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ESTIMATION OF HYDROCHLOROTHIAZIDE IN PURE AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of Hydrochlorothiazide in its pure form as well as in Pharmaceutical dosage forms. Chromatography was carried out on a ZORBAX, C-18, 150 x 4.6mm. 5µ using a mixture of Potassium dihydrogenortho phosphate and Acetonitrile (35:65%v/v) as the mobile phase at a flow rate of 0.8 mL/min, while the detection was done by UV at 270nm. The retention time of the drug was 2.414±0.001. The method produced linear responses in the concentration range of 20-60µg/ml of Hydrochlorothiazide. The method was found to be reproducible for analysis of the drug in Tablet dosage forms.

KEY WORDS: Hydrochlorothiazide, RP-HPLC, Method validation.

1.INTRODUCTION

Hydrochlorothiazide is frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and in the prevention of kidney stones. Hydrochlorothiazide is chemically described as 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide. It is also sometimes used for hypercalciuria, Dent's disease and Meniere's disease. For diabetes insipidus, the effect of thiazide diuretics is presumably mediated by a hypovolemia-induced increase in proximal sodium and water reabsorption, thereby diminishing water delivery to the ADH-sensitive sites in the collecting tubules and reducing the urine output. Thiazides are also used in the treatment of osteoporosis. Thiazides decrease mineral bone loss by promoting calcium retention in the kidney, and by directly stimulating osteoblast differentiation and bone mineral formation (Dvorak,2007). A literature survey revealed that only a few HPLC (Ashok kumar, 2011; Meyyanathan,2008;Kullai Reddy,2011) methods are available for the estimation of Hydrochlorothiazide. The authors now propose a new validated, sensitive and reproducible HPLC method for the determination of Hydrochlorothiazide and the Tablet dosage forms was also observed.

2.MATERIALS AND METHODS

Chromatographic conditions: A prominence HPLC system (Waters,YL 9100 HPLC System) with YL 9100 vacuum degasser Pump and with "Empower-II" software and UV- detector Dual Wavelength-Waters 2487 , Column ZORBAX, C-18, 150 x 4.6mm, 5µ . A 20µL, 2487 programmable auto sampler was used for sample injection. HPLC grade, a freshly prepared potassium dihydrogen ortho phosphate and Acetonitrile (35:65%v/v) was used as the mobile phase. The solvents was filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 0.8 mL/min. The column temperature was maintained at room temperature, the detection of the drug was carried out at 270nm.

Selection of mobile phase: The solution of Hydrochlorothiazide was injected into the HPLC system and run in different solvent systems. Different mobile phases containing methanol, water, acetonitrile and phosphate buffer in different proportions were tried and finally phosphate buffer(pH 2.5) and Acetonitrile (35:65 v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for Hydrochlorothiazide.

Preparation of Mobile Phase: Mobile phase comprised of 10 mM Potassium dihydrogenortho phosphate (Adjusted to P^H 2.5±0.05 with Ortho phosphoric acid), and Acetonitrile (35:65%v/v). Mobile phase was filtered through a 0.45-µm membrane filter, degassed with a helium spurge for 20 min and pumped from the respective solvent reservoir to the column (flow rate, 0.8 ml/min), which yield a column back pressure of 653-750 psi. Run time was set as 5 min, column was equilibrated for 60 min with mobile phase flowing through the system. Eluents were monitored at 270nm and data were acquired, stored and analyzed with the software "Empower-II" (Waters).

Selection of analytical wavelength: From the standard stock solution, further dilutions were prepared using mobile phase and scanned over the range of 200 – 400 nm and the spectrum was overlain. It was observed that 270nm is the λ_{max} for Hydrochlorothiazide and the wavelength suitable for Hydrochlorothiazide was preferred.

Checking the resolution of drug and material standard: The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Standard solution of Hydrochlorothiazide was injected to get the chromatogram. The retention time for Hydrochlorothiazide was found to be 2.414 min.

Preparation of Standard Solutions: A stock solution of Hydrochlorothiazide was prepared by dissolving Hydrochlorothiazide (100 mg) in a volumetric flask (100 ml) containing 25 ml of diluent(Mobile Phase), sonicated for 20 min and then made up to the volume with diluent. Working standard solution of Hydrochlorothiazide (100µg/ml) was prepared by suitable dilution of stock solution with diluent. Linearity solutions were prepared in diluents containing RS (20-60µg/ml). Each of these drug solutions (20µl) was injected into the column and the peak area and retention times were recorded.

Estimation of Hydrochlorothiazide in Tablets: Two commercial samples of the Tablets containing the drug were chosen for testing the suitability of the proposed method to estimate Hydrochlorothiazide Tablets. For this, weigh accurately quantity of the powdered contents of tablets equivalent to about 100mg of Hydrochlorothiazide into 100mL volumetric flask, add about 60mL of diluents, sonicate for about 30min and dilute to 20 ml with water and methanol. Filter through 0.45 micron filter. The contents of the flasks were made up to the volume with the mobile phase and mixed well. From the above stock, 40 µg/mL sample solution was prepared with mobile phase. Twenty micro liters of each of these solutions was then injected five times into the column. The mean peak area ratios of the drug to the five such determinations were calculated and the drug content in the tablets was quantified using the regression equation obtained for the pure sample.

3.RESULTS AND DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate RP-HPLC method for the analysis of Hydrochlorothiazide in bulk drug and pharmaceutical dosage forms. The retention time for Hydrochlorothiazide was 2.414minutes for a run period of 5 minutes (Fig.1 and Table1). Each sample was injected five times and the similar retention times were observed in all cases. The peak areas of different concentrations set up as above were calculated and average value for 5 such determinations are shown in Table-2. The peak area for drug solution was reproducible as indicated by low coefficient of variation(Fig.2).

A good linear relationship (r = 0.999) was observed between the concentration of Hydrochlorothiazide and the respective peak areas. The calibration graph was found to be linear in the range of 20-60 µg/mL, when the Hydrochlorothiazide solution was analyzed by the proposed RP-HPLC method. In intra and inter day variation studies, inter day and intraday precision was determined by analyzing the drug sample at three different concentration levels.

The results are presented in the form of %RSD which is below 1.00 (Tables 3,4 and 5) which shows that the proposed HPLC method was highly precise. The method was robust (Table 7) as observed from insignificant variation in the results of analysis by changes in flow rate, mobile phase composition and temperature separately.

The drug content in the Tablet was quantified using the proposed analytical method. The tablet was found to contain an average 101.755 of the labeled amount of drug (Table 6). The proposed reversed phase HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

Fig: 1. A typical chromatogram for Hydrochlorothiazide standard solution (40µg/ml)

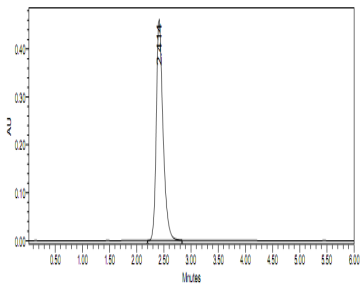

Fig 2: Linearity curve of Hydrochlorothiazide

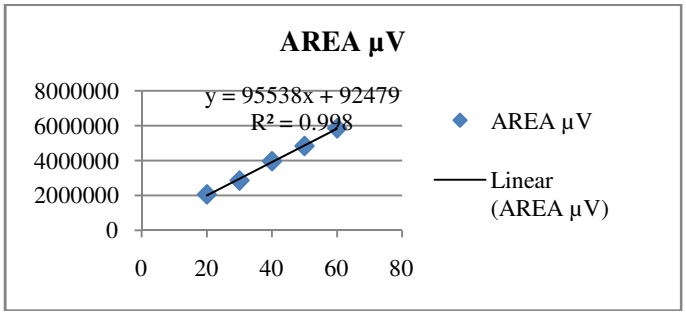

Table 1: parameters for typical chromatogram For standard Solution (40µg/ml)

RT(min)	2.414±.0.001
Peak area	3961749
Height	461852
Tailing factor	1.3
Plates	2028
HETP	0.0035
LOD (µg/ml)	0.03873
LOQ (µg/ml)	0.1162

Table 2: Calibration of the proposed method for

S.No.	Concentration (µg/ml)	Retention time (min)	Peak area	Peak height
1	20	2.415	2062353	238531
2	30	2.414	2858518	333151
3	40	2.414	3961749	461852
4	50	2.416	4837611	562723
5	60	2.416	5849687	677026

Table 3: Intra-day Precision

Concentration (µg/ml)	Peak Area	Mean (n=5)	S.D	% RSD
40	3989869	3990606	3303.6	0.08
40	3987777			
40	3991053			
40	3988291			
40	996042			

Table 4: Inter day precision

Concentration (µg/ml)	Peak Area	Mean (n=5)	S.D	% RSD
50	3989448	3990554.4	3463.461173	0.0867
50	3987491			
50	3991425			
50	3988256			
50	3996152			

Table 5: Recovery data

Amount pure drug Added µg/ml	Amount found µg/ml	±SD Mean found (n=5)	Mean Percent recovery	Percent RSD
20	20.007	0.0153	100.03	0.765
40	40.016	0.0020	100.04	0.005
60	60.103	0.0122	100.17	0.020

Table 6: Assay of Hydrochlorothiazide

Formulation	Label Claim	Amount found	% Amount found
Brand-1	25	25.53	102.15
Brand-1	25	25.34	101.36

Table 7: Results of the Robustness Study

Variables	Chromatographic Parameters			
	Retention Time (min)	Tailing Factor	Theoretical Plates	% Assay
70% Acetonitrile (More Organic)	2.375	1.3	2002.6	100.03
60% Acetonitrile (Less Organic)	2.471	1.3	2018	100.06
Flow Rate-0.9ml/min (more flow)	2.140	1.2	2120	99.99
Flow Rate-0.7ml/min (Less flow)	2.763	1.4	2146.2	100.07

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