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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF HYDROCHLOROTHIAZIDE IN TABLET FORMULATION BY USING RP-HPLC

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ABSTRACT
A reverse phase High Performance Liquid Chromatographic method was developed and validated for the quantitation of an Anti-Hypertensive drug Hydrochlorothiazide in bulk and pharmaceutical dosage form. The analysis was performed on Waters SUN FIRE C18 column (4.6mm x 50, 5µm) with a mobile phase composed of tri fluoro acetic acid (0.1%, acidic) and acetonitrile in gradient mode at a flow rate of 1.0 ml/min with detection of analyte at 225nm. The separation was achieved within 3.07min. The method validation parameters showed good results for linearity, accuracy, and precision. The calibration curve was linear in the concentration range 25-150 µg/ml with coefficient of correlation 0.999. The mean recovery was found to be 98.20%. The LOD and LOQ were found to be 3.07 µg/ml and 9.33 µg/ml. The proposed method was validated as per ICH guidelines for various parameters like accuracy, precision, linearity and robustness.

KEYWORDS: Hydrochlorothiazide (HCTZ), RP-Hplc, TRI Fluoro Acetic Acid Buffer (2.3 PH), Acetonitrile.

INTRODUCTION
Hydrochlorothiazide acts on the distal convoluted tubules and inhibits the Na+-cl- co-transporter system.[1] This action leads to a diuretic action and loss of potassium in the urine. The half-life of hydrochlorothiazide varies from 6 to 12 hours.[2] HCTZ is widely prescribed diuretic, used in congestive heart failure, hypertension, and edema.[3] It belongs to a class of drugs called as thiazide diuretics antihypertensive. Hydrochlorothiazide binds to and inhibits the carbonic enzyme anhydrase. It is frequently used alone or in combination with other medications for the treatment of hypertension, congestive heart failure, symptomatic edema, renal tubular acidosis, hypoparathyroidism, prevention of kidney stones and used in the treatment of osteoporosis.[4] Hydrochlorothiazide is available in tablet form rapidly absorbed orally are administered only by this route. Pharmacological effects begin in about 2 hours after an dose, and lasts for about 6 to 12 hours. Hydrochlorothiazide is not metabolized, and a majority is excreted in the urine unchanged. It also causes a loss of potassium and bicarbonate.[5] Hydrochlorothiazide, is a white, or practically white, crystalline powder which is slightly soluble in water, freely soluble in sodium hydroxide solution; sparingly soluble in methanol; insoluble in chloroform. Each tablet for oral administration contains 12.5 mg, 25 mg or 50 mg hydrochlorothiazide.

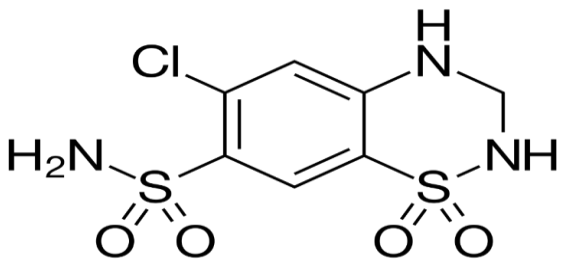

Fig. 1: Chemical structure of Hydrochlorothiazide.

The more lipid-soluble agents have larger volumes of distribution, lower rates of renal clearance and are longer acting. The protein binding is also variable. Most of the agents undergo little hepatic metabolism and are excreted as much. They are filtered at the glomerulus as well as secreted in the proximal tubule by organic anion transport tubular reabsorption depends on liquid solubility: the more soluble ones are highly reabsorbed duration of action. Literature survey reveals that a few HPLC methods, UV spectrophotometric[6], flow injection[7], LC-MS, method have been used .The objective of the present work was to develop simple, rapid, accurate, specific and economic RP-HPLC

stability indicating method.[8] The aim of the present work was to develop and validate a simple, fast and reliable gradient RP-HPL C18 method with UV detection for the determination of Hydrochlorothiazide in bulk and in tablet dosage forms. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, short elution time HCTZ, good precision (R.S.D. less than 2%) and high recovery (greater than 98%). Confirmation of the applicability of the developed method validated according to the International Conference on Harmonization (ICH)[8] for the determination of HCTZ in bulk and in tablet dosage form.

MATERIALS AND METHODS

Chemicals Hydrochlorothiazide (HCTZ) was obtained from jubilant generic (India), and was used as such without further purification. The commercial formulations available are Aquazide (25 mg) and were purchased from the local market.

Reagents Tri-fluoro acetic acid (FINAR), Acetonitrile (Honeywell), Water (Milli Q).

Instruments and Equipment WATERS HPLC, Model: Ailiance 2695, Photo diode array detector, with a Automated Sample injector. The output signal was monitored and integrated using Empower 2 software. A Sun fire C18 (4.6 x 50mm, 5 µm, Make: Waters), Weighing Balance, Sonicator, pH Meter, Filter Paper 0.45 microns.

Preparation of buffer Measure 1ml of Tri fluoro acetic acid in to a 1000mL beaker, dissolve and degassed with HPLC water. The pH of the solution is 2.3.

Preparation of mobile phase Mixed well Buffer: Acetonitrile in a ratio (90:10) and filtered through 0.2µ 6,6 Nylon membrane filter and degassed.

Preparation of Diluents Mixed well Milli-Q water: Acetonitrile (ACN) in a ratio (50:50).

Preparation of Blank Solution **Mixed well Milli-Q water: Acetonitrile (ACN) in a ratio (50:50).**

Preparation of standard solution Stock solution of HCTZ (0.5 mg/mL) was prepared by weighing 25 mg and dissolving in the diluents (Milli-Q water :ACN in a ratio 50:50) Standards solutions of HCTZ were prepared in the range of 25 µg/mL to 150 µg/mL by diluting the stock solution with diluents. The eluate was monitored at 225nm. Each solution was then injected into the column and the chromatograms were recorded.

Preparation of sample solution Ten tablets were weighed to get the average weight and then powdered. The fine powder, equivalent to 25 mg of HCTZ, was weighed and transferred into a 50 mL calibrated volumetric flask and dissolved using diluents. This mixture was sonicated (30 min) and then filtered through a 0.45 µm filter. After filtration, Aliquots solutions were prepared by taking 1mL into 20ml volumetric flasks, separately and made up to volume with diluents to yield concentrations of drug in range of linearity previously described. The amount of HCTZ was calculated from the related linear regression equations.

RESULTS AND DISCUSSION

Method Development: The method utilizing Tri fluoro acetic acid: Acetonitrile buffer of pH 2.3 as mobile phase in different ratios yielded sharp peak, whereas with TFA:ACN buffer in 95:5% v/v dilutions symmetric peak was obtained at flow rate 1.0mL/min and wavelength is 225nm.

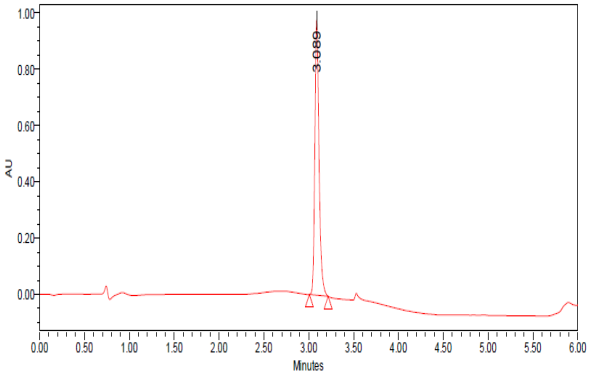

Fig. 2: Chromatogram of Hydrochlorothiazide.

Table. 1: Optimized Condition.

S. No.	Parameter Optimized	Optimized Condition
1	Instrument (HPLC)	Ailiance Waters 2996 PDA
2	Column	Waters SUN FIRE C18 (4.4 x 50 mm, 5µm)
3	Mode	Gradient
4	Mobile phase	Tri fluoro acetic acid : Acetonitrile
5	Column Oven	40 ºC
6	Auto sampler Temperature (ºC)	20 ℃
7	Flow rate	1.00 mL/min
8	Detector	Photodiode array
9	Temperature	Ambient room temperature
10	Detection wavelength	225nm
11	Injection volume	5µl
12	Retention time (RT)	3.07 ± 0.05 min
13	Run time	6.00 min

Validation Validation of HPLC method was in compliance with recommendations of the ICH Guidelines.

Linearity For all methods, 6-point calibration curve were prepared on single day. The results obtained were used to calculate the equation of the line by using linear regression by the least square method.

Table. 2: Linearity Area.

S. NO	Conc.	Area
1	25	813620
2	50	1722110
3	75	2613801
4	100	3498206
5	125	4298446
6	150	5259279
Correlation Coffecient		0.9999

Procedure for calibration curve: Prior to injection of the drug solutions, the column was equilibrated with the mobile phase flowing through the systems. The chromatographic separation was achieved using a mobile phase consisting of Tri fluoro acetic acid: Acetonitrile (95:5 v/v) at a flow rate of 1.0mL/min. The eluent was monitored using UV detection at a wavelength of 225 nm. The column was maintained an ambient temperature (25°C) and an injection volume of 5 µl of each of standard and sample solutions were injected into the HPLC system to get the chromatograms. The retention time and peak areas of the drug were recorded. The calibration curve for the HPLC analysis was constructed by plotting the peak area of normalization of HCTZ on x axis against concentration on y-axis.

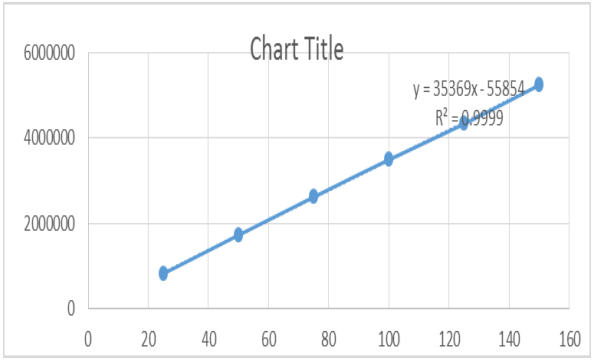

Fig. 3: Linearity Plot of HCTZ.

Accuracy: The accuracy of an analytical method is the closeness of the test results to the true value. It has been determined by application of the analytical procedure to recovery studies, where known amount of standard HCTZ (50%, 100%, and 150%) is spiked into the pre-analyzed amount of formulation of concentration 100µg/ml.

Table. 3: Accuracy.

% Accuracy	conc ppm	Area	Mean Area	Sample wt (mg)	Amount added (µg)	Amount recoverd (µg)	% Recovery	Mean % Recovery	STD Dev Recovery	% RSD Recovery
50%	50	1666622	1671374	150.8	**1508.00**	1480.88	98.20	**98.48**	0.25	**0.26**
		1674929				1488.26	98.69			
		1672571				1486.17	98.55			
100%	100	3362599	3382004	150.8	**3016.00**	2987.84	99.07	**99.64**	0.65	**0.65**
		3377638				3001.21	99.51			
		3405776				3026.21	100.34			
150%	150	5126747	5127115	150.8	**4524.00**	4555.38	100.69	**100.70**	0.122	**0.121**
		5133516				4561.39	100.83			
		5121082				4550.34	100.58			

Precision (system and method): The precision of the system was evaluated by carrying out 6 independent injection of standard. The % RSD of peak area of the standard was found to be 0.33.

Table. 4: System Precision.

Standard Inhection	RT(Min)	Std. Area
INJ-01	3.09	3185986
INJ-02	3.08	3170956
INJ-03	3.08	3158134
INJ-04	3.09	3179351
INJ-05	3.09	3186415
INJ-06	3.08	3177576
MEAN	**3.09**	**3176403.00**
SD	**0.01**	**10635.48**
%RSD	**0.18**	**0.33**

Table. 5: Method Precision.

Sample	Injection	Area	Avg. Area	Retention Time	% Assay
1	INJ-01	3458017	3448530	3.07	95.0
	INJ-02	3439043		3.07	
2	INJ-01	3474376	3475769	3.05	95.8
	INJ-02	3477161		3.05	
3	INJ-01	3454870	3453771	3.07	95.2
	INJ-02	3452671		3.07	
4	INJ-01	3458515	3455544	3.07	95.2
	INJ-02	3452573		3.07	
5	INJ-01	3449514	3451427	3.07	95.1
	INJ-02	3453340		3.07	
6	INJ-01	3448375	3448663	3.07	95.0
	INJ-02	3448351		3.07	
Mean			3455617		95.2
SD			10253.35		
%RSD			0.296715		

Table. 6: Intermediate Precision (Diff. Analyst).

S No.	Injection	Std. Area	Test Area		Avg. Test Area
			Inj.1	Inj.2	
1	INJ-01	3678344	3527403	3552001	**3539702**
2	INJ-02	3664857	3539790	3565949	**3552870**
3	INJ-03	3678221	3554409	3554147	**3554278**
4	INJ-04	3673712	3548711	3559829	**3554270**
5	INJ-05	3702518	3554932	3554829	**3554881**
6	INJ-06	3718767	3465247	3500145	**3482696**
	MEAN	**3686069.83**			**3539783**
	SD	**20315.71**			**28559.16**
	%RSD	**0.55**			**0.806806**

Robustness: Robustness of the method reflects the reliability of an analysis with respect to deliberate variations in the method parameters. Here, the flow rate and mobile phase composition were slightly changed to lower and higher sides of the actual values to find if the change in the peak area and retention time were within limits. The results obtained with changes in the parameters on a 100µg/mL solution.

Table. 7: Increase Flow (1ml-1.1ml).

Standard	RT(Min)	STD Area	Test Inj.	Avg. Area
INJ-01	2.90	3283648	1	3150656
INJ-02	2.91	3278800	2	3159221
INJ-03	2.90	3280025		
INJ-04	2.91	3288138		
INJ-05	2.92	3310044		
INJ-06	2.91	3283825		
MEAN	**2.91**	**3287413.33**		**3154938.50**
SD	**0.01**	**11562.90**		**6056.37**
%RSD	**0.20**	**0.35**		**0.19**

Table. 8: Decrease Flow (1ml-0.9 ml).

Standard	RT(Min)	STD Area	Test Inj.	Avg. Area
INJ-01	3.31	4042487	1	3914596
INJ-02	3.30	4010770	2	3921164
INJ-03	3.31	4042599		
INJ-04	3.32	4061306		
INJ-05	3.31	3984734		
INJ-06	3.32	4010969		
MEAN	**3.31**	**4025477.50**		**3917880**
SD	**0.01**	**28117.58**		**4644.28**
%RSD	**0.22**	**0.70**		**0.12**

System suitability parameters System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation has been completed. The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Asymmetry (A), LOD(µg/mL) and LOQ(µg/mL) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of HCTZ in pharmaceutical formulations was validated or not.

Table. 9: System Suitability.

Parameters	Obtained values
Theoretical Plates	7459.85
Asymmetry	1.51
Tailing factor	1.51
LOD(µg/mL)	3.07
LOQ(µg/mL)	9.33

CONCLUSION

The proposed method was found to be simple, precise, accurate, rapid and specificity for determination of Hydrochlorothiazide from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Hydrochlorothiazide in pure form and its dosage form and also can be used for dissolution or similar studies.

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