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Der Pharmacia Sinica, 2011, 2 (3): 123-130

Der Pharmacia Sinica

ISSN: 0976-8688
CODEN (USA): PSHIBD

Spectrophotometric method for simultaneous estimation of Valsartan and Hydrochlorothiazide in combined tablet dosage form

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ABSTRACT

The simple, accurate and precise UV spectrophotometric method has been developed for the simultaneous estimation of Valsartan and Hydrochlorothiazide in combined tablet dosage form. Two wavelengths 250 nm and 270 nm were selected for the estimation of Valsartan and Hydrochlorothiazide by simultaneous equation respectively. The linearity range lies between 6-36 µg /mL for Valsartan and 2-12 µg/mL for Hydrochlorothiazide at their respective wavelengths. Water and acetronitrile is used as diluent in equal proportion. The results were analyzed by simultaneous equation method that confirms the accuracy and reproducibility of the said method. Statistical analysis proves that proposed method was validated as per ICH guidelines.

Keywords: Valsartan, Hydrochlorothiazide, Simultaneous equation method, ICH guidelines.

INTRODUCTION

Valsartan (VAL), (**Fig. 1**) an orally active, specific angiotensin II receptor blocker [1] acting on the AT_1 receptor subtype, is chemically N-(1-oxopentyl)-N-[[2 '-(1H-tetrazol-5-yl)[1,1 '-biphenyl]-4-yl]methyl]-L-Valine. Its empirical formula is $C_{24}H_{29}N_5O_3$ and molecular weight 435.5 gm/mole. Hydrochlorothiazide (HCTZ), (**Fig. 2**) a thiazide diuretic [2], is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide. Its empirical formula is $C_7H_8ClN_3O_4S_2$ and molecular weight 297.73 gm/mole.

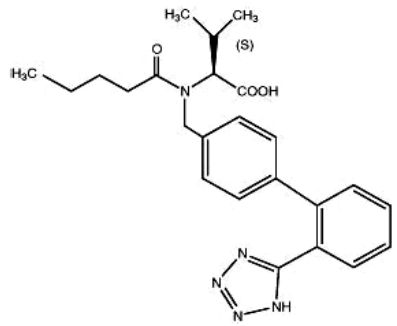

Fig.1 Structure of VAL

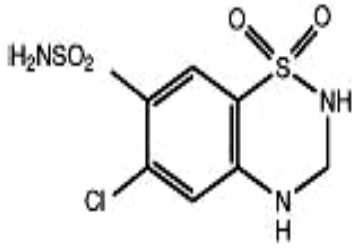

Fig.2 Structure of HCTZ

The applicability of this combination is particularly in patients likely to need multiple drugs to achieve their blood pressure goals. Hence this is widely used combination in treatment of hypertension.

A detailed literature survey revealed various reported methods for analysis of VAL and HCTZ in pharmaceutical preparation. That includes HPLC [3], LCMS [4], HPTLC [5], UV [6, 7, 8, 9]. However, this present literature only stimulated us to work out the simultaneous equation method for estimation of VAL and HCTZ in pharmaceutical preparation as no such method was reported.

MATERIALS AND METHODS

Instrument:
A Shimadzu UV spectrophotometer – 2450, Software Version- 2.2 was used. Balance Shimadzu Uni Bioc and Ultrasonicator of ENERTECH Electronics Pvt. Ltd were used.

Materials:
VAL and HCTZ as bulk drugs were obtained from Cadila Healthcare Ltd, Ahemdabad (Gujarat) as gift sample while Co-Diovan tablet (Novartis Pharma) is used as formulation. HPLC grade Acetronitrile of Merck, India Ltd. and RO water is used as solvent.

Diluent:
Mixture of Water and Acetronitrile is used in equal proportion.

Preparation of Stock Solution:
10 mg of VAL and 10 mg of HCTZ were weighed and transferred separately to 100 mL volumetric flasks. Each drug was dissolved in 50 mL diluent then sonicated for 15 min. The volume was made up to the mark with diluent to afford conc. of 100 µg/mL.

Selection of Analytical Wavelengths:
From the stock solutions, transferred separately1.6 mL of VAL to 10 mL volumetric flasks and 0.25 mL of HCTZ to 10 mL volumetric flask, volume was adjusted to the mark with diluent, to get the concentration of 16 µg/mL of VAL and 2.5 µg/mL of HCTZ respectively. Both the drug

solutions were scanned separately between 200 nm to 400 nm. The overlain spectra of both drugs were recorded are shown in **Fig. 3.**

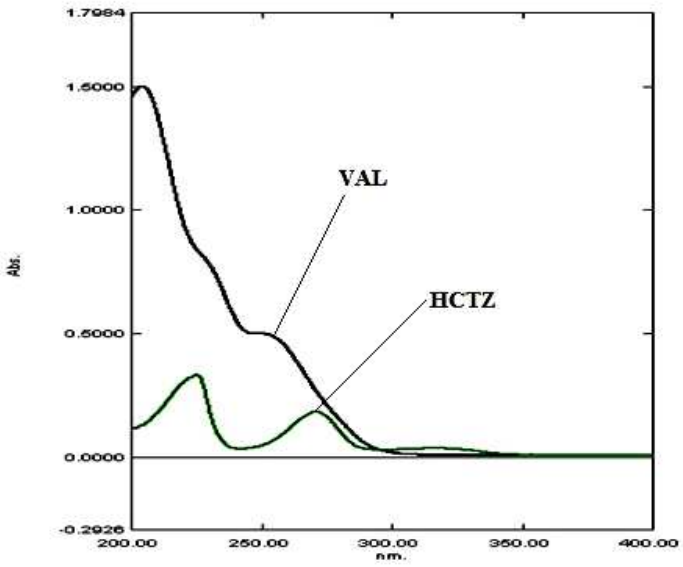

Fig.3 Overlain spectra of VAL and HCTZ

From overlain spectra, wavelengths 250 nm and 270 nm for VAL and HCTZ respectively were selected for analysis of both drugs using simultaneous equation method.

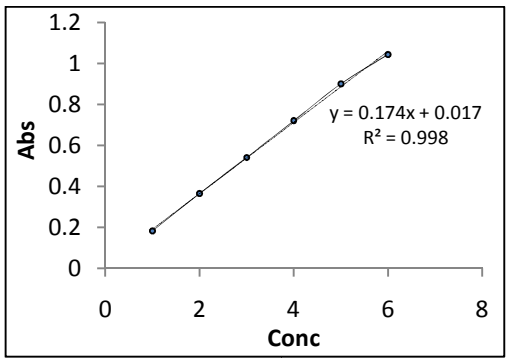

Fig. 4 Calibration Curve of VAL

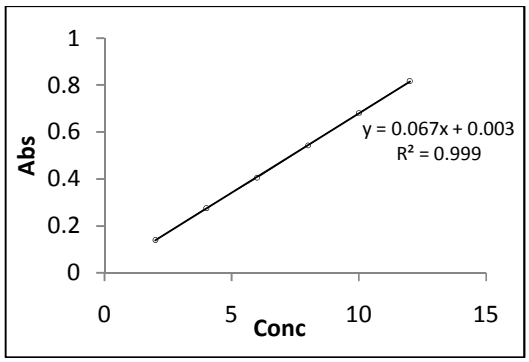

Fig. 5 Calibration Curve of HCTZ

Calibration curve and linearity:

From the stock solution of 100 µg/mL, working standard solutions of drugs were prepared by appropriate dilution and were scanned in entire UV range to determine the λ max. Standard solutions were prepared having concentration 6-36 µg/mL for VAL and 2-12 µg/mL for HCTZ. The absorbance of these standard solutions was measured at 250 nm and 270 nm and calibration curves were plotted. Two simultaneous equations (variables C1 and C2) were formed using these absorptivity coefficient values are given in **Table 1**. Such linearity ranges are selected, as the

marketed formulation for VAL and HCTZ respectively have strength of 160 mg and 25 mg. The calibration curve was plotted as concentration *vs* absorbance for VAL and HCTZ (**Fig. 4 & 5**) [10, 11, 12].

Table 1: Absorptivity Values of VAL and HCTZ

Sr. no.	VAL (250nm)	HCTZ (250nm)	VAL (270nm)	HCTZ (270nm)
1	305	100	145	700
2	305	97.5	145.833	690
3	300.555	101.666	143.333	675
4	300.416	98.75	145.833	678.75
5	300.333	101	145.666	681
6	290	100.833	145.833	680.833
Mean	300.21	99.95	145.24	684.26
SD	5.48	1.56	0.99	9.15

A set of two simultaneous equations were framed using these absorptivity coefficient values as….

C_{VAL} = A2 ay1 – A1 ay2/ ax2 ay1 – ax1 ay2 ……..…. (1)

C_{HCTZ} = A1 ax2 – A2 ax1/ ax2 ay1 – ax1 ay2…….... (2)

Where, **A1** and **A2** are absorbance of mixture at 250 nm and 270 nm; **ax1** and **ax2** are absorptivities of VAL at 250 nm and 270 nm respectively; **ay1** and **ay2** are absorptivities of HCTZ at 250 nm and 270 nm respectively. **C_{VAL}** and **C_{HCTZ}** are concentrations of VAL and HTZ in mixture.

By rearranging equations **1 and 2, C_{VAL}** and **C_{HCTZ}** can be obtained as,

$$C_{VAL} = \frac{A2 \times 99.95 \; - A1 \times 684.26}{190908.97} \qquad \text{……} \qquad (3)$$

$$C_{HCTZ} = \frac{A1 \times 145.24 \; - A2 \times 300.21}{190908.97} \qquad \text{……} \qquad (4)$$

Application of proposed method for physical laboratory mixture:

Mixture of VAL and HCTZ was prepared by dissolving 10 mg, diluted with 50 ml of diluent, sonicating it for 15 min and then make up the volume up to 100 mL to afford the concentration of 100 µg/mL. From the stock solution of VAL, 1.6 mL of VAL solution was transferred to 10 mL of volumetric flask and diluted up to the mark to get concentration of 16 µg/mL of VAL; and from the stock solution of HCTZ, 0.25 mL of HCTZ solution was transferred to 10 mL of volumetric flask and diluted up to the mark to get final concentration of 2.5 µg/mL of HCTZ. The solution was scanned in the range of 200 – 400 nm, absorbance of the sample solutions (A_1 and A_2) were recorded, at 250 nm and 270 nm respectively against blank. The concentrations (C_{VAL} and C_{HCTZ}) in sample solution were determined; by using equation 3 and 4, results are given in **Table 2**.

Table 2: Analysis of Physical Laboratory Mixture

Sr. no.	Concentration found (µg/mL)	
	VAL	HCTZ
1	16.15	2.52
2	16.18	2.51
3	16.18	2.53
4	15.99	2.51
5	16.19	2.5
6	16.16	2.49
Mean	16.14	2.51
% RSD	0.46	0.56

Application of proposed method for analysis of tablets:

Tablet equivalent to 25 mg of HCTZ and 160 mg of VAL was taken into 100 mL volumetric flask. To that diluent was added, shaken for 15 min and volume was make up with diluent. 5mL was solution pipetted out and diluted up to 100 mL to afford the concentration of 16 µg/mL VAL and 2.5µg/mL HCTZ. Clear solution was obtained by filtration through Whattman filter paper-41. Solution was scanned within 200-400 nm and absorbance of sample solution (**A_1** and **A_2)** was recorded at 250nm and 270nm respectively against blank. The concentrations of the two drugs in sample solutions (C_{VAL} and C_{HTZ}) were determined, using equation 3 and 4. The results are tabulated in **Table 3**.

Table 3: Analysis of Tablet

Sr. no.	Drug	Concentration taken (mg/mL)	Concentration found (mg/mL) n=5	Concentration found (%)	% RSD
1	VAL	160	161.4	100.85	0.46
2	HCTZ	25	25.1	100.4	0.56

Validation of proposed method:

The proposed method was validated as per the ICH guidelines for various parameters like accuracy, precision, ruggedness, repeatability, Limit of Detection, Limit of Quantitation etc. [13]

Accuracy:

It was done by recovery study using standard addition method at 80%, 100% and 120% level; known amount of standard VAL and HTZ was added to pre–analyzed sample (16 µg/mL of VAL and 2.5 µg/mL of HTZ) and subjected them to the proposed method. Results of Recovery studies were shown in **Table 4**.

Table 4: Accuracy

Sr. no.	Initial concentration (µg/mL)		Concentration of excess drug added to analyte (µg/mL)		Concentration found (µg/mL) n=3		% Recovery		% RSD	
	VAL	HCTZ	VAL	HCTZ	VAL	HCTZ	VAL	HCTZ	VAL	HCTZ
1	16	2.5	12.8	2	29.09	4.51	101	100.2	0.099	0.679
2	16	2.5	16	2.5	32.07	5	100.3	100	0.690	0.692
3	16	2.5	19.2	3	35.6	5.51	101.1	100.1	0.197	0.922

Precision:
Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

Variation of results within the same day (intraday), variation of results between consecutive days (inter day) were analyzed. Intraday precision was determined by analyzing 12.5 µg/mL, 25 µg/mL and 37.5 µg/mL of VAL and 2 µg/mL, 4 µg/mL, 6 µg/mL of HCTZ for three times within the day. Inter day precision was determined by analyzing above mentioned concentrations of both the drugs daily for three consecutive days and results are given in **Table 5**.

Table 5: Precision

Sr. no.	Drug	Concentration taken (µg/mL)	Intraday n=3		Interday n=3	
			Concentration found (µg/mL)	% RSD	Concentration found (µg/mL)	% RSD
1	VAL	16	16.13	0.75	16.16	0.11
		22.4	22.86	0.35	22.49	0.55
		28.8	28.86	0.51	29.09	0.09
2	HCTZ	2.5	2.51	0.8	2.51	0.22
		3.5	3.49	0.4	3.53	0.29
		4.5	4.48	0.55	4.49	0.17

Ruggedness:
Ruggedness was determined by two different analyst by preparing sample solution of VAL (16 µg/mL) and HCTZ (2.5 µg/mL) from stock solution using similar operational and environmental conditions and results are given in **Table 6**.

Table 6: Ruggedness

Sr. no.	Drug	Concentration Found (%) ± RSD	
		Analyst-I	Analyst-II
1	VAL	100.9 ± 0.51	101 ± 0.48
2	HCTZ	99.6 ± 0.65	100.2 ± 0.65

Repeatability:
Six test sample solutions containing 16µg/mL of VAL and 42.5µg/mL of HCTZ were scanned over the range of 200-400 nm and absorbance are measured at 250nm and 270nm, concentrations were determined with the help of proposed method and %RSD was calculated and results are given in **Table 2**.

Limit of Detection and Quantification (LOD & LOQ):
The LOD and LOQ were estimated from the standard calibration curve. It is calculated using the formula i.e. LOD = 3.3 X σ/S and LOQ = 10 X σ/S where, σ is the standard deviation of the response and S is slope of the calibration curve.

RESULTS AND DISCUSSION

UV spectrophotometric method for VAL and HCTZ using simultaneous equation was developed. VAL showed absorbance maxima at 250 nm and HCTZ at 270 nm. Linearity was observed in the

concentration range of 6-36 µg/mL for VAL and 2-12 µg/mL for HCTZ. Correlation coefficient was found to be 0.998 and 0.999 at 250 and 270 nm respectively. The proposed method was applied for the determination of VAL and HCTZ in the marketed dosage and estimated as 100 % and 100.4 %.

The recovery of drug was determined at 80, 100 and 120 % levels. The percentage recovery was from 100 to 101.1 % for VAL and 100 to 100.2 % for HCTZ. Precision, Ruggedness, Repeatability was performed as per ICH guidelines, results shows that % RSD < 2 % i.e. within the limit. LOD and LOQ were found to be 0.013, 0.39 for VAL and 0.053, 0.16 for HCTZ respectively. The summary of parameters performed is given in **Table 7**.

Table 7: Summary of parameters

Sr. no.	Parameters	VAL	HCTZ
1	λ max (nm)	250	270
2	Linearity range (µg/mL)	6-36	2-12
3	Correlation coefficient (r^2)	0.998	0.999
4	Slope	0.174	0.067
5	Intercept	0.017	0.003
6	Limit of Detection (µg/mL)	0.013	0.053
7	Limit of Quantification (µg/mL)	0.39	0.16
8	Accuracy (%)	100.2	100
9	Interday Precision (%RSD)	0.25	0.22
10	Intraday Precision (%RSD)	0.53	0.58
11	Repeatability (%RSD)	0.46	0.56
12	Ruggedness Analyst-I (%)	0.51	0.65
13	Ruggedness Analyst-II (%)	0.48	0.65

CONCLUSION

The developed simultaneous spectrophotometric method is found to be simple, precise, specific, and accurate and can be used for routine analysis of VAL & HCTZ. The developed method was validated as per ICH guidelines. Statistical analysis proves that the method is repeatable and selective for the analysis of VAL and HCTZ in combination as a single drug in bulk as well as in pharmaceutical formulations.

Acknowledgement
Authors are thanks to Cadila Healthcare Ltd., Ahemdabad, India for providing the gift samples of Valsartan and Hydrochlorothiazide.

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