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SIMULTANEOUS ESTIMATION OF VALSARTAN AND HYDROCHLOROTHIAZIDE IN FIXED DOSE COMBINATION IN UV SPECTROPHOTOMETRY

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ABSTRACT

Valsartan (VAL) and Hydrochlorothiazide (HTZ) are used in combination in treatment of Hypertension. Two simple, accurate, precise, economical and reproducible UV spectrophotometric methods have been developed for the estimation of Valsartan and Hydrochlorothiazide in Pharmaceutical formulation. Method I- Absorption ratio method (Q-analysis) using two wavelengths, 265nm (isobestic point at which both the drugs exhibit absorbance) 249nm (λ_{max} of Valsartan) and Method II- Area under Curve method. For the second method Area under the Curve in the range of 249 - 259nm and 261-281nm was selected for the analysis of Valsartan and Hydrochlorothiazide respectively. Linearity for detector response was observed in the concentration range of 2-24μg/ml & 2-14μg/ml for Valsartan and Hydrochlorothiazide respectively. The results of analysis have been validated statistically and by recovery studies the value of standard deviation was satisfactory and recovery studies ranging from 99.54 - 99.97 % for Valsartan and 99.75 - 101.04 % for Hydrochlorothiazide were indicative of the accuracy and precision of the proposed method The proposed methods were successfully applied for the determination of Valsartan and Hydrochlorothiazide in commercial pharmaceutical preparation. All two methods were validated statistically as per ICH guidelines.

INTRODUCTION: Valsartan (VAL) is chemically (S)-N-(1-Oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl]-4-yl]methyl]-L-valine, is an orally active specific angiotensin II receptor blocker effective in lowering blood pressure in hypertensive patients [1].

Hydrochlorothiazide (HTZ) is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs, which decreases active sodium reabsorption and reduces peripheral vascular resistance [2]. It is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide-1, 1-dioxide.

VAL and HTZ are official in USP-NF2007 [6, 8] and IP 2007 [3], BP 2004 [5], respectively. Extensive literature survey revealed that few UV spectrophotometric methods [9-11], RP-HPLC methods [12-17] and HPTLC methods [18-21] has been reported. Single drug estimation of VAL by RP-HPLC method has been reported [22-30].

Since, no spectrophotometric method is reported for simultaneous estimation of VAL and HTZ in combination by these two methods therefore, the present work describes successful attempt to estimate both these drugs simultaneously by two simple UV spectrophotometric methods (Q-Analysis method and

Area under curve method). The proposed methods were optimized and validated as per ICH guidelines [7].

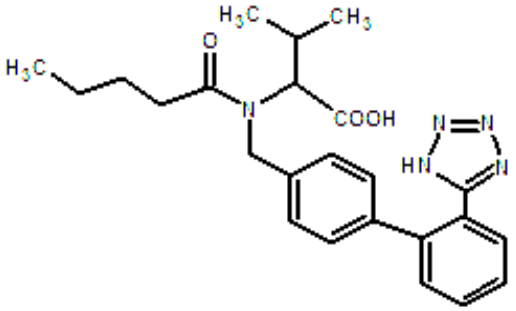

FIG. 1: STRUCTURE OF VAL

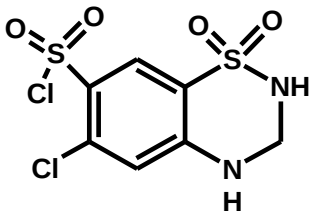

FIG. 2: STRUCTURE OF HTZ

MATERIAL AND METHODS: Instrumentation: For the present study JASCO double beam UV/Visible spectrophotometer (Model V-530) was used with slit width fixed at 2nm, equipped with spectra manager software (Version 1.5). Pair of 1-cm matched quartz cells were used to measure the absorbance of solution. The samples were weighed on electronic analytical balance (Contech Model CB-50) (**Table 1**).

TABLE 1: INSTRUMENTS

Name	Model	Manufacturer
UV-Visible spectrophotometer	V-530	Jasco
Electronic analytical balance	CB-50	Contech

Materials: VAL and HTZ drug samples were kindly supplied as gift samples by Lupin Pharmaceuticals, J & K India. The Pharmaceutical dosage form used in the present study was Valent –H tablets (Lupin Pharmaceuticals Ltd).Assay was carried out on tablets containing label claim, each film coated tablet contains 12.5mg of HTZ and 80 mg of VAL.

Preparation of Stock Solutions: Accurately weighed 10 mg of VAL & 10 mg of HTZ was transferred to 100ml of volumetric flask and was dissolved separately in 0.1 M NaOH, sonicated for 3 mins, to give the standard stock solution of 100µg/ml respectively. Aliquots were prepared by using 0.1 M NaOH in the increasing concentration range.

Experimental methods:

Method I -

Absorption ratio/Q-analysis method: In this method absorbances are measured at two wavelengths, one being the λmax of Valsartan and other being a wavelength of absorptivity of the Hydrochlorothiazide. Then absorbance of both drugs was recorded on selected wavelengths. Concentrations of Valsartan & Hydrochlorothiazide were calculated by using following equations.

$$C_{VAL} = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_1}{a_{X1}} \quad \text{---------- (1)}$$

$$C_{HTZ} = \frac{Q_M - Q_X}{Q_Y - Q_X} \times \frac{A_2}{a_{X1}} \quad \text{---------- (2)}$$

Where, Q_M is ratio of absorbances A_1 and A_2 of mixture at λ_1 and λ_2 (isobestic point wavelength) Q_X is ratio of absorptivities ax_1 and ax_2 at λ_1 and λ_2. Q_Y is ratio of absorptivities ay_1 and ay_2 at λ_1 and λ_2. C_{VAL} and C_{HTZ} are concentrations of Valsartan & Hydrochloro-thiazide respectively.

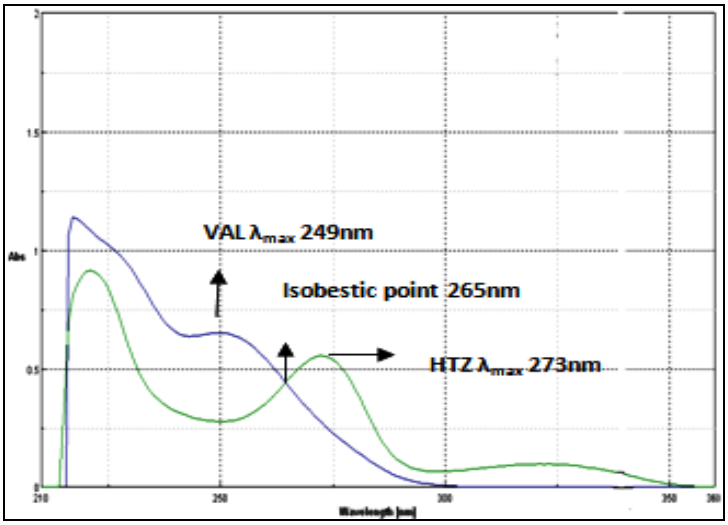

FIG. 3: OVERLAIN SPECTRA OF VAL & HTZ SHOWING ISOBESTIC POINT

METHOD II -

Simultaneous equation using area under the curve method: For the selection of analytical wavelength standard solutions of VAL & HTZ were prepared and series of dilutions of standard solutions of VAL and HTZ were prepared by using 0.1N NaOH and were scanned from 400 to 200 nm.

From the spectra of drug obtained after scanning of standard solution of VAL and HTZ, area under the curve in the range of 249-259nm and 261-281nm was selected for the analysis respectively. After optimization, the concentration of 12.8 µg/ml of VAL as standard 1 and 2 µg/ml of HTZ as standard 2 were found to give best results. The absorbance of each resulting solution was measured at 249-259 nm and 261-281 nm. The absorptivity values calculated. The calibration curve was plotted with concentration v/s area under the curve and regression equation was calculated.

Determination of Absorptivity values: (Table 2)

$$\text{Absorptivity} = \frac{\text{Absorbance}}{\text{Concentration of that component in gm/l}}$$

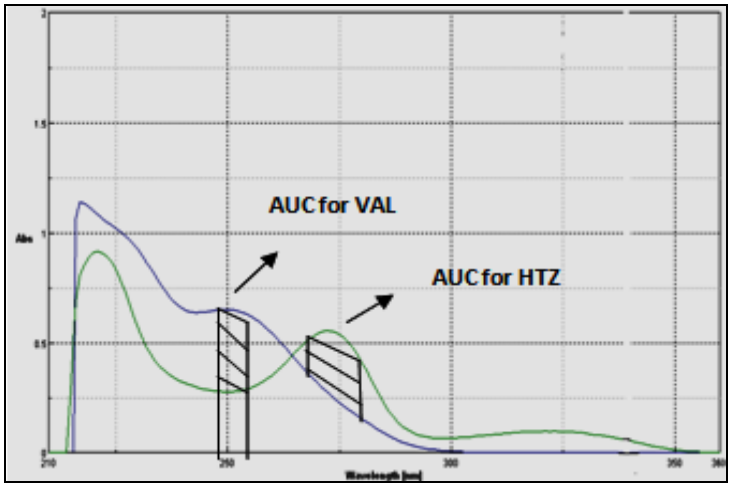

FIG. 4: OVERLAIN SPECTRA OF VAL & HTZ SHOWING AREA UNDER THE CURVE

Concentration of VAL and HTZ was calculated using following formula;

$$C_{VAL} = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1ay_2}$$

$$C_{HTZ} = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2}$$

Where,

C_{VAL} = Concentrations of VAL,

C_{HTZ} = Concentrations of HTZ,

A_1 = Area at 239-249 nm,

A_2 = Area at 261-281 nm,

ax_1 = Absorptivity value of HTZ at 239-249 nm,

ax_2 = Absorptivity value of HTZ at 261-281 nm,

ay_1 = Absorptivity value of VAL at 239-249 nm,

ay_2 = Absorptivity value of VAL at 261-281 nm.

TABLE 2: ABSORPTIVITY VALUES OF VAL AND HTZ

Drug	Absorptivity at 249-259nm	Absorptivity at 261-281nm
VAL	0.043(ax_1)	0.036(ax_2)
HTZ	0.080(ay_1)	0.0510(ay_2)

Analysis of Tablet Formulation: For the estimation of drugs in the commercial formulations, twenty tablets containing 80 mg of VAL and 12.5 mg of HTZ were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drugs, quantity of powder equivalent to 1.28 mg of VAL and 0.2 mg of HTZ was transferred to 100 ml volumetric flasks and dissolved in sufficient quantity of 0.1N NaOH. It was sonicated for 30mins and volume was made up to obtain a stock solution of 12.8µg/ml of VAL (maintaining 2 µg/ml of HTZ).

This solution was then filtered through Whatmann filter paper # 42. Further dilutions were made from this stock solution to get required concentration. The concentration of both VAL and HTZ was determined by measuring absorbances of sample solutions in wavelength range of 249nm-259nm (for VAL) and 261nm-281nm (for HTZ) using equation 3 and 4 Results of tablet analysis are shown in **Table 3**. The assay procedure was repeated six times (n=6).

TABLE 3: RESULT OF MARKETED FORMULATION ANALYSIS

Method	Drug	Amt of drug taken (µg/ml)	% of drug estimated*	%RSD (±SD)
Q- analysis	VAL	12.8	99.89±0.75	0.840
Area under curve	VAL	12.8	99.44±0.55	0.155
Q- analysis	HTZ	2	99.94±0.66	0.531
Area under curve	HTZ	2	101.40±0.87	0.991

*Average of six determinations

Validation: The method was validated according to ICH guidelines to study linearity, accuracy, precision, LOD and LOQ.

Linearity: The measurement of linearity was evaluated by analyzing different concentrations of the standard solution of VAL and HTZ. For both the methods, the Beer law was obeyed in the concentration range 2-24 µg/ml and 2-14 µg/ml for VAL and HTZ respectively. The Absorbance was plotted against the corresponding concentrations to obtain the calibration graphs.

Accuracy (Recovery studies): To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery was calculated for VAL and HTZ, by both the methods (**Table 4**).

TABLE 4: RESULTS OF RECOVERY STUDIES

Recovery level	Drug	Conc. of drug Level taken in µg/m	Conc. of drug Level taken in µg/ml	Method I			Method II		
				% Recovery	SD	%RSD	% Recovery	SD	%RSD
80	VAL	9.6	7.68	99.54	0.054	0.624	99.23	0.066	0.720
100	VAL	9.6	9.6	99.84	0.069	0.753	100.02	0.390	0.570
120	VAL	9.6	11.52	99.97	0.089	0.982	100.08	0.240	0.333
80	HTZ	1.5	1.2	99.75	0.042	0.568	99.84	0.026	0.321
100	HTZ	1.5	1.5	99.97	0.075	0.510	99.92	0.042	0.630
120	HTZ	1.5	1.8	101.04	0.083	0.960	100.33	0.681	0.951

Precision: The reproducibility of the proposed methods was determined by performing tablet assay at different time intervals on same day (Intra-day precision) and on three different days (Inter-day precision) (**table 5**).

Limit of Detection and Limit of Quantitation: The LOD and LOQ were separately determined based on calibration curve. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines were used to calculate the LOD and LOQ (**table 5**).

The detection limit (LOD) may be expressed as:

$LOD = 3.3\ \sigma/S$

The quantitation limit (LOQ) may be expressed as:

$LOQ = 10\ \sigma/S$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

TABLE 5: RESULTS OF VALIDATION PARAMETERS

Parameter	Method I		Method II	
	VAL	HTZ	VAL	HTZ
Linearity (µg/ml)	2-24	2-14	2-24	2-14
Correlation coefficient (r^2)	0.9992	0.9998	0.9990	0.9995
Interday precision	0.63	0.75	0.19	1.48
Intraday precision	0.42	0.39	0.33	1.59
LOD (µg/ml)	0.0024	0.033	0.0013	0.040
LOQ (µg/ml	0.0063	0.039	0.0054	0.0164

RESULTS AND DISCUSSION: The present work provides an accurate, reproducible, sensitive method for the simultaneous analysis of VAL & HTZ in bulk and tablet formulation. Linear relationships between drug concentrations were obtained over the range of at 2-24 µg/ml & 2-14 µg/ml for VAL and HTZ respectively. Under experimental conditions described assay of tablet, linearity, accuracy studies and precision, LOD and LOQ were estimated. Correlation coefficient was found to be > 0.998.

The results of commercial tablet formulation are presented in Table 3. Results of accuracy studies are presented in Table 4. The % assay was found to be 99.44 - 99.89% for VAL and 99.94-101.40 for HTZ, S.D. and R.S.D. for six determinations of tablet sample, by both the methods, was found to be less than 2.0 indicating the precision of both the methods. No interference was observed from the pharmaceutical adjuvants.

CONCLUSION: The two spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of Valsartan and Hydrochlorothiazide in bulk and formulation.

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