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METHOD DEVELOPMENT, VALIDATION AND STABILITY INDICATING STUDIES OF HYDROCHLOROTHIAZIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV-SPECTROSCOPY

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Keywords:

Hydrochlorothiazide (HTZ), Area under curve, Q-absorbance ratio method, Stability studies

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ABSTRACT: Development of UV spectrophotometric method for the estimation of Hydrochlorothiazide was done by Q-Absorbance ratio method and area under curve method and stability indicating studies using methanol as solvent. In the present research, we have made an attempt to develop a simple, specific, accurate, precise and reproducible method for the estimation of hydrochlorothiazide by UV spectrophotometric method, the method includes area under curve method (Method I) and Q- absorbance Ratio method (Method II). The wavelength is 272 nm λ_{max} of drug was selected for Method I, and for Q-absorbance Ratio method (Method II) 258 nm an iso-absorptive wavelength and 272 nm were selected for estimation of Hydrochlorothiazide and the drug follow Beer's law over the concentration range of 1-6 µg/ml. The % recovery of the drug was found to be nearly 100 % representing the accuracy of the proposed methods. LOD and LOQ values of hydrochlorothiazide were Found to be 0.136, 0.411, 0.181, 0.556, 0.134, 0.404 at 272 nm, 258 nm and 242 nm respectively, validation of the proposed methods was carried out for its precision, accuracy, specificity and ruggedness according to ICH guidelines. The present validated method was successfully applied for determination of hydrochlorothiazide in bulk and pharmaceutical dosage form.

INTRODUCTION: Hydrochlorothiazide was a first line diuretic drug. Chemically: 6-Chloro-1,1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide. It belongs to thiazides class of diuritics, it reduces blood volume by acting on kidneys to reduce sodium reabsorption in the distal convoluted tubule. Thiazides increase the reabsorption of calcium. It was suppose to lower peripheral vascular resistance.

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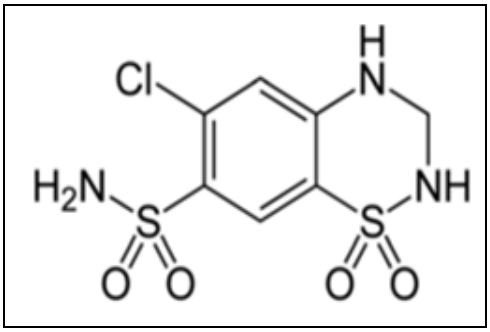

FIG. 1: HYDROCHLOROTHIAZIDE (HTZ)

MATERIALS AND METHODS [1-7]:

Chemicals and Reagents: Hydrochlorothiazide procured from the KP laboratories, commercial pharmaceutical preparation Hydrochlorothiazide, manufactured by INTA pharmaceuticals, 20mg of Hydrochlorothiazide was collected from local

market, methanol analytical grade was procured from Quietens India Pvt Ltd.

Instrumentation: The proposed method was carried on a shimadzu UV-Visible Spectro-photometer (UV-1800 series). All the products were weighed on digital balance (Shimadzu), a fast clean ultra sonicator was used for degassing the solvent.

Selection of Solvents: On the basis of the solubility studies methanol was selected as solvent for method development.

UV-SPECTROSCOPY:

Preparation of Standard Solutions: Weigh accurately 10mg of Hydrochlorothiazide into a 100ml volumetric flask, add 10ml of solvent and shake well to dissolve the drug completely. Make up the volume to 100ml with solvent to get 100µg/ml of Hydrochlorothiazide.

Preparation of Sample Solution: 20 Tablets were taken, crushed into fine powder. Accurately weighed powder sample equivalent to 10mg of Hydrochlorothiazide powder and transferred to 100ml volumetric flask, dissolved in sufficient solvent and filtered through Whatman filter paper. The filtrate was made up to volume of 100ml with solvent get 100µg/ml of Hydrochlorothiazide.

Determination of λ_{max}: Standard solutions of Hydrochlorothiazide was prepared and scanned in UV- spectrophotometer in the range of 200-400nm to determine the λ_{max}. The λ_{max} of Hydrochlorothiazide found to be 272nm.

Method Development [8-12]:

Q-Absorbance Ratio Method: According to Q-absorption ratio method, at selected wavelength was used for the ratio of absorption. One was at iso-absorptive point and other one was at the λmax, the concentrations were calculated by using the equation.

$$C_x = \{(Q_m-Q_y)/ (Q_x-Q_y)\}* (A_1/ax_1)$$

$$C_y = \{(Q_m-Q_x)/ (Q_y-Q_x)\}* (A_1/ay_1)$$

Area Under the Curve Method: Hydrochlorothiazide was scanned between 200-400nm and found 272nm as λmax for estimation using area under curve method. Aliquotes of 1-6 µg/ml solutions was prepared using methanol as solvent and measured absorbance of drugs at λmax.

$$C^M = X^N_{\lambda1-\lambda2} AUC_{\lambda3-\lambda4} - X^N_{\lambda3-\lambda4} AUC_{\lambda1-\lambda2} / X^N_{\lambda1-\lambda2} = X^M_{\lambda3-\lambda4} - X^N_{\lambda3-\lambda4} X^M_{\lambda1-\lambda2}$$

$$C^N = X^M_{\lambda1-\lambda2} AUC_{\lambda3-\lambda4} - X^M_{\lambda3-\lambda4} AUC_{\lambda1-\lambda2} / X^N_{\lambda1-\lambda2} = X^M_{\lambda3-\lambda4} - X^N_{\lambda3-\lambda4} X^M_{\lambda1-\lambda2}$$

Validation of the Method [13-14]: Validation was done by UV-VIS Spectroscopic method according to International Conference on Harmonization (ICH) guidelines. Different parameters were studied for validation: they are linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ).

Linearity: The method was validated according to International conference on Harmonization guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte. Calibration curve was generated with appropriate volume of working standard solution for UV and with the range of 1-5 respectively. The linearity was determined by using unweighted data in the least square regression method.

Accuracy and Precision: The precision of the product was validated by intermediate precision (inter-day) and repeatability (intra-day) and reported as %RSD for a statistically remarkable number of replicate measurements. The intermediate precision was carried out by comparing the assay in three different days and the results were reported as standard deviation and %RSD. Accuracy was the percent of analyte recovered from assay by addition known amount, for the measurement of accuracy data from nine determinations over three concentration levels covering the specified range were validated.

Robustness: Robustness of the method was validated by making minute changes in the chromatographic conditions, such as composition mobile phase ratio, flow rate and wavelength.

LOD and LOQ: Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentration of Hydrochlorothiazide. The LOD and LOQ values were calculated by using the following formula:

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10\times \sigma/S$$

Where σ = the standard deviation of the response, S = Slope of calibration curve.

RESULTS AND DISCUSSION:

TABLE 1: Q-ABSORBANCE RATIO METHOD VALUES OF HTZ

Concentration (µg/mL)	HTZ 258nm	HTZ 272nm
1	0.16	0.18
2	0.261	0.349
3	0.365	0.498
4	0.481	0.657
5	0.589	0.798

TABLE 2: AREA UNDER CURVE OF HTZ

Concentration(µg/mL)	HTZ 272nm
1	0.07873
2	0.16242
3	0.26275
4	0.31423
5	0.39782
6	0.465434
Mean	0.2401
SD	0.1697

Linearity: A series of solutions in the concentration range of 1-6µg/mL of Hydrochlorothiazide from the stock solutions were prepared. These solutions were scanned in the range of 200-400 nm and the absorbance was noted at the λ_{max} of 272nm for HTZ.

TABLE 3: LINEARITY OF HTZ

Concentration(µg/ml)	Absorbance
1	0.07873
2	0.16242
3	0.26275
4	0.31423
5	0.39782
6	0.465434

TABLE 4: INTRADAY PRECISION

Concentration	272nm	258nm	242nm
3	0.473	0.476	0.386
3	0.472	0.480	0.393
3	0.471	0.487	0.396
3	0.470	0.484	0.401
3	0.573	0.485	0.402
Mean	0.472	0.485	0.399
SD	0.0012	0.0059	0.0048
%RSD	0.2472	1.1906	1.2656

TABLE 5: INTER DAY PRECISION

Concentration	272nm	258nm	242nm
3	0.473	0.473	0.386
3	0.472	0.474	0.387
3	0.471	0.475	0.388
3	0.473	0.476	0.389
3	0.472	0.477	0.402
Mean	0.473	0.475	0.390
SD	0.0010	0.0014	0.059
%RSD	0.1925	0.1935	0.1574

TABLE 6: ROBUSTNESS OF HTZ

Drug	Changes in wavelengths (±1nm)	Absorbance
HTZ	273	0.07764
	274	0.07765
	275	0.07766
	276	0.07767
	277	0.07768

TABLE 7: LOD AND LOQ OF HTZ

Parameter	Hydrochlorothiazide		
	Methods -A		Method -B
	272nm	242nm	258nm
LOD	0.136	0.134	0.181
LOQ	0.411	0.404	0.556

TABLE 8: ACCURACY OF HTZ

Methods	Amount taken	Amount found	%Recovery
Method A	50	0.141	99.6
	100	0.149	99.8
	150	0.231	100.1

Method B	50	0.146	99.5
	100	0.147	100.2
	150	0.236	100.3

TABLE 9: FORCED DEGRADATION STUDIES

Stress degradation condition	Area under curve	% Degradation	Active drug process after degradation (%)
Standard drug	3.867	0	100
Acid Degradation	1.724	64.26732675	57.73267326
Base degradation	0.833	79.9669968	30.0330034
Oxidative degradation	0.251	61.50825084	44.49174916
Photo stability degradation	0.261	85.80858087	14.19141913

CONCLUSION: The proposed UV Spectrophotometric methods are simple, fast, sensitive, accurate, precise, less time-consuming and economic. All the parameters were observed within the limits, validation of the proposed methods was carried out for its accuracy, precision, specificity and ruggedness according to ICH guidelines.

The stability studies have been developed for the estimation of Hydrochlorothiazide. The use of this method has proved to be a smart strategy to provide both environmental and economic benefits. The present validated method was successfully applied for determination of hydrochlorothiazide in bulk and pharmaceutical dosage form.

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CONFLICT OF INTERESTS: The authors declare that there exist no conflicts of interests regarding the publication of this manuscript.

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