ESTIMATION OF VALSARTAN AND HYDROCHLOROTHIAZIDE IN PHARMACEUTICAL DOSAGE FORMS BY ABSORPTION RATIO METHOD

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ABSTRACT A new absorption ratio method was developed and validated for the determination of valsartan and hydrochlorothiazide in tablets. Calibration curves for valsartan and hydrochlorothiazide over concentration range of 2-20 µg/ml were plotted and molar absorptivity for both the drugs were calculated at both the wavelengths of 270.5 nm (λ-max of hydrochlorothiazide) and 231.5 nm (iso- absorptive point). The results of analysis have been validated statistically and by recovery studies. The value of standard deviation was satisfactory and recovery studies ranging from 99.05-102.23% for valsartan and 97.42-100.22% for hydrochlorothiazide were indicative of the accuracy and precision of the proposed method. The results of the assay are in good agreement with the label amount. The method was found to be simple, rapid, and accurate and can be adopted in routine analysis of these drugs in formulations. Due to these attributes, the proposed method could be used for routine analysis of these drugs in combined dosage forms.

INTRODUCTION

Valsartan, (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]. A number of high performance liquid chromatographic (HPLC) methods are available for separation and quantification of valsartan from pharmaceutical dosage forms [2]. Hydrochlorothiazide 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 [3]. It is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide-1,1-dioxide, and was successfully used as one content in association with other drugs [4],[5],[6],[7],[8],[9] in the treatment of hypertension. Simultaneous determination of both drugs is highly desirable as this would allow more efficient generation of clinical data and could be more cost-effective than separate assays. There are very few methods appearing in the literature for the simultaneous determination of valsartan and hydrochlorothiazide in tablets. Since these methods were based on UV-derivative spectrophotometry, HPLC and HPTLC [10],[11],[12]. A literature survey has revealed there is no absoption ratio method for analysis of VAL and NEB in pharmaceutical preparations. The present work describes a validated absorption ratio method for simultaneous determination of these drugs in tablets
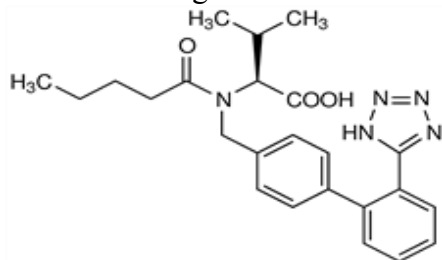

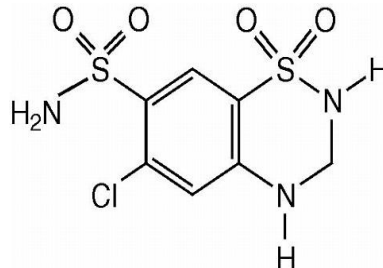**Fig. I Structure of Valsartan**   **Fig. II Structure of Hydrochlorothiazide**

MATERIAL AND METHOD

A double-beam UV-Visible spectrophotometer, model UV-1800 (Shimadzu, Japan) having two matched cells with 1-cm light path wavelength accuracy of ± 0.5 nm with automatic wavelength correction with a pair of 10 mm quartz cells. A Sartorius electronic analytical balance (CP224S) was used for weighing the sample. An ultrasonic cleaner (Frontline FS 4) was used for sonicating the tablet powder. Valsartan (VAL) and Hydrochlorthiazide (HCTZ) from Torrent Pharmaceuticals Ltd and Methanol -AR grade (Finar laboratories) were used in the study.

Preparation of standard stock solutions

Standard Stock solutions (100µg/ml) of VAL and NEB were prepared by dissolving separately, 5 mg of drug in 50 ml volumetric flask and dilute up to the mark with methanol.

Determination of iso-absorptive point and wavelength of maximum absorbance

The working standard stock solutions of VAL and HCTZ were scanned in the range of 200 to 400 nm against methanol as a blank. Iso- absorptive point was found at 231.5 nm. (Figure III).
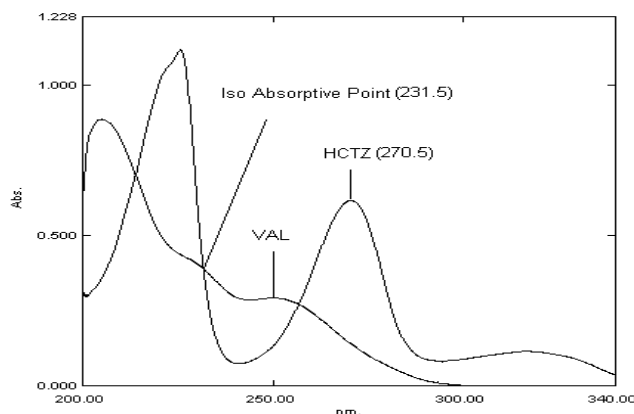

Figure: III Overlay spectra of VAL and HCTZ showing iso-absorptive point at 231.5

Preparation of Sample solution from tablet dosage form

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 80 mg of VAL and 5 mg of HCTZ into 100 ml volumetric flask and diluted to 100 ml with methanol. This solution is sonicated for 20 minutes. The solution was filtered through Whatman filter paper No. 41. Transfer 1 ml of solution into 10 ml volumetric flask and dilute to the mark with methanol. Then transfer 2 ml of solution into 10 ml volumetric flask and dilute to the mark with methanol to get a final concentration 16 µg/ml of VAL and 2.5 µg/ml of HCTZ.

Calibration curve (Linearity)

A calibration curve was plotted over a concentration range of 2-20 μg/ml for both VAL and HCTZ. Accurately measured standard stock solution of VAL (0.2, 0.4, 0.6, 0.8, 1.2, 1.6, 2 ml) and standard stock solution of HCTZ (0.2, 0.4, 0.6, 0.8, 1.2, 1.6, 2 ml) were transferred to a separate series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbance of each solution was measured at both the wavelength 231.5 nm and 270.5 nm. Calibration curves were constructed for VAL & HCTZ by plotting absorbance versus concentrations at both wavelengths. Each reading was average of three determinations.
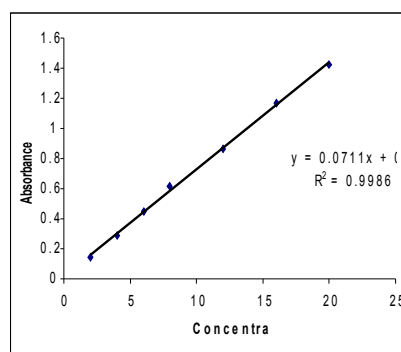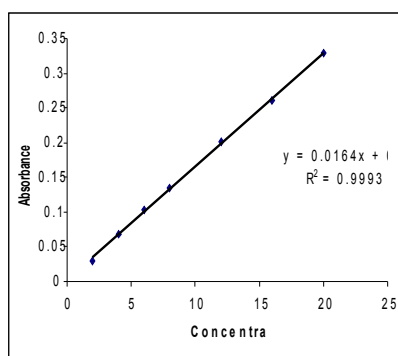

Figure: IV Calibration curve at 276.5 nm   **Figure: V. Calibration curve of VAL at 270.5 nm**
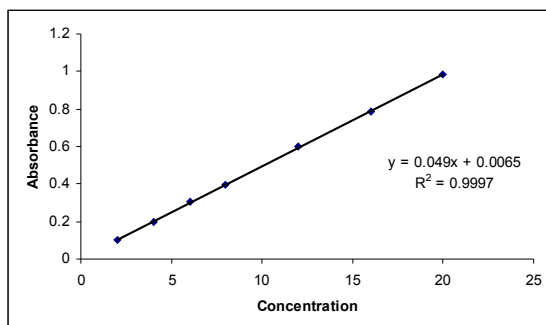

Figure: VI Calibration curve of HCTZ at 270.5 nm (Iso-absorptive point)

Estimation of VAL and HCTZ from pharmaceutical dosage form

The absorptivity coefficients of these two drugs were determined using calibration curve equation. The concentration of VAL and HCTZ were determined using the following simultaneous equations.

$$C_X = \frac{(Q_M - Q_Y) \times A_1}{(Q_X - Q_Y) \times aX_1} \quad \text{AND} \quad C_Y = \frac{A_1}{aX_1 - C_X}$$

Where, A1 & A2 are the absorbance of the mixture at 231.5 nm & 270.5 nm respectively; aX_1 and aY_1 are absorptivity of VAL and HCTZ respectively at 231.5 nm; aX_2 and aY_2 are absorptivity of VAL and HCTZ respectively at 270.5 nm; $Q_M = A_2/A_1$, $Q_X = aX_2/aX_1$ and $Q_Y = aY_2/aY_1$.

 Method of validation

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation proportional to the concentration of analyte in samples within a given range. The range of analytical method is the interval between upper and lower level of analyte including levels that have been demonstrated to be determining with precision and accuracy using the method. The linear response of VAL and HCTZ were determined by analyzing five independent levels of the calibration curve in the range of 2 - 20 μg/ml. Result should be expressed in terms of Correlation co-efficient.

 Precision

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, Relative standard deviation or coefficient of variance of a series of measurements.

Repeatability (Precision on replication)

It is a precision under a same condition (Same analyst, same apparatus, short interval of time and identical reagents) using same sample. Method precision of experiment was performed by preparing the standard solution of VAL (10 µg/ml) and HCTZ (10 µg/ml) for six times and analyzed as per the proposed method. Percentage relative standard deviation (%RSD) or coefficient of variation (CV) was not more than 2%.

Intermediate precision (Reproducibility)

It expresses within laboratory variations as on different days analysis or equipment within the laboratory. Variation of results within same day is called Intra-day precision and variation of results amongst days called Inter-day precision. The Intra-day precision (C.V) was determined for standard solution of VAL and HCTZ (2 - 20 µg/ml) for five times at the same day. The Inter-day precision (C.V) was determined for standard solution of VAL and HCTZ (2 - 20 µg/ml) for five days.

Accuracy (% Recovery)

Accuracy of an analysis is determined by systemic error involved. It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (VAL 16 µg/ml and HCTZ 2.5 µg/ml) with three different concentrations of standards (VAL 1,2,3 µg/ml and HCTZ 1,2,3 µg/ml).

Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions.Limit of detection can be calculated using following equation as per ICH guidelines.

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

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines.

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

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

RESULTS AND DISCUSSION

In this method, the standard stock solutions of VAL and HCTZ were prepared in methanol. Calibration curves for VAL and HCTZ over concentration range of 2 - 20 µg/ml were plotted and molar absorptivity for both the drugs were calculated at both the wavelengths of 270.5 nm (λ-max of HCTZ) and 231.5 nm (iso- absorptive point). It is evident from the spectra of VAL and HCTZ that these drugs obey the Lambert-beer's law at all the wavelength. Calibration curve of VAL and HCTZ at 270.5 are shown in figure IV and V respectively, while calibration curve at 231.5 nm (iso- absorptive point) is shown in figure VI. The optical and regression characteristics and validation parameters are reported in Table III. The assay was performed according to the experimental conditions previously described. The linearity of the calibration graphs and adherence of the system to Beer's law were validated by the high value of the correlation coefficient and the intercept value. The good recoveries with the standard addition method (Table I) prove the good accuracy of the proposed methods.

Table I: Data of recovery study of VAL and HCTZ

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D (n=3)
VAL	16	1	17.38	102.23 ± 1.32
	16	2	17.21	101.16 ± 0.73
	16	3	18.82	99.05 ± 0.94
HCTZ	2.5	1	3.41	97.42 ± 1.32
	2.5	2	4.51	100.22 ± 0.85
	2.5	3	5.81	97.81 ± 1.47

Precision

For evaluation of precision, repeatability of the results for a concentration of 10 µg/ml was evaluated by 6 replicate determinations. For evaluation of intermediate precision, the results over the concentration range 2 - 20 µg/mL was evaluated by 5 replicate determinations to estimate intraday variation and another 5 replicate determinations on different 5 days to estimate interday variation. The coefficients of variation (CV) values at these concentration levels were calculated.

Limit of Detection

The limit of detection of the drug was found as in the text. LOD was found to be 0.484µg/ml at Iso-absorptive point. LOD for VAL and HCTZ was found to be 0.628 µg/ml and 0.413 µg/ml respectively at 270.5.

Limit of Quantification

The limit of quantification of the drug was found as in the text. LOQ was found to be 1.469 µg/ml at Iso-absorptive point. LOQ for VAL and HCTZ was found to be 1.902 µg/ml and 1.251 µg/ml respectively at 270.5.

Application to the pharmaceutical dosage form

The proposed validated method was successfully applied to determine VAL and HCTZ in bulk powder and in tablet dosage forms. Results are given in Table II. No interference of the excipients with the peaks of interest appeared, hence the proposed method is applicable for the routine simultaneous estimation of VAL and HCTZ in pharmaceutical dosage form

Table II: Application of the proposed method to the pharmaceutical dosage forms

Formulation	VAL			HCTZ		
	Amount labeled (mg)	Amount found (mg)	% Amount Found S.D. (n=3)	Amount labeled (mg)	Amount found (mg)	% Amount Found S.D. (n=3)
Brand I	80	81.52	101.90 ± 1.47	12.5	12.32	98.56 ± 1.46
Brand II	80	80.59	100.73 ± 1.51	12.5	12.39	98.23 ± 1.56
Brand III	80	81.72	102.15 ± 1.68	12.5	12.87	102.96 ± 1.74

**Table III: Optical and Regression characteristics and validation parameters of
Q Absorbance ratio method for analysis of VAL and HCTZ**

Parameters	Isoabsorptive Point	VAL (270.5)	HCTZ (270.5)
Beer's Law Limit (µg/ml)	2-20	2-20	2-20
Absorptivity	499	165	731
Regression equation ($y^* = mx + c$) Slope (m) Intercept (c)	0.049 0.0065	0.0164 0.0019	0.0711 0.0165
Correlation Coefficient (r^2)	0.9997	0.9993	0.9986
Standard Deviation (S.D)	0.0072	0.0042	0.0089
Relative Standard Deviation (RSD or %CV)	1.1397	1.5362	1.3632
LOD (µg/ml)	0.484	0.628	0.413
LOQ (µg/ml)	1.469	1.902	1.251
Precision Intra-day (n=5) (% CV) Inter-day (n=5) (% CV)	0.81-1.72 0.56-1.82	0.62-1.71 0.71-1.69	0.63-1.92 0.67-1.82

CONCLUSION

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, selective, robust and rapid and can be applied successfully for the estimation of VAL and HCTZ in bulk and in pharmaceutical formulations without interference and with good sensitivity.

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