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Validated Stability Indicating Spectroscopic Method for Estimation of Degradation Behaviour of Valsartan and Hydrochlorothiazide in Tablet Formulation

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ABSTRACT:
Purpose: A simple, accurate and preciseUV spectrometric method has been developed for the simultaneous determination of valsartan and hydrochlorothiazide in tablet dosage form. ***Methods***: The employed was simultaneous determination of the valsartanandhydrochlorothiazide in tablet dosage form. Spectra of valsartan and hydrochlorothiazide in methanol and water (50:50) show λ max at 250.0 nm and 271.4 nm respectively. Valsartan and hydrochlorothiazide are subjected to various stress conditions like acid, alkali, thermal and photolytic degradation. ***Results***: Beer's law obeyed in concentration range of 4-24µg/ml for VAL and 0.5-3 µg/ml for HCT at their respective wavelengths. The proposed method was successfully applied to tablet dosage form for determination of both drugs. The percentage recovery of valsartan and hydrochlorothiazide were found to be 100.19% and 99.51% respectively. ***Conclusion***: A novel accurate and precise stability indicating spectroscopic method has been developed for estimation of valsartan and hydrochlorothiazide.

Keywords: Valsartan, Hydrochlorothiazide, Stability, Spectrophotometric method, ICH.

INTRODUCTION:

Chemicallyvalsartan is N-(1-oxopentyl)-N-[[2 '-(1H-tetrazol-5-yl)[1,1- biphenyl]-4-yl] methyl]-L-Valine1(Figure 1).Valsartan is an angiotensin II receptor blocker it is used to treat a variety of cardiac diseases such as, hypertension, diabetic nephropathy and heart failure. Valsartan lowers blood pressure by competing angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure decreasing effects of angiotensin II.[1-6]Chemically Hydrochlorothiazide is 6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide (Figure 2). Hydrochlorothiazide is a thiazide diuretic decreases the reabsorption of electrolytes from the renal tubules by inhibiting the

sodium-chloride symporter in the distal convoluted tubule (DCT) and finally decreases the osmotic gradient and water reabsorption throughout the nephron. It increases excretion of water and electrolytestogether with metallic element, potassium, chloride, and metals. It has been used in the treatment of many disorders as well as swelling, hypertension, diabetes insipidus, and hyperparathyroidism.[7-9]

OnlyUV spectrometric[8],RP-HPLC[12] method has been found to be reported for the simultaneous determination of valsartan and hydrochlorothiazide in tablet dosage formand no stability indicating spectroscopic method has been developed for estimation of valsartan and hydrochlorothiazide. Therefore, the objective of present work is to develop a simple, accurate and precise stability indicating UV spectrometric method for the simultaneous determination of valsartan and hydrochlorothiazide in tablet dosage form.

MATERIALS AND METHODS

2.1 Reagents and Chemicals:

Methanol AR grade was obtained from S. D. Fine-chemicals Ltd. (India). Standard bulk sample ofvalsartan and hydrochlorothiazide were obtained as a gift sample from Umedica Lab Pvt. Ltd. Mumbai, India. Marketed formulation Valent-H tablet containing HCT 12.5 mg and VAL 80 mg was purchased from local market.

2.2 Instruments:

A Shimadzu model UV-1800 double beam UV-Visible spectrophotometer attached with computer operated software UV probe 2.0 with spectral width of 2 nm, with a pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Analytical weighing balance (AA-2200) and ultrasonic bath (HMG India: CD-4820) were used during the study.

2.3 Preparation of stock standard solution:

Accurately weighed quantity of about 10 mg of bothdrugswas dissolved in methanol: water (50:50) and diluted to 100 ml to obtain 100 µg /ml and further diluted to obtain 10 µg /ml of both drug.

2.4 Selection of Analytical Wavelengths:

Appropriate dilutions were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200nm.Valsartan and Hydrochlorothiazide showed absorbance maxima at 250 nm and at 271.4 nmrespectively (Figure 3).

2.5Estimation of VAL and HCT in their Combined Tablet Dosage Form:

Twenty tablets were weighed accurately; the average weight was determined and then triturated to a fine powder. Powder equivalent to 80 mg of valsartanand 12.5 mg of Hydrochlorothiazide was weighed and transferred to a 100 ml volumetric flask that of methanol: water (50:50 v/v) was added and sonicated for 20 min to dissolve the active ingredients. Volumewas made up to 100 ml with methanol: water (50:50 v/v)and filtered through What man filter paper no. 41 to give the stock solution containing 800µg/ml of VAL and 125 µg/ml of HCT. From this sample solution, by further dilution technique 16 µg/ml and 2.5 µg/ml concentrations of valsartan and hydrochlorothiazide obtained respectively. Concentrations of valsartan and hydrochlorothiazide in the tablet formulation were calculated using equation (3) and (4).The analysis procedure was repeated six times. The results of marketed tablet formulation are given in Table 1. Two equations are made primarily based upon the actual fact that at λ1 and λ2 the absorbance of the mixture is that the total of the individual absorbance of X and Y.

At λ1, $A1 = ax1bcx + ay1bcy$…… (1)

At λ2, $A2 = ax2bcx + ay2bcy$……. (2)

For the measurements in 1 cm cells, b =1

Rearranging above equations,

$$c_x = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \quad \text{.....(3)}$$

$$c_y = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2} \quad \text{.....(4)}$$

Where,

A_1 and A_2 =Absorbances of diluted mixture at λ1 and λ2

Cx and CY = Concentration of component X and Y, g/100 ml in final solution

ax_1 and ax_2= Absorptivity of component X at λ_1 and $\lambda_2$respectively.

ay_1 and ay_2= Absorptivity of component Y at λ_1 and $\lambda_2$respectively

2.6 Method Validation:

The proposed method was validated for accuracy, precision, linearity, limits of detection (LOD) andlimits of quantification (LOQ). The method validation was performed as per ICH guidelines.[10]

2.6.1 Linearity:

Stock solutions of valsartan and hydrochlorothiazide were prepared by dissolving 10 mg of valsartanand 10 mg hydrochlorothiazide separately dissolved in water: methanol (50:50 v/v) and then the volume was adjusted to 100 ml with water: methanol (50:50 v/v) separately. Stock solutions were subsequently diluted with same solvent to get 4-24µg/ml and 0.5-3 µg/mlfor valsartan and hydrochlorothiazide respectively. Then the absorbance of these diluted solutions were measured at 250 nm (λ1) for valsartan and 271.4 nm (λ2) for hydrochlorothiazide by using double beam UV spectrophotometer against a blank of water: methanol (50:50, v/v). Average of six replicates readings was taken and tabulated.[10] (Optical characteristics and other parameters data is reported in Table4).

2.6.2Precision:

The repeatability was evaluated by assaying 6 times the sample solution prepared for assay determination. Precision of the method was evaluated by interday and intraday variation studies. In intraday studies, working solutions of standard and sample were analyzed thrice in a day and percentage relative standard deviation (% RSD) was calculated. In the interday variation studies, working solution of standard and sample were analyzed on two consecutive days and percentage relative standard deviation (% RSD) was calculated. The data is reported in Table 6.

2.6.3Accuracy Study:

To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels (80%, 100% and 120%) as per ICH guidelines. Recovery studies were carried out by applying by proposed method to a drug sample to which known amount of standard valsartan and hydrochlorothiazide corresponding to 80%, 100% and 120% of label claim had been added. The data is reported in Table 7.

2.6.4 LOD& LOQ:

ICH guideline describes several approaches to determine the limit of detection (LOD) and the limit of quantification (LOQ). These include visual evaluation, signal to noise ratio, and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3σ/S and 10σ/S criterions, respectively. Where, σ is the standard deviation of the γ-intercepts of the regression lines and S is the slope of the calibration curve.[10]The data are reported in Table 8.

FORCED DEGRADATION STUDY:

Forced degradation study was carried out by exposing test solution to different strengths of hydrochloric acid (0.1 N to 1.0N), sodium hydroxide (0.1N to 1.0N),Neutral,Thermal (80^{o}C), UV light radiation as per ICH guideline (Q1, R2). The data are reported in Table 9.

RESULTS AND DISCUSSION

4.1Analytical Method Validation:

The UV method for simultaneous estimation of VAL and HCT was developed using methanol: water (50:50) as solvent. The linearity was observed in the concentration ranges of 4-24 µg/ml and 0.5-3 µg/ml with coefficients of correlation r^2= 0.9989 and r^2= 0.9972 for VAL and HCT at 250 nm and 271.4 nm respectively. The accuracy of method was determined by standard addition method. Accuracy study was carried out at three levels i.e. 80, 100& 120% of labelled claims as per the ICH guidelines. The mean recovery was found to be 100.19 %and 99.51 % for VAL and HCT in Valent –H tablet respectively, indicating that the method has required accuracy and there was no interference by excipients present in tablets.Mean assay values in Valent –H tablet were found to be 101%and 98.48%for of valsartan and hydrochlorothiazide respectively. The RSD value below 2% indicated that the method has required precision. The limit of quantitation of of valsartan and hydrochlorothiazide were found to be 2.8145µg/ml and 0.5584µg/ml respectively. Limit of detection was found to be 0.92880µg/ml and 0.18427µg/ml for of valsartan and hydrochlorothiazide respectively. Hence, it can be successfully applied for routine estimation for of valsartan and hydrochlorothiazide in quality control laboratories.

4.2 Forced Degradation study:

The stress degradation studies showed that of valsartan and hydrochlorothiazide undergoes degradation in acidic, alkaline, neutral, photolytic and dry heat condition

CONCLUSION:

A simple, rapid, accurate, and precise stability-indicating UV Spectrophotometric method has been developed and validated in accordance to the ICH guidelines showing linearity, accuracy, precision, selectivity, stability and system suitability for the routine determination of valsartan and hydrochlorothiazide in tablet dosage form. Stress degradation results of show that the method is selective and stability indicating. The proposed method has the ability to separate the drug from their degradation products, related substances and excipients found in tablet dosage form.

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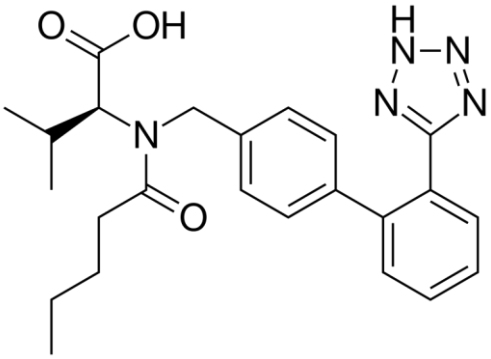

Figure 1. Structure of Valsartan

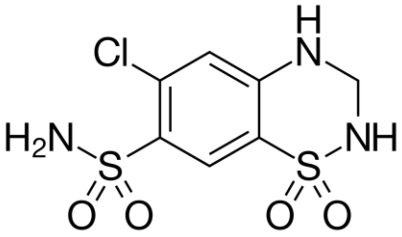

Figure2. Structure of Hydrochlorothiazide

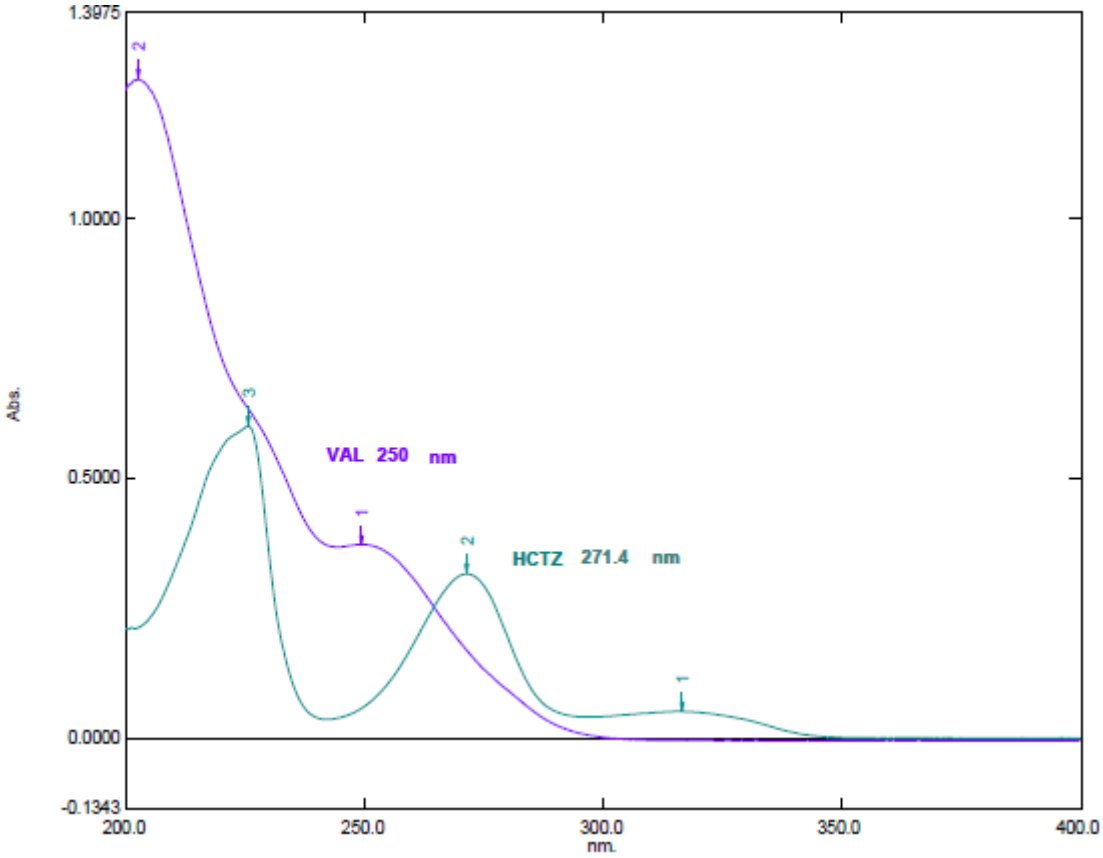

Figure3. Overlain spectra of VAL and HCT

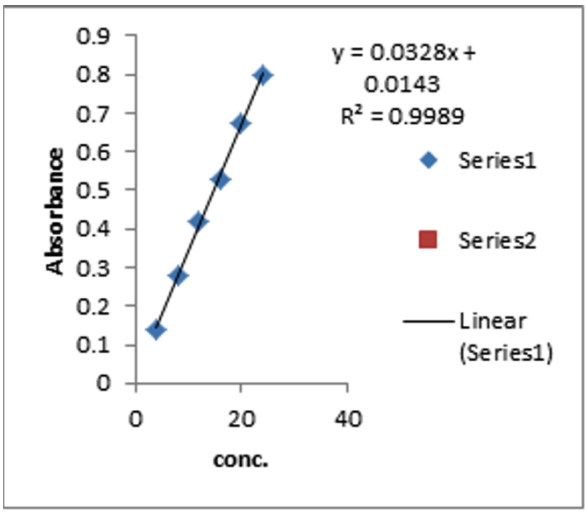

Figure 4. Linearity Curve of Valsartan

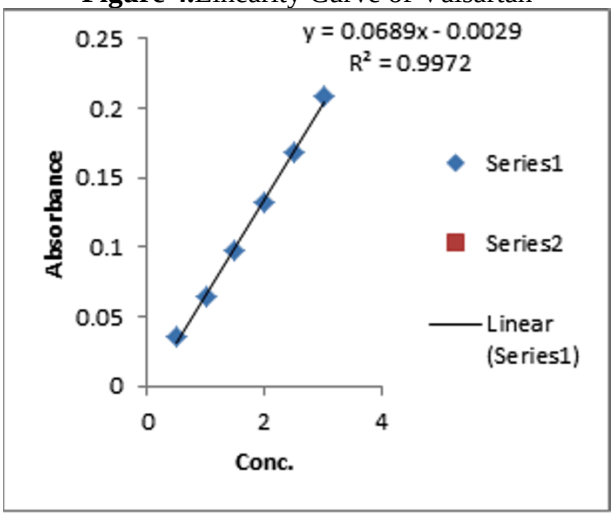

Figure 5. Linearity Curve of Hydrochlorothiazide

Sr. no.	Label claim (mg/ tab)		Amount of drug found (mg/tab)		% Label Claim	
	VAL	**HCT**	**VAL**	**HCT**	**VAL**	**HCT**
1	80	12.5	80.77	12.4	100.96	99.20
2	80	12.5	80.62	12.25	100.77	98.00
3	80	12.5	80.95	12.3	101.18	98.40
4	80	12.5	80.75	12.49	100.93	99.92
5	80	12.5	80.62	12.25	100.77	98.00
6	80	12.5	80.8	12.31	101.00	98.48
				Mean	100.93	98.67

	SD	0.1548	0.7550
	% RSD	0.1533	0.7652

Table 1: Analysis of tablet formulation

* Indicates average of six determinations

Table 2: Linearity study data of VAL

Sr. No.	Conc. (µg/ml)	Absorbance at 250 nm
1	4	0.141
2	8	0.277
3	12	0.421
4	16	0.528
5	20	0.673
6	24	0.80

Table 3: Linearity study data of HCT

Sr. No.	Conc. (µg/ml)	Absorbance at 271.4 nm
1	0.5	0.036
2	1	0.064
3	1.5	0.098
4	2	0.132
5	2.5	0.168
6	3	0.208

Table 4: Optical characteristics and other parameters:

Parameters	VAL	HCT
λ_{max}i.e. selected wavelength (nm)	250	271.4
Linearity range (µg/ml)	4-16	0.5-3
$y = mx + c$	-	-
Slope (m)	0.0328	0.0689
Intercept (c)	0.0143	0.0029
Regression coefficient (R^2)	0. 9989	0. 9972

Table 5: Repeatability Data

No. of Sample	% Recovery	
	VAL	HCT

1	99.21687	97.47899
2	99.57831	99.71989
3	99.39759	100.8403
4	100.1205	101.9608
5	100.8434	100.2801
6	100.8434	99.71989
Mean*	100	100
SD *	0.719873	1.49276
%RSD*	0.719873	1.49276

* Indicates average of six determinations

Table 6: Precision data of marketed formulation

Samples	**Intraday**		**Interday**	
	VAL	**HCT**	**VAL**	**HCT**
1	97.77	98.04	97.49	98.04
2	97.951	98.60	100.30	99.72
3	98.13	99.16	101.56	101.40
Mean*	97.95	98.59	100.0602	99.71989
SD*	0.180723	0.560224	1.639838	1.680672
%RSD*	0.184502	0.568182	1.638851	1.685393

* Indicates average of three determination

Table 7: Results of recovery studies

Level of (%) Recovery	**Amount present (mg/tab)**		**Amount of standard added (mg)**		**Total amount Recovered (mg)**		**% Recovery***	
	VAL	**HCT**	**VAL**	**HCT**	**VAL**	**HCT**	**VAL**	**HCT**
80	80	12.5	64	10	145.3	22.3	100.90	99.47
100	80	12.5	80	12.5	160.19	24.82	100.11875	99.28
120	80	12.5	96	15	175.22	27.44	99.55681818	99.78
						Mean*	100.19	99.51
						SD*	0.5519	0.2067
						% RSD*	0.5509	0.2078

*Each value is the mean of three observations

Table 8: Summary of validation parameters

Parameters	**VAL**	**HCT**

Linearity Range (µg/mL)	4-24	0.5-3
Correlation coefficient (r^2)	0. 9989	0. 9972
Precision (%RSD)		
Intraday	0.184502	0.568182
Interday	1.638851	1.685393
Accuracy (%)		
80% ± RSD	101.62 ± 3286	98.41 ± 0.3234
100% ± RSD	100.24 ± 0.5681	98.59 ± 0.5602
120% ± RSD	99.03 ± 0.3249	99.53 ± 0.3234
Repeatability (%RSD)	0.719873	1.49276
LOD (µg/mL)	0.92880	0.18427
LOQ (µg/mL)	2.8145	0.5584
Solution stability	Stable for 24 hrs.	Stable for 24 hrs.

Table 9: Summary of results of stress degradation studies

Sr.no.	Condition	% Degradation		% Assay	
		VAL	HCT	VAL	HCT
1	Acid hydrolysis (0.1 N HCL, 80°c, 6 hrs)	32.08	19.64	67.91	80.35
2	Base hydrolysis (0.5M NaOH, 80°c, 6 hrs)	20.89	12.26	79.16	87.73
3	Neutral hydrolysis (80°C, 6 hrs 6 hrs)	22.33	18.09	77.66	81.90
4	Photolytic degradation (UV Rays, 2 hrs)	10.94	16.53	89.05	83.46
5	Thermal degradation (80°C, 6 hrs)	23.37	16.83	46.62	93.16