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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OFHYDROCHLOROTHIAZIDE IN TABLET BY HPLC TECHNIQUE

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ABSTRACT

An HPLC method was developed and validated for the determination of hydrochlorothiazide in bulk and pharmaceutical formulation. The method was optimized selecting chromatographic conditions of 50:50 Diphosphate hydrogen: water, Inertsil column (ODS-3 250 mm × 4.6 mm 5 μm), 20 μL injection volume, flow rate of 1 mL/min at ambient temperature (40°C), and 272 nm. The method was validated giving good precision (RSD% < 1), acceptable linearity (≥ 0.9978), and low LOD and LOQ (0.5 and 1.7 μg/mL, resp.) on both columns. Successful application on pharmaceutical dosage tablet form gave high recovery of 99.93%. The method was compared with official BP and other reported methods. The proposed method is economic, simple, and rapid and hence can be employed for routine analysis in quality control laboratories.[1,2,4]

KEYWORD: Hydrochlorothiazide, Method Development, HPLC, Validation.

INTRODUCTION

Hydrochlorothiazide is used alone or in combination with other medications to treat high blood pressure. Hydrochlorothiazide is used to treat edema (fluid retention; excess fluid held in body tissues) caused by various medical problems, including heart, kidney, and liver disease and to treat edema caused by using certain medications including estrogen and corticosteroids. Hydrochlorothiazide is in a class of medications called diuretics ('water

pills'). It works by causing the kidneys to get rid of unneeded water and salt from the body into the urine. High blood pressure is a common condition and when not treated, can cause damage to the brain, heart, blood vessels, kidneys and other parts of the body. Damage to these organs may cause heart disease, a heart attack, heart failure, stroke, kidney failure, loss of vision, and other problems. In addition to taking medication, making lifestyle changes will also help to control your blood pressure. These changes include eating a diet that is low in fat and salt, maintaining a healthy weight, exercising at least 30 minutes most days, not smoking, and using alcohol in moderation.

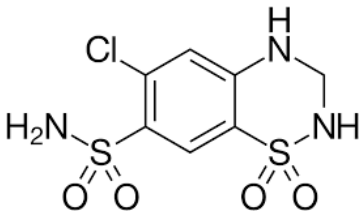


Fig: Structure of Hydrochlorothiazide.

Hydrochlorothiazide directly inhibits the sodium chloride cotransporter located on the apical membrane of the distal convoluted tubules in the kidney. The distal convoluted tubule is responsible for reabsorbing approximately 5% to 10% of the sodium in the kidney.[9] This inhibition increases the concentration of sodium that moves to the collecting ducts by preventing sodium resorption in the distal convoluted tubules. Hydrochlorothiazide reduces the sodium-potassium ATPase pump's activity on the basolateral surface by preventing sodium from crossing the tubular lumen. This action prevents the movement of sodium and water into the interstitial space.

In adults, the pharmacological effects of hydrochlorothiazide commence within 2 hours, peak after 4 hours, and persist for approximately 6 to 12 hours.[13] As the kidney is the primary excretory route for the medication, patients with renal impairment may exhibit a prolonged half-life and increased plasma concentration. Although no dosage adjustment is recommended for impaired renal function, the medication is unlikely to be effective in cases of severe renal impairment with a creatinine clearance of less than 10.

Duration of action

Hydrochlorothiazide increases excretion of sodium and chloride in approximately equivalent amounts. Natriuresis may be accompanied by some loss of potassium and bicarbonate. After oral use diuresis begins within 2 hours, peaks in about 4 hours and lasts about 6 to 12 hours.

MATERIAL AND METHODS

METHOD DEVELOPMENT

Analytical method development and validation play important roles in the discovery development and manufacture of pharmaceuticals. These methods used to ensure the identity, purity, potency, & performance of drug product There are many factors to consider when developing methods. The initially collect the information about the analyte's physiochemical properties (piker, log P, solubility) and determining which mode of detection would be suitable for analysis in case of UV detection). The majority of the analytical development effort goes in validating a HPLC–method. There are many steps involve in method development which are.

- Physicochemical properties of drug
- Set up HPLC conditions
- Sample preparation
- Method optimization
- Validation of developed method

METHOD VALIDATION

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for its intended use. The methods validation process for analytical procedures begins with the planned and systematic collection by the applicant of the validation data to support analytical procedures. All analytical methods that are intended to be used for analyzing any clinical samples will need to be validated. The validation of analytical methods is done as per ICH.

VALIDATION PARAMETER

The following are typical analytical performance characteristics which may be tested duringmethods validation

- Accuracy
- Precision
- Repeatability
- Intermediate precision
- Linearity
- Detection limit
- Quantitation limit

- Specificity
- Robustness
- System suitability determination

FORMULATION DETAILS OF HYDROCHLOROTHIAZIDE

Manufactured by : Sun pharma laboratories ltd.

Batch no : GTE0350A

Mfg. Date : Apr 2023

Exp Date : Mar 2026

Process of analysis

Phosphate Buffer (pH 6.0): 6.08 gm of Di-potassium hydrogen Orthophosphate dissolved in1000 ml of purified water. Adjust the pH to 6.0 with Orthophosphoric acid.

Mobile Phase- Phosphate Buffer (pH 6.0): Methanol: Acetonitrile (60:40:60). Mixed, Sonicated and filtered through 0.45-micron nylon filter paper.

Chromatographic Condition

Column: 250 x 4.6mm, 5 µm, Agilent Eclip C18

Wavelength: 267 nm

Flow Rate: 1.0 ml / min.

Injection Volume: 20 µl

Column Oven Temperature: 40 ^{0}C

Run time: 7 min

Mobile Phase: Phosphate Buffer: Methanol: Acetonitrile (60:40:60)

STANDARD PREPARATION

Weigh accurately and transfer about 25 mg hudrochlorothiazide Standard in 50ml volumetric flask. Add about 30ml of mobile phase. Sonicate for 5 min. Allow the solution to attend room temperature and dilute upto mark with mobile phase. Dilute 5ml of solution to 50ml volumetric flask.[7]

SAMPLE PREPARATION

Crush and make the powder of 20 tablets at a time in mortal. Mix to uniform and perform assay from it. Weigh accurately and transfer sample powder containing about 50mg of hydrochlorothiazide in 100ml volumetric flask. Add about 70ml mobile phase and sonicate for 20 min. Allow to attend room temp. Dilute up to the mark with mobile phase. Filter through whatman filter paper No.41.Filter paper disposed first 10ml of filtrate. Further dilute 5ml of this solution to 50ml with mobilephase. And inject.[7]

SYSTEM SUITABILITY CRITERIA

1) % RSD for retention time of replicates of standard preparation should not be more than 1.0 %.
2) % RSD for area of replicates of standard preparation should not be more than 2.0 %
3) Theoretical plates for all standards injections should not be less than 2000. Report Theoretical plates of first replicate of standard preparation.
4) Tailing factors for all standard injections should not be less than 2.0. Report tailing factor offirst replicate of standard preparation.

Calculate Content of hydrochlorothiazide (mg/Tablet) for both sample preparations.

Calculate Content of hydrochlorothiazide (mg/Tablet) for both sample preparations independently by using formula

$$\frac{(\text{SPL area})}{\text{Average area of 5 STD replicates}} \times \frac{W1}{100} \times \frac{10}{100} \times \frac{100}{W2} \times \frac{P}{100} \times W3(gm)$$

For % Assay = (mg / per Tablet) (100) / (Label Claim i.e., 40)

Were,

W1 = Weight of Febuxostat standard taken in mg for standard prep.

W2 = Weight of sample taken in gm for Sample preparation

W3 = Average weight of Tablets in grams

P = % Purity of Hydrochlorothiazide on as such basis.

Acceptance Criteria

Assay of hydrochlorothiazide in should be between 25.5 to 27.5 mg per Tablet.

% Assay of hydrochlorothiazide should be between 90.0% to 110.0%.

described below

DEVELOPMENT OF HPLC ANALYTICAL METHOD

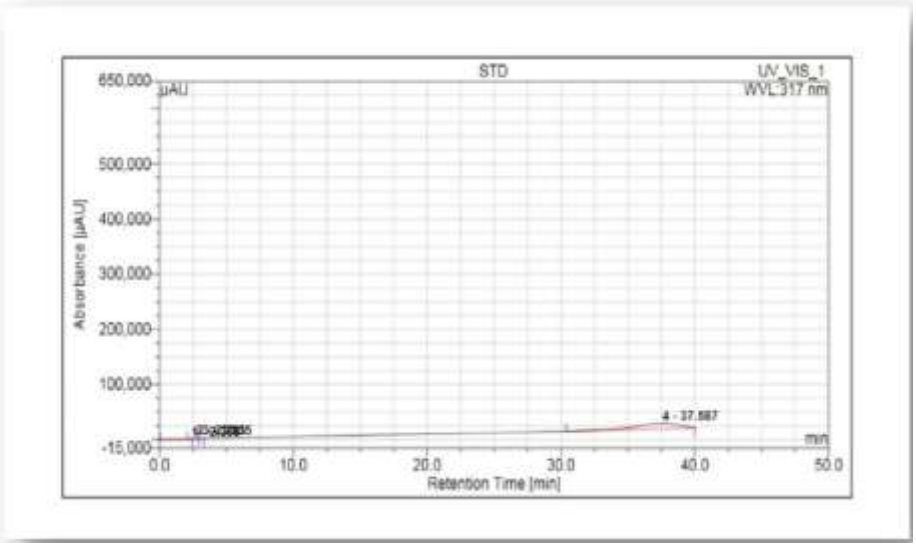
HPLC Used: Low Pressure Quatrernary Gradient

(Make-Shimadzu, Model-LC2010)

HPLC column- 250 x 4.6nm, 5um, Agilent Eclips C18.

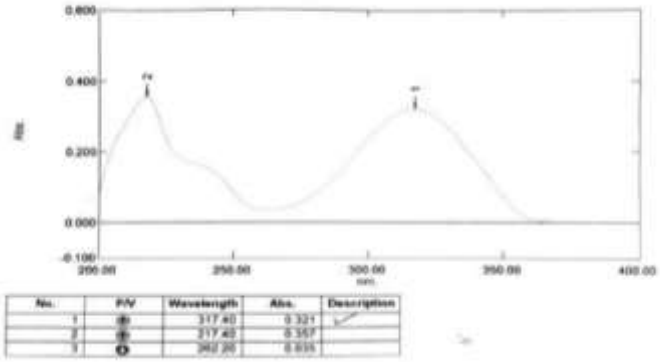
Sr no.	Chemical used	Make	Grade
1	Potassium hydrogen	Fisher scientific	SQ grade
2	acetonitrile	merck	Hplc grade
3	Orthophospric acid	rankem	AR grade
4	Purified water	--	Mili Q

METHOD DEVELOPMENT DETAILS



TRAIL 1 In these trial, same column was employed but the mobile phase was changed to mobile phase consisting of 20 mM Potassium Di-hydrogen Orthophosphate (pH 3): Acetonitrile (60:40 %v/v) at a flow rate of 1.0 mL/min was used on an Agilent Eclipse C18 column (250 x 4.6) 5-micron column at ambient temperature.

Following changes are done.



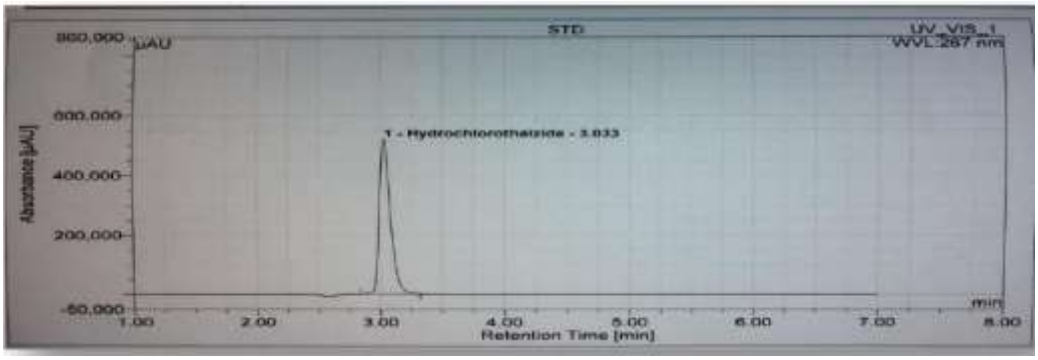
1) Methanol solvent is introducted in some portion of acetonitrile.
2) Buffer: ACN: methanol. (60:40:60).
3) Conc of salt increased from 20 to 30 mM
4) pH of buffer is shifted from 6.0 to 6.5.
5) Column length is decreased. From 250 to 150 cm, Lambda max is confirmed by ultravioletspectroscopy. spectra is observed as above.[11]

METHOD VALIDATION

A) SPECIFICITY AND SYSTEM SUITABILITY

Specificity demonstrated by observing interference of mobile phase (Diluent).

System suitability parameters (%RSD of area, Retention time, Theoretical Plates and Tailing factor) demonstrated by injecting standard preparation in replicate.1

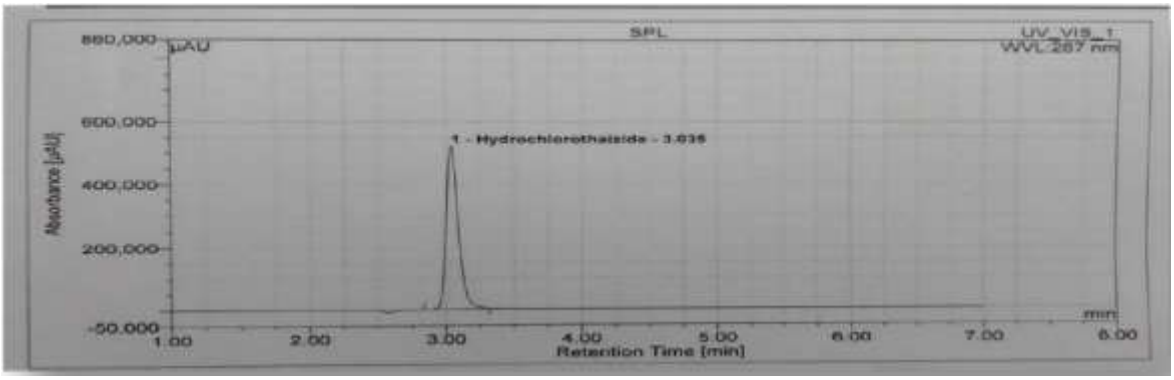


Std Inj No.	Retentiontime	Area	Theoreticalplates	Tailing factor
1	3.033	54227.888	5768	1.46
2	3.032	54569.469	5762	1.21
3	3.033	53923.506	5851	1.35
Average	0.030%	0.635%	0..756%	

A) PRESCISION

a) REPEATABILITY

The repeatability was demonstrated by preparing the standard solution at 40 ppm concentration and six independent consecutive sample preparation at 40 ppm. System suitability found within limit. Relative standard deviation of assay value for six preparations found within 2 %.



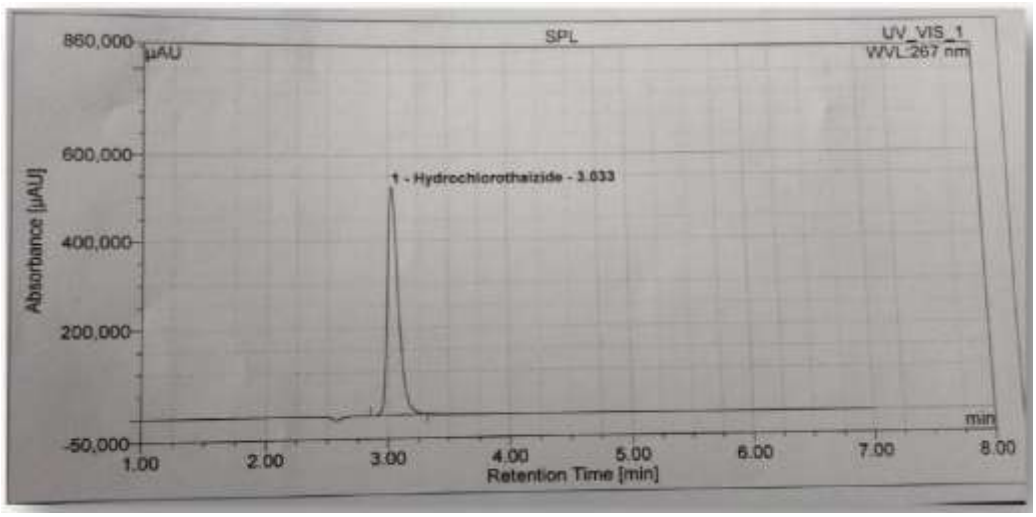
Sample	RT	Area	% Assay
1	3.035	53746.431	100.06
2	3.034	54163.654	100.93
3	3.035	53687.219	101.47
4	3.035	53839.548	100.10
5	3.035	53780.629	101.31

Sample	RT	Area	% Assay
1	3.035	53746.431	100.06
2	3.034	54163.654	100.93
3	3.035	53687.219	101.47
4	3.035	53839.548	100.10
5	3.035	53780.629	101.31

%RSD=0.08

Conclusion of repeatability- % RSD for repeatability found 0.08 which well within limit therefore method is repeatable**.**

B) INTERMEDIATE PRECISION: The Intermediate Precision was demonstrated by preparing the standard solution at 40 ppm concentration and six independent consecutive sample preparation at 40 ppm. by other person on other day with other set of chemicals. System suitability found within limit. Relative standard deviation of assay value for six preparations found within 2%. 19% Variation of average assay values obtained via repeatability andintermediate precision found within 3 %.



Std Inj No.	Retention time	Area	Theoretical plates	Tailing factor
1	3.033	53963.207	5914	1.43
2	3.034	54193.186	5915	1.44
3	3.035	53711.206	5942	1.43
4	3.035	53828.929	5963	1.43
5	3.033	53813.693	5914	1.44
6	3.034	53978.227	5958	1.44

Sample	RT	Area	% Assay
1	3.033	53963.207	100.98
2	3.034	54193.186	101.55
3	3.035	53711.206	100.45
4	3.035	53828.929	100.63
5	3.033	53813.693	100.77
6	3.034	53978.277	100.78

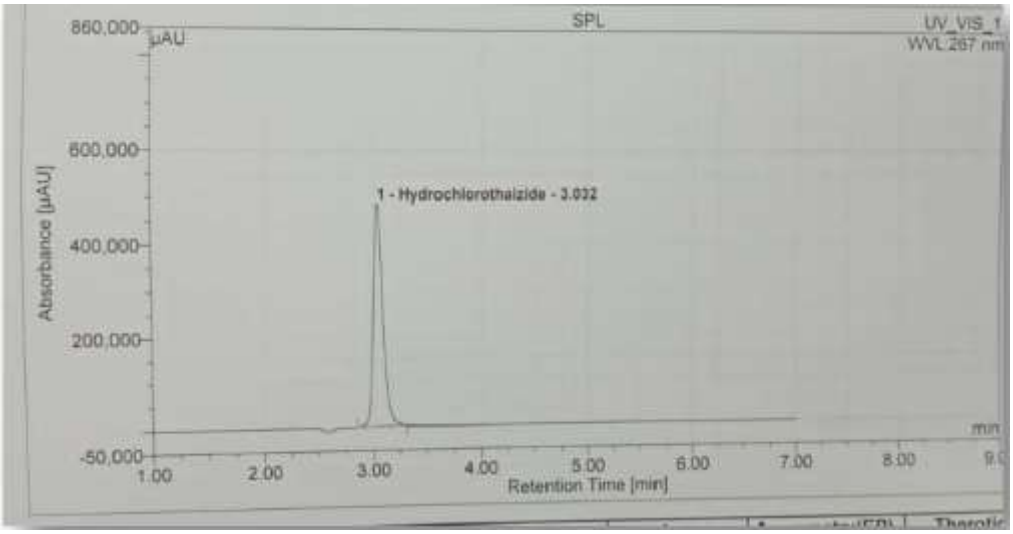
Conclusion of Intermediate precision

% RSD for 6 replicate independent analysis by changing various objects found 0.52 % whichwell within limit therefore method is intermediately reputable.

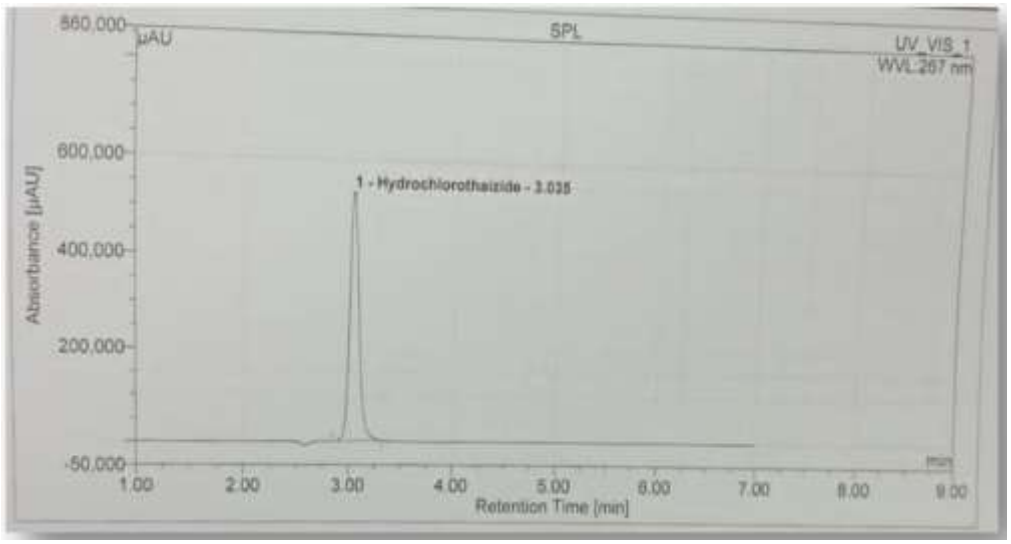
% RSD for 12 observations (6 of repeatability and 6 of intermediate precision) found 0.93 %which well within limit therefore Based on both experiment Method found Precise.

c) ACCURACY

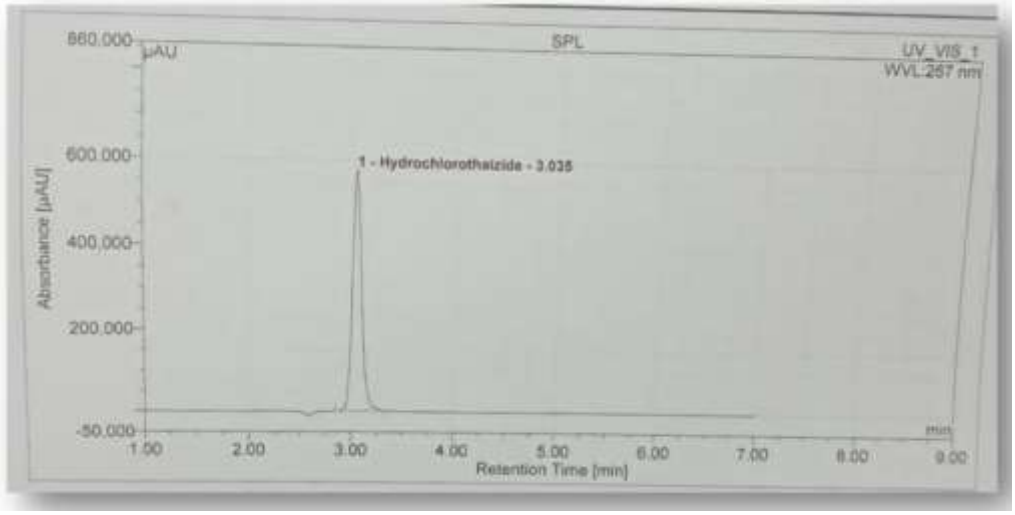
To determine the accuracy of the method, recovery studies were carried out in triplicate by using different concentrations of pure drug in the pre analyzed samples with 3 different concentrations of sample that consists of 80 %, 100 % and 120 % of the pure drug. The accuracy was expressed as the percentage analytes recovered.



ACCURACY SAMPLE 80%



ACCURACY SAMPLE 100%

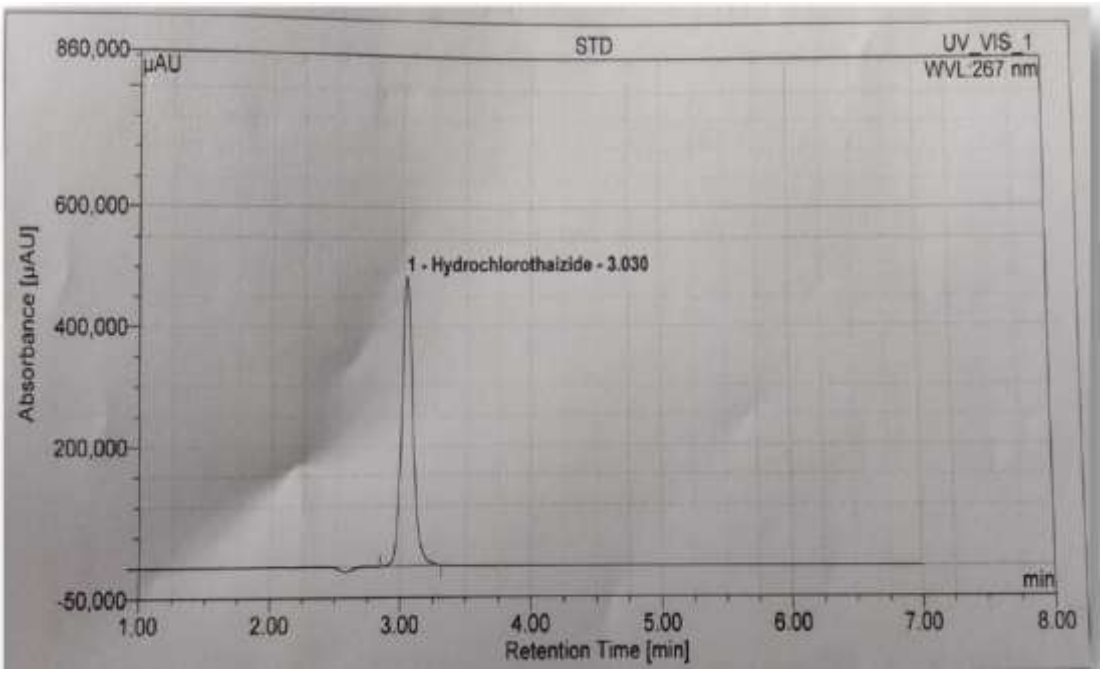


ACCURACY SAMPLE 120%

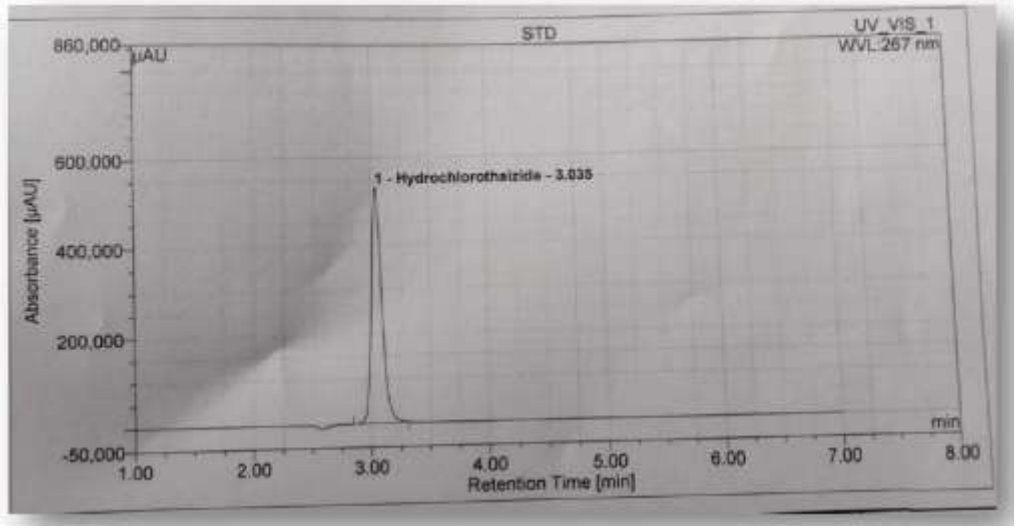
Sample	Area Hydrochlorothiazide	% Assay Hydrochlorothiazide
Spl-80%	49265.1883	91.62
Spl-80%	49568.1254	92.19
Spl-80%	49684.5478	92.40
Spl-100%	54435.4601	101.24
Spl-100%	54265.1254	100.92
Spl-100%	54821.5648	101.96
Spl-120%	59611.824	110.87
Spl-120%	59589.2546	110.83
Spl-120%	59354.2654	110.39

D) LINEARITY AND RANGE

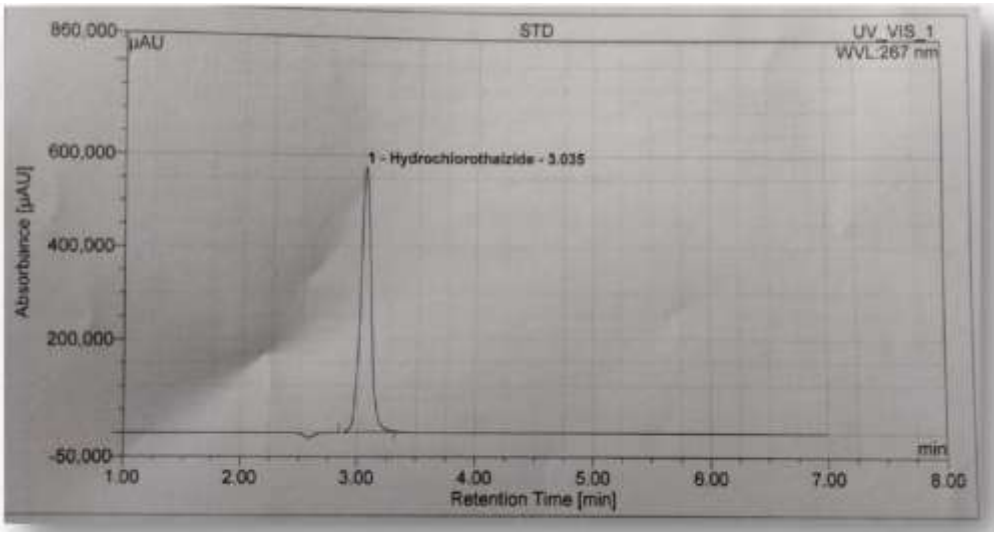
From the standard stock solution, the various dilutions of hydrochlorothiazide in the concentration of 160.0, 200.0, 240.0 ppm three level standard solutions of each were prepared. The solutions were injected using 20 µL injection volumes into the chromatographic system at the flow rate of 1.0 ml/min and the effluents were monitored at 267 nm, chromatograms were recorded. Calibration curve of hydrochlorothiazide was obtained by plotting the peak area ratio versus the applied concentrations of hydrochlorothiazide by using average of each sample. The linear correlation coefficient (R2) was found to be 1.000 & %y intercept is -0.0034 %.



LINEARITY STD 80%

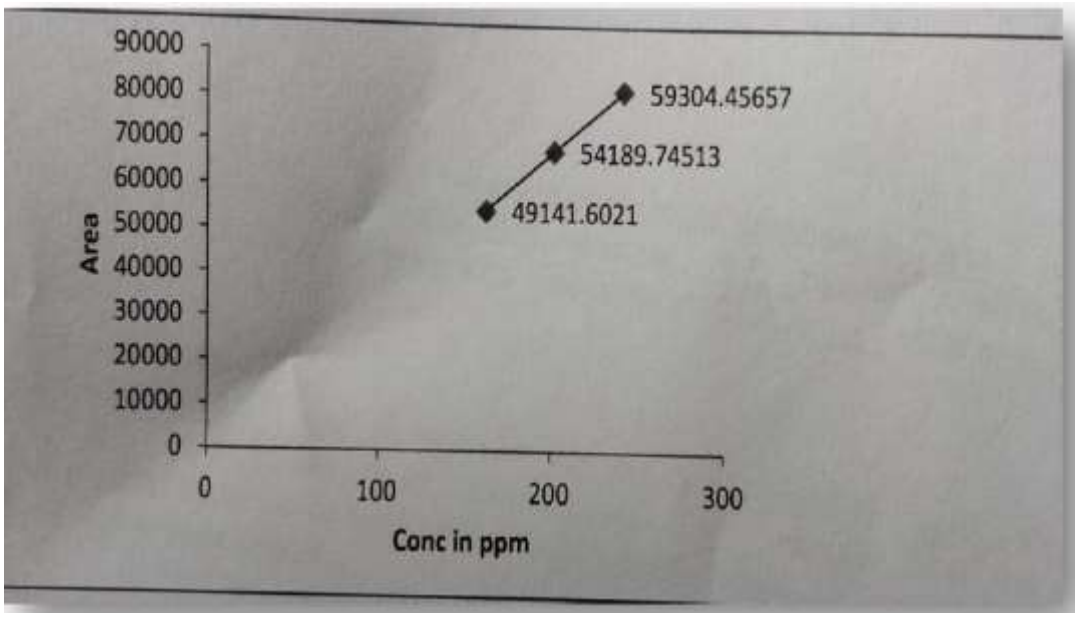


LINEARITY STD 100%



LINEARITY STD 120%

Sr No.	Conc.ppm	Area	Average
1	163.0	49034.360	49141.6021
2	163.0	49265.188	
3	163.0	49125.258	
4	201.00	54179.074	54189.74513
5	201.00	54265.154	
6	201.00	54125.007	
7	240.00	59332.009	59304.45657
8	240.00	59125.125	
9	240.00	59456.235	



Correlation-1.000

% Y intercept= -0.0035

Conclusion – Method found Linear within the range 80 % to 120 % of working level.

E) LIMIT OF DETECTION AND LIMIT OF QUANTITATION(LOD & LOQ)

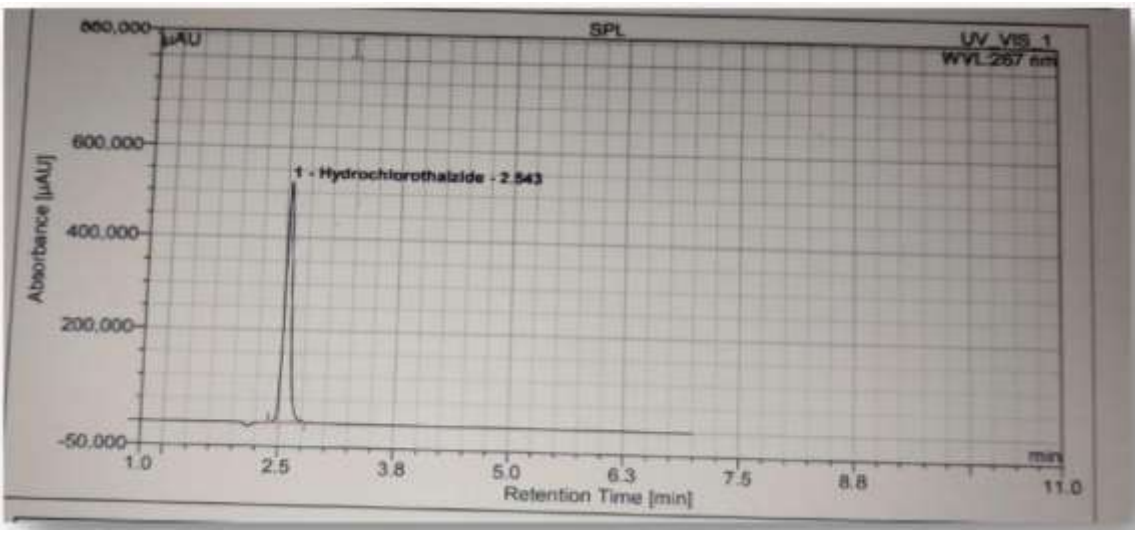
The limit of detection and limit of quantification means the lowest concentration of analytes in the sample are detected and quantified. LOD and LOQ was found as listed below

Table of Limit of Detection & Limit of Quantitation.

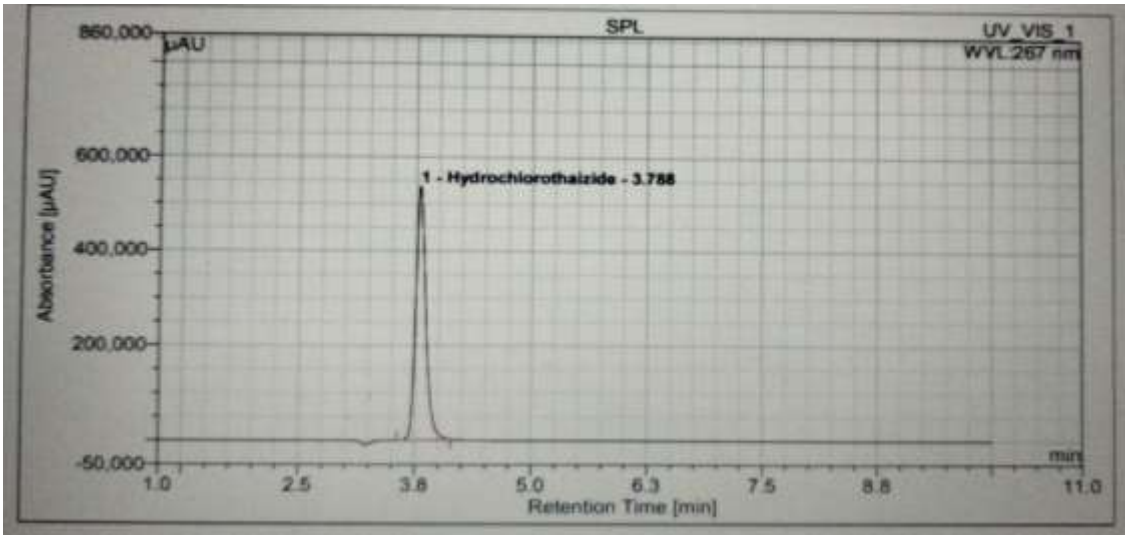
Parameter	Obtained value
LOD	7.1 ppm
LOQ	25 ppm

F) ROBUSTNESS

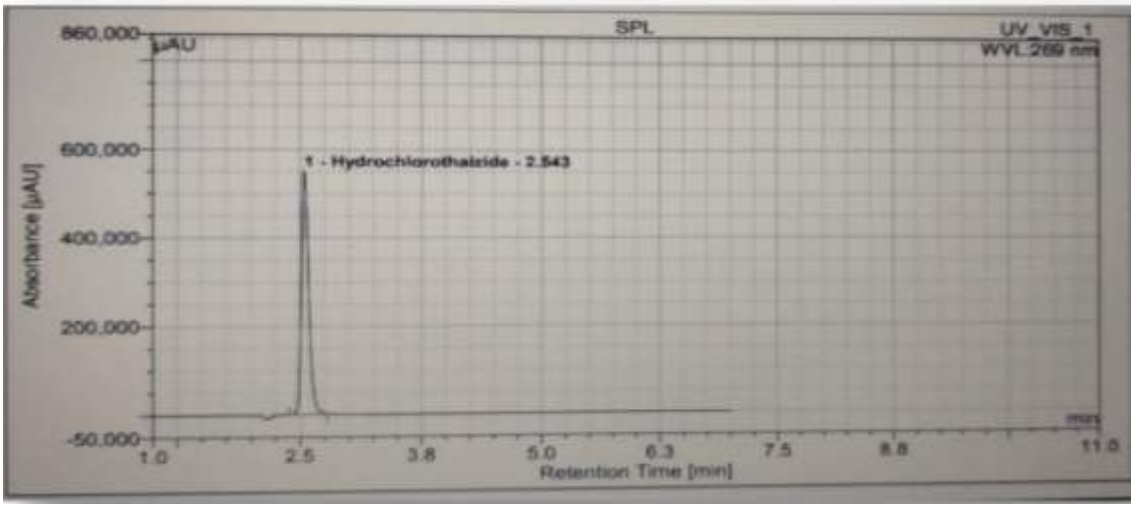
Robustness of the method was determined by intentionally changing some operating conditions such as flow rate and wavelength. The flow rate as per the developed method is 1.0 ml / min. It has been purposely changed to 0.8 ml/min and 1.2 ml/ min and the chromatogram was developed as well as the wavelength of developed method is 267 nm. It has been purposely changed to 265 nm and 269 nm and the chromatogram was developed.



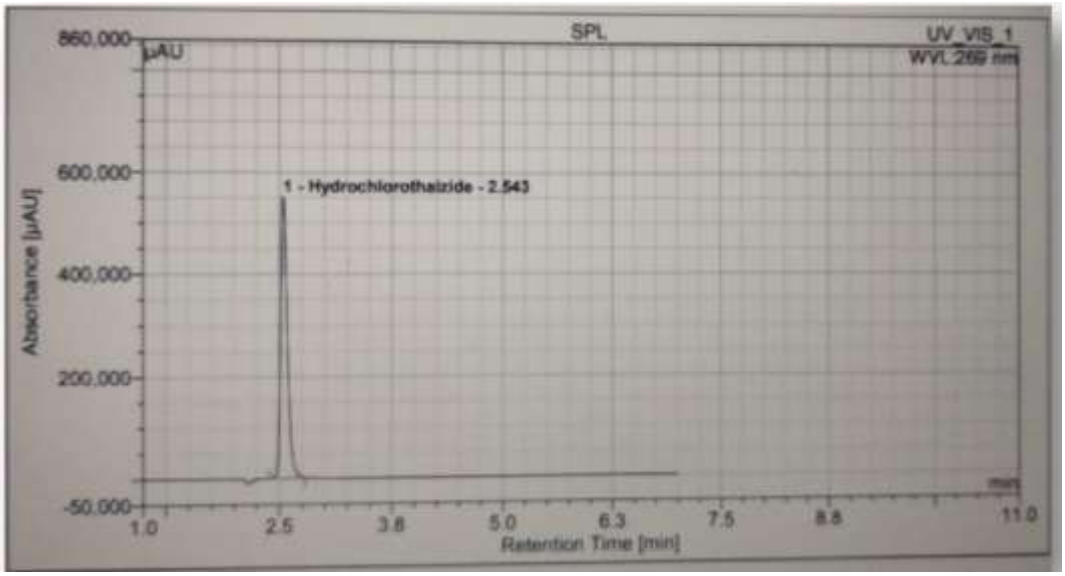
SPL 1.2ml



SPL 0.8ml



SPL Wavelength =265nm



SPL Wavelength=269nm

Parameter	% Assay	Cumulative % RSD with repeatability
	Hydrochlorothiazide	Hydrochlorothiazide
Change in flow rate 0.8ml	100.46	0.53
Change in flow rate 1.2ml	100.48	0.53
Change in wavelength256nm	100.63	0.52
Change in wavelength269nm	100.43	0.53

Conclusion – Method found Robust.

RESULT AND DISCUSSION

This developed and validated HPLC assay method of hydrochlorothiazide is reliable and economical. Using Phosphate buffer (pH 6.0): Acetonitrile (60:40) as a Mobile phase and 1.0 mL/min flow rate at room temperature, chromatographic separation was obtained ona (4.6 x 150 mm 3.5 um Agilent Eclips C18) C18 column. The injection Volume is 20 microliter, column temperature 40^0 C and the run time is 7 minutes. The wavelength of detection is set to 317 nm. The linear correlation coefficient (R2) was found to be 1.000, with a 0.0035 percent y intercept. The detection limit was determined to be 0.0056 ppm while the quantification limit was found to be 0.018 ppm. hydrocholothiazide was proven to be 99.85 % pure. The requirements for repeatability and precision have been met. For the determination of hydrochlorothiazide in pharmaceutical dosage forms, the approach is simple, specific, precise, durable, and accurate (tablets).

CONCLUSION

This analytical method used for determination of assay of hydrochlorothiazide from drug formulations like tablet. This HPLC method shows all results within acceptance criteria for the analytical parameters such as Specificity and system suitability, Linearity and Range, Precision, Accuracy and Robustness. Hence method stands validated and can be used for assay analysis of hydrochlothiazide from drug formulations. I suggest you can also try this method for bio-analysis of hydrochlorthiazide.

ACKNOWLEDGEMENT

At the very outset, I fail to find adequate words, with limited vocabulary at my command, to express my emotions to “Dear God”, & Chatrapati Shivaji Maharaj whose eternal blessings, divine presence, and masterly guidance helps me to fulfill all my goals.

It’s not easy to express my emotions in words especially when I have to say thanks to my guide Prof. Avinash M. Bhagwat (Asst. Prof. Dept. of Pharmaceutical Chemistry Yspm,s YTC, Satara), Dr. Ajit Akal, and Sonali T.Dhumal (Managing Director and Assistance, Insta Vision Laboratory, Satara)for his inspiring guidance, affectionate encouragement, and never-ending enthusiasm; without which this research work would not have seen the light of the day.

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Last but not least I am humbly grateful to all those people who directly or indirectly played the role of a catalyst to bring out the lovely reaction of this research.

Thank you to all for Your Help!

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