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## DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR THE ESTIMATION OF HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORMS

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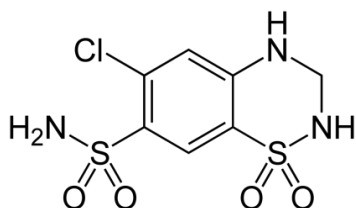
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**ABSTRACT:** An accurate, precise and reproducible high performance liquid chromatographic method was developed for quantitative estimation of hydrochlorothiazide in tablet dosage forms. Chromatographic separation of the drug was achieved on a Kromasil C<sub>18</sub> column (150 x 4.6 mm; 5 $\mu$ ) using a mobile phase consisting of phosphate buffer (pH 2.5) and acetonitrile (50:50 v/v) at a flow rate of 0.6 mL/min. The drug in the eluate was monitored at 254 nm. The retention time obtained for the drug was 3.47 min. The calibration curve was linear over the range of 20-60  $\mu$ g/mL of the drug. The performance of the method was validated according to ICH guidelines. The method could be applied for determination of hydrochlorothiazide in its tablet dosage forms without any interference from excipients or endogenous substances. The proposed method is suitable for routine quality control analysis of hydrochlorothiazide.

**INTRODUCTION:** Hydrochlorothiazide<sup>1, 2</sup> (6-chloro- 1,1-dioxo- 3,4-dihydro-2H- 1,2,4-benzothiadiazine -7-sulfonamide) (**Figure 1**) is a first-line diuretic drug, which acts by inhibiting the kidneys' ability to retain water. It is frequently used in the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and for the prevention of kidney stones.



**FIGURE 1: CHEMICAL STRUCTURE OF HYDROCHLOROTHIAZIDE**

A literature survey revealed that many analytical methods have been reported for the determination of hydrochlorothiazide in pharmaceutical dosage forms and in biological samples using HPLC<sup>3-9</sup>, HPTLC<sup>10</sup> and spectrophotometry<sup>11-14</sup> techniques.

We have developed a new accurate and precise RP-HPLC method with short retention and run times for the determination of hydrochlorothiazide in bulk samples and in tablet dosage forms. The developed method has been validated as per ICH<sup>15</sup> guideline.

### MATERIALS AND METHODS:

**Drugs, chemicals and solvents:** Potassium dihydrogen phosphate and *ortho*- phosphoric acid were purchased from Qualigens Chemicals Limited. HPLC grade acetonitrile was purchased from JT Baker Limited (Germany). HPLC grade water was prepared by using Millipore Milli-Q system. The reference sample of Hydrochlorothiazide was obtained from Hetero Drugs Ltd. (Hyderabad, India).

**Equipment and Chromatographic conditions:** A Waters Alliance liquid chromatograph (model 2695) monitored with Empower2 data handling system and fitted with a Kromasil C<sub>18</sub> column (150 x 4.6 mm; 5 $\mu$ m) and a Diode array detector (model 2996) was

employed for this study. The mobile phase used consisted of a mixture of Phosphate buffer (pH 2.5) and acetonitrile in the ratio of 50:50 v/v. All chromatographic runs were carried out in isocratic elution mode with a flow rate of 0.6 mL/min. The detector wavelength was set at 254 nm. The sample injection volume was 20  $\mu$ L.

**Preparation of the Buffer:** Phosphate buffer was prepared by dissolving 2.85 g of potassium dihydrogen phosphate in 1000 mL of water and adjusting the pH to 2.5 using *ortho*-phosphoric acid. The solution was filtered through a 0.45 $\mu$  filter and sonicated.

**Preparation of the Mobile phase and the Diluent:** The above phosphate buffer and acetonitrile were mixed together in the ratio of 50:50 v/v and employed as the mobile phase. The same solution was also used as the diluent for preparing drug dilutions.

**Preparation of the Working Standard Solution of Hydrochlorothiazide:** About 100 mg of hydrochlorothiazide was accurately weighed and transferred into a 100 mL volumetric flask. 70 mL of the diluent was added to it and sonicated to dissolve the drug. The volume was made up with a further quantity of the diluent and the solution mixed well. This was used as the standard stock solution. 2.0 mL of the stock solution was transferred into a 50 mL volumetric flask, made up to volume with the diluent to get a concentration of 40  $\mu$ g/mL of hydrochlorothiazide. This was used as the working standard solution.

**Calibration Curve:** Solutions containing different concentrations of hydrochlorothiazide were prepared from the working standard solution covering different concentration levels including the working concentration. A volume of twenty microlitres of each concentration was injected into the HPLC system. Five replicate runs were performed at each concentration level. The detector response was read at 254 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak area at each concentration level was calculated. The relevant linearity graph of the mean peak area over concentration was plotted.

**Estimation of the Drug from its Tablet Dosage Form:** Twenty tablets of Aquazide-12.5 (Sun Pharma) were weighed and ground to a fine powder. An amount

equivalent to about 100 mg of hydrochlorothiazide was transferred into a 100 mL volumetric flask and to it 70 mL of the diluent was added and sonicated for 20 min. Then further diluent was added to make up the volume and mixed. A portion of the above solution was filtered through a 0.22  $\mu$  membrane filter (discarding the first few mL of the filtrate).

2.0 mL of this filtrate was transferred into a 50 mL volumetric flask containing 30 mL of the diluent. The volume was made up to 50 mL with the diluent and mixed well. Six replicate injections of were analyzed on the column. The mean of the peak areas of the drug was found out and the drug content in the formulation was calculated by using the regression equation obtained for the pure drug.

**RESULTS AND DISCUSSION:** During the method optimization studies various columns with C<sub>18</sub> and C<sub>8</sub> packing were tested for an ideal separation of the drug using different mobile phases and different chromatographic conditions. Finally, the following conditions were found to be optimum after evaluating the column efficiency by parameters like theoretical plates and tailing factor.

**TABLE 1: OPTIMIZED CONDITIONS FOR THE PROPOSED HPLC METHOD**

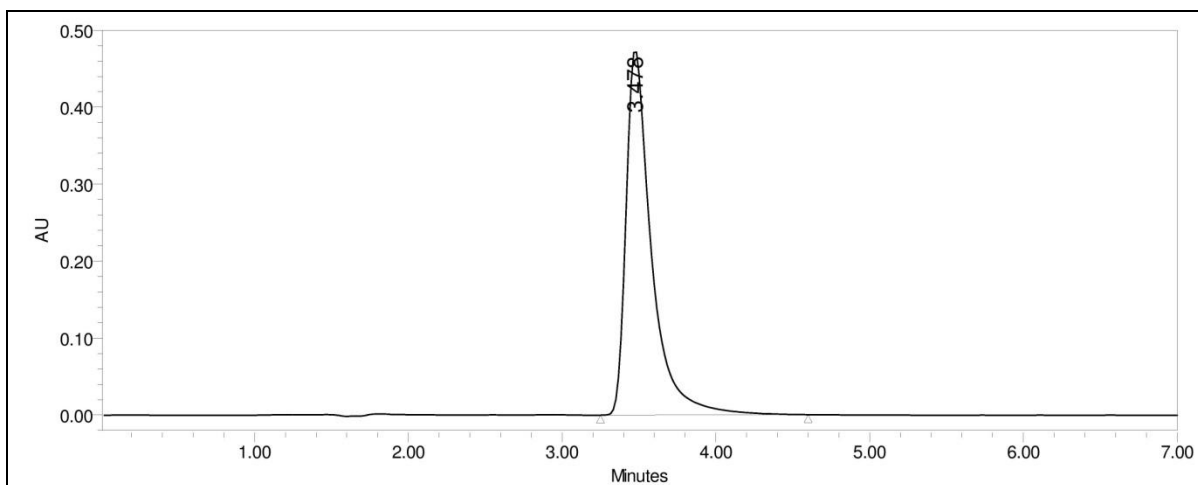
<b>Stationary Phase:</b>	Kromasil C <sub>18</sub> (150 x 4.6 mm, 5 $\mu$ m)
<b>Mobile Phase:</b>	Phosphate buffer : Acetonitrile =50:50 v/v
<b>Flow Rate:</b>	0.6 mL/min
<b>Column Temperature:</b>	26°C
<b>Injection Volume:</b>	20 $\mu$ L
<b>Detection Wavelength:</b>	254 nm
<b>Run Time:</b>	7.0 min
<b>Retention time of the drug:</b>	3.475 min

Optimum wavelength was selected by scanning the standard solution of the drug in 200 - 400 nm region in a UV-Vis spectrophotometer. Using the above mobile phase, good base line separation for the hydrochlorothiazide peak was achieved. Under the above optimized conditions, the retention time for hydrochlorothiazide was found to be 3.475 min (**Figure 2**).

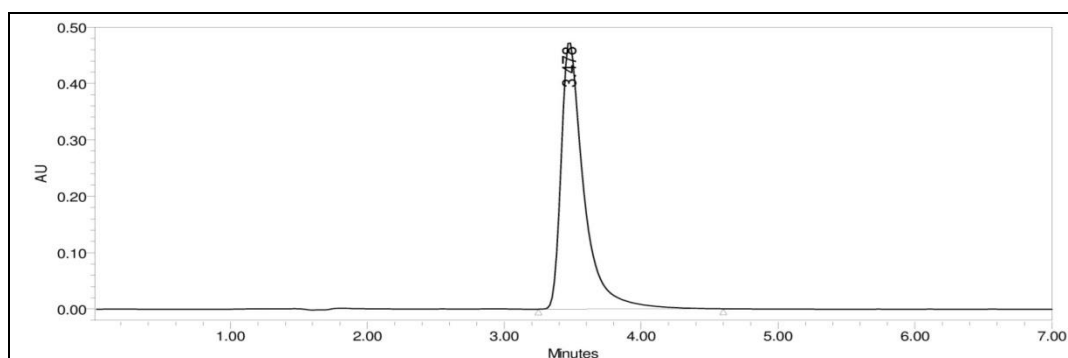
**Specificity:** The chromatograms obtained for the samples of tablet dosage forms demonstrate that no interfering peaks were found at the retention time of hydrochlorothiazide due to the excipients (**Figure 3**).

**Linearity:** The regression of the plot was computed by least squares method and is shown in **Figure 4**. The calibration curve (n=5) constructed for the drug was

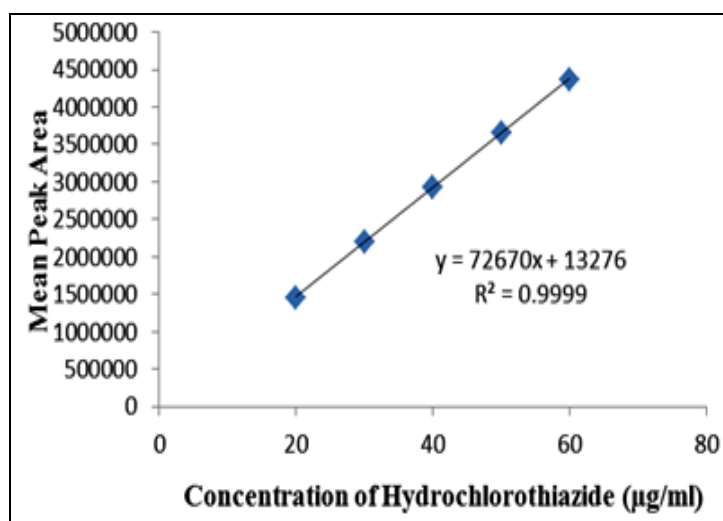
linear over the concentration range of 20-60 µg/mL. The correlation coefficient is greater than 0.99 and the %RSD for each concentration studied was less than 2.



**FIGURE 2: REPRESENTATIVE CHROMATOGRAM OBTAINED FOR HYDROCHLOROTHIAZIDE.**



**FIGURE 3: REPRESENTATIVE CHROMATOGRAM OBTAINED FROM ANALYSIS OF HYDROCHLOROTHIAZIDE FROM TABLET DOSAGE FORM**



**FIGURE 4: LINEARITY PLOT FOR HYDROCHLOROTHIAZIDE**

**Accuracy and Precision:** The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in **Table 2**.

The high percentage of recovery indicates that the proposed method is highly accurate. The precision of the method was demonstrated by inter-day and intra-day variation studies. Six replicate injections of sample solutions were made and the percentage RSD was calculated and presented in **Table 3**. From the data obtained the developed RP-HPLC method was found to be precise.

**System Suitability Parameters:** System suitability parameters were studied with six replicates of standard sample solution and the corresponding values are presented in **Table 4**.

**Method Suitability:** The commercial tablet formulation, Aquazide 12.5, was analyzed by the proposed method and the average percent recovery was found to be 99.95. The value is in good agreement with the labeled amount, which confirms the suitability of the method for the analysis of hydrochlorothiazide in pharmaceutical dosage forms.

TABLE 2: ACCURACY DATA OF THE PROPOSED METHOD

Analyte	Amount of the analyte taken ( $\mu\text{g/mL}$ )	Mean recovery ( $\mu\text{g/mL}$ ) $\pm$ SD	% Mean recovery $\pm$ SD
Hydrochlorothiazide	32	32.02 $\pm$ 0.021	100.08 $\pm$ 0.065
	40	40.07 $\pm$ 0.087	100.18 $\pm$ 0.222
	48	48.16 $\pm$ 0.058	100.33 $\pm$ 0.121

TABLE 3: PRECISION DATA OF THE PROPOSED METHOD

S. No.	Intra-day precision	Inter-day precision
1	2919388	2919388
2	2927821	2932167
3	2917852	2924339
4	2930963	2919975
5	2920636	2929636
6	2930286	2930872
<b>Average</b>	2924491	2926063
<b>SD</b>	5857.229	5616.817
<b>%RSD</b>	0.200282	0.191958

TABLE 4: SYSTEM SUITABILITY PARAMETERS OF THE PROPOSED METHOD

Parameter	Value
Retention time (min)	3.478
Tailing factor	1.76
Theoretical plates	2313.667
HETP	0.0648

**CONCLUSION:** The proposed RP-HPLC method is sensitive, precise and accurate and can be used for the routine quality control analysis for the determination hydrochlorothiazide in its tablet dosage forms.

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