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Research Article

A VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF HYDROCHLOROTHIAZIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

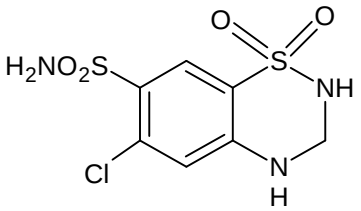
A RP-HPLC method has been developed and validated for the estimation of hydrochlorothiazide in bulk and pharmaceutical dosage form. A RP-HPLC isocratic separation was achieved on C18 column (250×4.6 mm i.d., 5µm) utilizing a mobile phase comprising of methanol and acetonitrile in the ratio of 30: 70(v/v) and the eluents from the column were detected using a variable wavelength detector at 271nm. The proposed method has permitted the quantification of hydrochlorothiazide in the linearity range of 20-100µg/ml and the flow rate was maintained at 0.6ml/min. The column was maintained at ambient temperature and the complete separation was achieved for hydrochlorothiazide in an overall analytical run time of approximately 10 minutes. The retention time of hydrochlorothiazide was found to be 4.76 minutes. The limit of detection and limit of quantification were found to be 1.16 and 3.53 µg/ml, respectively. The percentage recovery was found to be in between 98.52 to 100.18. The method was found to be suitable for the routine quality control analysis of hydrochlorothiazide in bulk drug and formulation. The method was validated as per ICH guidelines.

Key Words: Hydrochlorothiazide, RP-HPLC, ICH guidelines, Validation.

Introduction

Hydrochlorothiazide (HCTZ) is a thiazide class diuretic drug. This reduces the volume of the blood, decreasing blood return to the heart. Hydrochlorothiazide is often used in the treatment of hypertension, congestive

heart failure, symptomatic edema and the prevention of kidney stones. The recommended dose of hydrochlorothiazide for treating high blood pressure is hydrochlorothiazide 25 mg to 50 mg per day.



IUPAC name: 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-Benzothiadiazine-7-sulfonamide

Figure 1: Chemical Structure of Hydrochlorothiazide

Literature survey reveals that a few analytical methods like HPTLC, HPLC and some spectroscopic methods have been reported for the estimation of HCTZ alone and in combination with other drugs.

Materials and Methods

Materials

Hydrochlorothiazide was obtained as a gift sample. The commercially available tablet, "Hydrazide" 25mg (Cipla Ltd.) containing 25 mg of HCTZ was procured from the local market and used for analysis. Acetonitrile, methanol and water used were of HPLC grade and purchased from Merck specialties private limited, Mumbai, India. All the glassware employed in the study was cleaned with hot water, followed by acetone and dried in hot air oven whenever required.

Instrumentation

Analysis of samples were performed by using JASCO PU 2080 HPLC system, a variable wavelength programmable UV/VIS detector with precision loop injector (Rhenodyne, 20 µl). The data was processed by using BORWIN 6.0 software. All samples were filtered through 0.45 µm membrane Millipore filtration apparatus with vacuum pump.

Chromatographic Conditions

The isocratic method was employed with the mobile phase consisting of 30 volumes of methanol and 70 volumes of acetonitrile. The chromatographic column used was a HiQ Sil C-18 Column with dimensions of 250×4.6 mm with 5µm particle size. The column was maintained at ambient temperature and detection was

performed at a wavelength of 271 nm. Prior to injection of analyte, the column was equilibrated for 30-40 mins with mobile phase. The injection volume was 20 µL. Methanol was used as diluent for preparation of solutions.

Preparation of mobile phase

The HPLC grade solvents of methanol and acetonitrile were used for the preparation of mobile phase in the ratio of 30:70 (v/v). The contents of the mobile phase were filtered before use through a 0.45µm membrane filter, degassed by sonication and pumped from the solvent reservoir to the column at a flow rate of 0.6 ml/min throughout the analysis.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving the 10mg of HCTZ in 10 ml methanol to obtain primary stock solution of 1000µg/ml. From the primary stock solution; 5ml of the solution was pipette out and diluted up to 10ml with methanol to get final concentration of 500 µg/ml.

Analysis of tablet formulation

Twenty tablets were accurately weighed and triturate thoroughly to get fine powder. The powder equivalent to 25mg of HCTZ was weighed and transferred into 25ml volumetric flask. The contents of the flask were dissolved in the 10ml of the methanol with the aid of ultrasonication for 10mins. The solution was filtered through whatmann filter paper no. 41 and volume was made up to 25ml with methanol. From the resultant solution, further dilutions were prepared with methanol to get final concentration of HCTZ. The absorbances were measured at selected wavelengths and the concentration of the analyte was determined with the equation obtained from calibration curve. The results of assay of tablets are shown in Table 3.

Table 3: Analysis of tablet formulation.

Formulation	Concentration (ppm)	Percentage of drug estimated	Statistical analysis
Tab. Hydrazide	40	97.65	Mean- 98.82 %RSD-1.03
	40	99.27	
	40	99.55	

Procedure:

A mixture of methanol and acetonitrile in the ratio of 30:70v/v was prepared. The solvent mixture was filtered through a 0.45µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.6ml/min. The column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30min prior to the injection of the drug solution. The detection of the drug was monitored at 271nm. The run time was set at 8min. Under these optimized chromatographic conditions the retention time obtained for the drug was 4.767min. A typical chromatogram showing the separation of the drug is given in Figure 2.

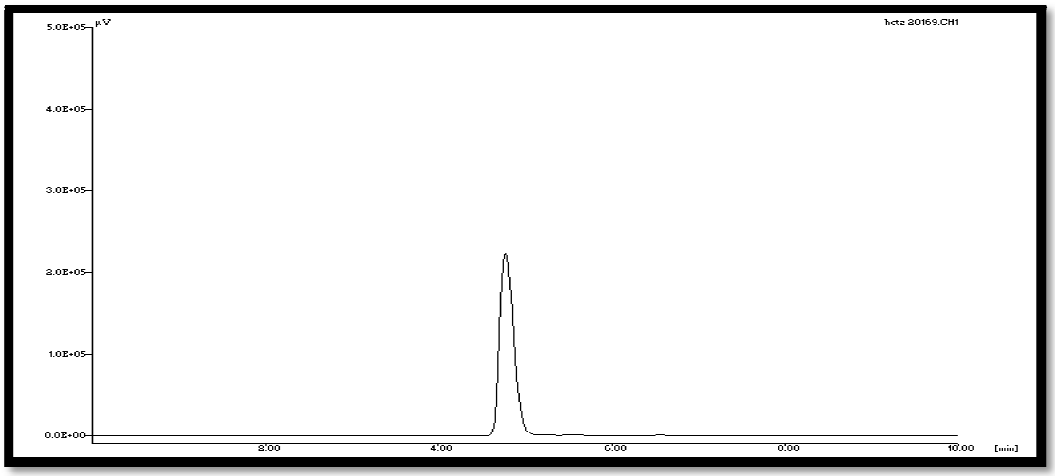


Figure 2: Typical chromatogram of Hydrochlorothiazide.

Preparation of calibration graph:

Preparation of Level – I (20,40, 60,80,100 µg/ml): 0.4, 0.8, 1.2, 1.6 and 2ml of stock solution has taken in 10ml of volumetric flask diluted up to the mark with diluent.

Procedure:

Injected each level into the chromatographic system and measured the peak area. Plotted a graph of peak area versus concentration and calculated found to be linear in the concentration range of 20-100µg/ml of the drug (Figure 3).

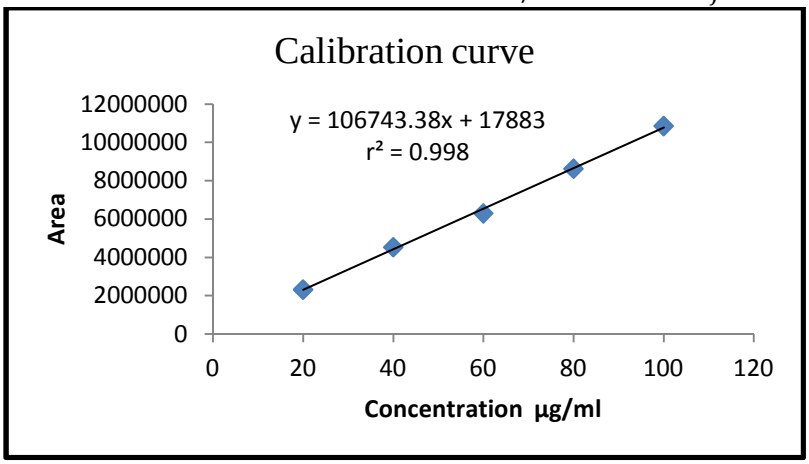


Figure 3: Calibration curve of Hydrochlorothiazide.

The relevant data are furnished in Table-1. The regression equation of this curve was computed. The regression equation was later used to estimate the amount of HCTZ in tablet dosage forms.

Validation of the proposed method:

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification and robustness parameters were studied systematically to validate the proposed HPLC method for the determination of hydrochlorothiazide.

LOD and LOQ

In this study, LOD and LOQ were based on the standard deviation of the response (σ) and the slope of the corresponding curve (S) using the following equation:

$$LOD = 3.3\sigma/S, \quad LOQ = 10\sigma/S$$

Where, σ is the standard deviation of the response of blank,

S is the slope of calibration curve.

The LOD and LOQ of HCTZ were found to be 1.16 µg/ml and 3.53 µg/ml respectively.

Precision

Precision is the measure of how close the data values to each other for a number of measurements under the same analytical conditions. Precision of the method was determined by performing interday variation, intraday variation and repeatability studies. Three replicate injections of the specific standard (3 different

concentrations) at various time intervals on the same day were injected into the chromatograph and the value of % RSD was found to be within the limits. In inter-day precision, same standard concentrations were injected on different days and % RSD was also found to be within the limits for HCTZ. In repeatability study, six determinations of the fixed concentration of HCTZ were analyzed separately. The results of precision data are given in Table 2.

Table 2: Precision Data.

	Fortified amount (ppm)	**Mean Area found**	**Amount found** (ppm)	**% RSD**
Intraday (n = 3)	40	4273410	39.86	0.78
	60	6360658	59.42	0.61
	80	8636985	80.74	0.25
Interday (n = 3)	40	4300931	40.12	0.04
	60	6361769	59.43	0.33
	80	8605241	80.44	0.14
Repeatability (n = 6)	40	4233645	39.49	0.88

Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered and the resolution was evaluated. The flow rate of the mobile phase was 0.6 ml/min. To study the effect of flow rate on resolution, it was changed by 0.1 units from 0.5 to 0.7ml/min. The effect of percent organic strength on resolution was studied by varying composition of mobile phase by 2 units from methanol: acetonitrile (28:72 v/v) to methanol: acetonitrile (32:68 v/v). The results are given in Table 4.

Table 4: Results of Robustness Study.

Chromatographic conditions	**Normal**	**Variation**	**Drug amount**	**Rt**
Mobile phase (methanol : acetonitrile)	30 : 70	28 : 72	40.32	4.8
		32 : 68	39.86	4.8
Flow rate(ml/min)	0.6	0.5	43.55	5.75
		0.7	42.89	4.1

Specificity

Marketed formulation were analyzed to determine the specificity of the optimized method in the presence of excipients and it was observed that single peak for HCTZ (Rt 4.7 min) were obtained under optimized conditions (Figure 4), showing no interference from excipients and impurities. Also the peak area were compared with the standard and % purity calculated was found to be within the limits.

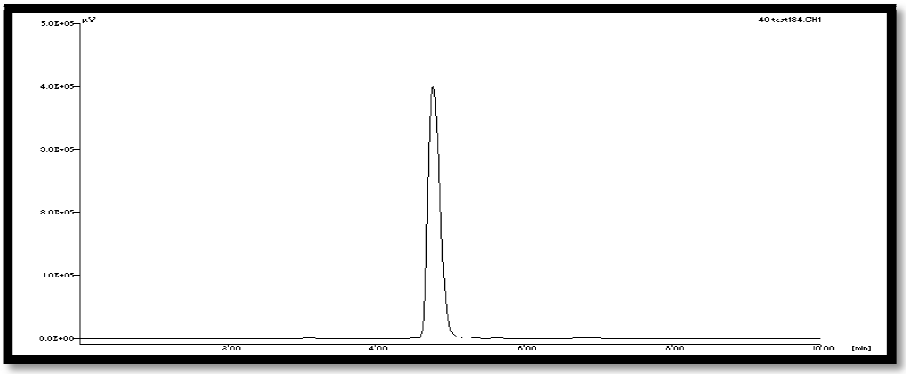


Figure 4: Chromatogram of tablet formulation.

Accuracy (Recovery studies)

The accuracy of the proposed method was determined by calculating the recoveries of HCTZ by the standard addition method. It was determined by preparing solutions of different concentrations at 80%, 100% and 120% of 60µg/ml in which the amount of marketed formulation was kept constant (30 µg/ml) and the amount of pure drug was varied that is 10µg/ml, 30µg/ml and 50µg/ml for 80%, 100% and 120% respectively. The amount of HCTZ recovered was estimated by applying obtained values to the regression line equation. The results of % recovery are given in Table 5.

Table 5: Results of % Recovery Study.

Concentration % of spiked level	Amount of drug added (ppm)		Area found	Amount of pure drug found (ppm)	% Recovery	Statistical analysis of % recovery
	Pure drug	Formulation				
80% sample 1	18	30	5113670	17.73	98.54	Mean- 98.52 %RSD-0.36
80% sample 2	18	30	5106980	17.67	98.16	

80% sample 3	18	30	5119962	17.79	98.87	
100% sample 1	30	30	6399437	29.78	99.28	Mean- 99.45 %RSD-0.32
100% sample 2	30	30	6398673	29.77	99.25	
100% sample 3	30	30	6416972	29.94	99.82	
120% sample 1	42	30	7712496	42.08	100.20	Mean- 100.18 %RSD-0.23
120% sample 2	42	30	7722193	42.17	100.41	
120% sample 3	42	30	7707068	41.97	99.84	

Results and discussions

A simple RP-HPLC method has been developed for determination of HCTZ. The method was optimized to provide a good separation of the component (acceptable theoretical plates) with a sufficient sensitivity and suitable peak symmetry in a short run. For this purpose, the analytical column, solvent selection, mobile phase composition, flow rate, and detector wavelength were studied. The chromatographic separation was achieved using an RP C18 column. Our experiments using methanol along with acetonitrile (HPLC grade) as mobile phase was eluted the HCTZ in a significant shorter retention time of 4.7 mins. Therefore, we selected methanol and acetonitrile in the ratio of 30:70 (v/v) as a mobile phase. The method has many advantages, e.g., simplicity, isocratic conditions, and absence of buffers in the mobile phase that could damage the chromatographic column and equipment. Under these conditions, the retention time of HCTZ was about 4.7 min, with a good peak shape (peak symmetry). The optimized chromatographic conditions are given in Table 1.

Table 1: Optimized Chromatographic Conditions.

Parameters	Optimized conditions
Chromatograph	HPLC (Jasco with UV detector)
Column	HiQ sil C-18 HS (5 µm; 250 × 4.6 mm)
Mobile phase	Methanol : acetonitrile (30 : 70v/v)
Flow rate	0.6 ml/min
Detection wavelength	271 nm
Injection volume	20 µl

Conclusion

A validated RP-HPLC analytical method has been developed for the determination of HCTZ in bulk and dosage form. The proposed method was simple, accurate, precise, specific and suitable to use for the routine analysis of HCTZ in either bulk API powder or in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS and GC–MS. These methods are complicated, costly and time consuming rather than a simple HPLC-UV method.

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