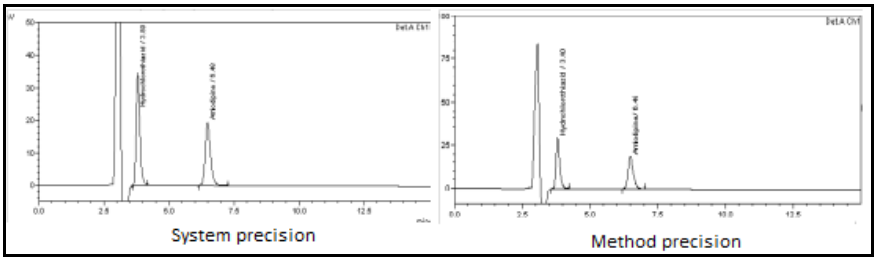

Fig. 2: Chromatogram of system precision and method precision

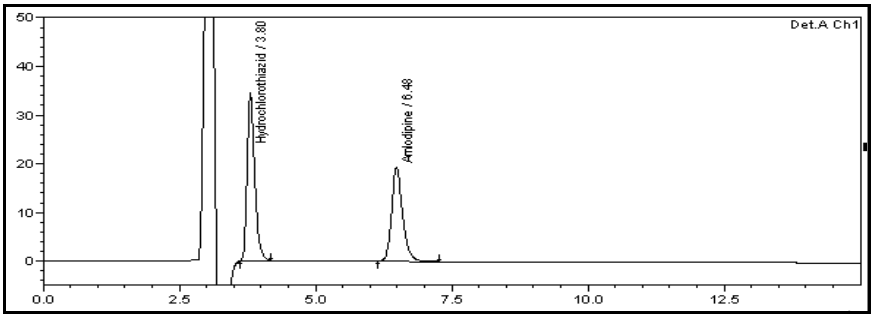

Fig. 4: chromatogram of linearity

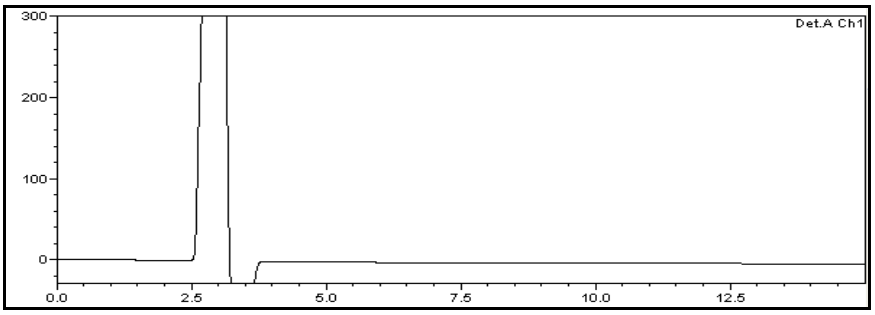

Fig. 5: Chromatogram of specificity

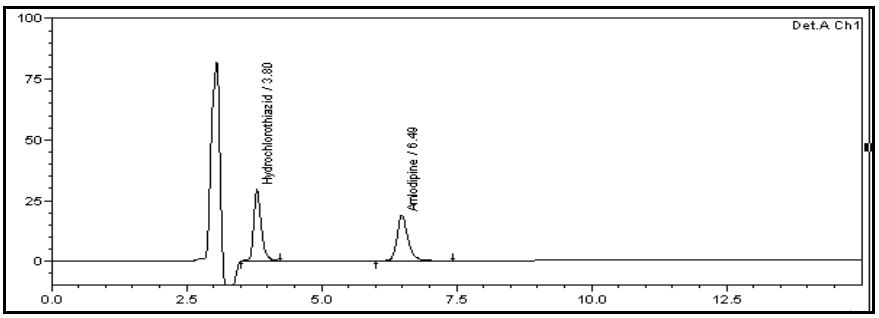

Fig. 6: Chromatogram of solution stability of HCT and Amlo

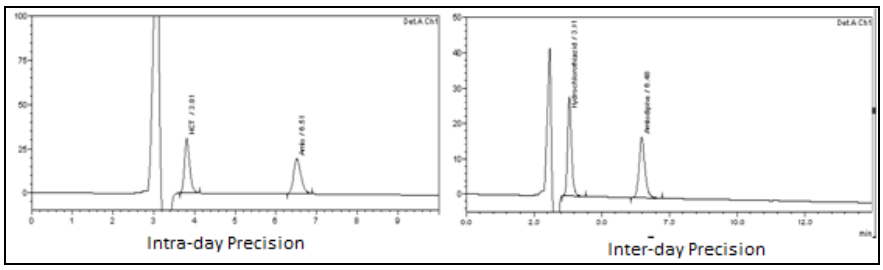

Fig. 3: Intra and inter day precision

RESULTS AND DISCUSSION

Optimum solubility of both the drugs was obtained in the methanol. Spectral study showed that the λmax 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 the 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 the developed method for standard and sample are mentioned in fig. 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 fig. 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 the method is reproducible (table 2 and fig. 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 (fig. 4). These were represented by a linear regression equation as follows, HCT (r2=0.998), Amlo = (r2=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 (fig. 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 ≤2. Hence the proposed method is accurate. The method is rugged by the 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 min for eluting both HCT and Amlo. In stability studies, 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 (fig. 6) [7-9].

CONCLUSION

Statistical analysis result showed that the proposed procedure has showed good precision and accuracy. The method completely validated and shows the satisfactory result for the all method parameter. The developed method was found with good stability over the 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.

CONFLICT OF INTERESTS

Declared None

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