Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2012, 4 (2):593-599
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

An ecofriendly estimation of valsartan and hydrochlorothiazide in pharmaceutical dosage form by absorption ratio method

Tanushree Banerjee[1*], Bhaskar Banerjee[1] and Angshu Banerjee[2]

[1]RKDF College of Pharmacy, Bhopal (M.P.)
[2]Sri Balaji College of Pharmacy, Jaipur (Raj.)

ABSTRACT

A new, simple, ecofriendly, economical and accurate absorption ratio method was developed and validated by using hydrotropic solubilizing agent (8M ammonium acetate solution) for the determination of valsartan and hydrochlorothiazide in tablets. Hydrotropy is one of the solubility enhancement techniques, which enhance solubility of poorly water-soluble drugs to many folds with use of hydrotropes. In the present investigation, hydrotropic solution of ammonium acetate (8M) was employed as solubilizing agent to solubilize valsartan and hydrochlorothiazide (poorly water-soluble drugs) fine poder and tablet dosage form. 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 (l-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.

Keywords: Valsartan, Hydrochlorothiazide, ecofriendly, hydrotropic agent.

INTRODUCTION

The term hydrotropic has been used to designate the increase in solubility of poorly water soluble drugs in concentrated solutions of hydrotropic agents .A huge number of poorly water soluble drugs have been solubilized by use of various hydrotropic solutions.[1-10]

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[11]. A number of high performance liquid chromatographic (HPLC) methods are available for separation and quantification of valsartan from pharmaceutical dosage forms[12]. 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[13]. 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[14-19] in the treatment of hypertension. There are very few methods appearing in the literature for the simultaneous determination of valsartan and hydrochlorothiazide in tablets based on UV-spectrophotometry, HPLC and HPTLC[20-22]. A literature survey has revealed there is no absoption ratio method using hydrotropic solubilizing agent for analysis of Valsartan and Hydrochlorothiazide in pharmaceutical preparations. The present work describes a validated absorption ratio method for simultaneous determination of

these drugs in tablets. Here calibration curve method was employed by using hydrotropic agent for the estimation of Valsartan in pure and tablet dosage forms. In the present investigation, hydrotropic solublizing agent, 8 M ammonium acetate was employed to solubilize Valsartan and hydrochlorothiazide fine powder and its tablet dosage form to carryout spectrophotometric analysis.

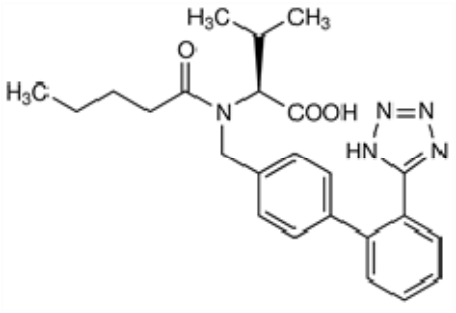


Fig. I Structure of Valsartan

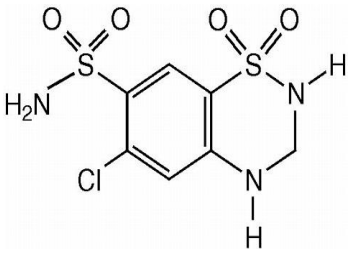


Fig. II Structure of Hydrochlorothiazide

MATERIALS AND METHODS

Instrumentation chemicals

Shimadzu UV-Visible spectrophotometer (model UV-1800) with 1cm matched quartz cells was used for spectrophotometric analysis.A Sartorius electronic analytical balance (CP214S) was used for weighing the sample.cyclo mixer REMI Instrument Limietd,Centrifuje Remi Instrument Limited. Valsartan and Hydrochlorthiazide procured from Torrent Pharmaceuticals Ltd. All chemicals and reagents used were of analytical grade and purchased from Merck Chemicals, India.

Preparation of standard stock solutions

Standard Stock solutions of Valsartan and Hydrochlorothiazide were prepared by dissolving separately, 50 mg of drug in 40 ml of 8M ammonium acetate solution and final volume was adjusted with distilled water in 100ml of volumetric flask. From the above solution 10ml of solution was taken and diluted to 50ml with distilled water to get a solution containing 100 µg/ml of each drug.

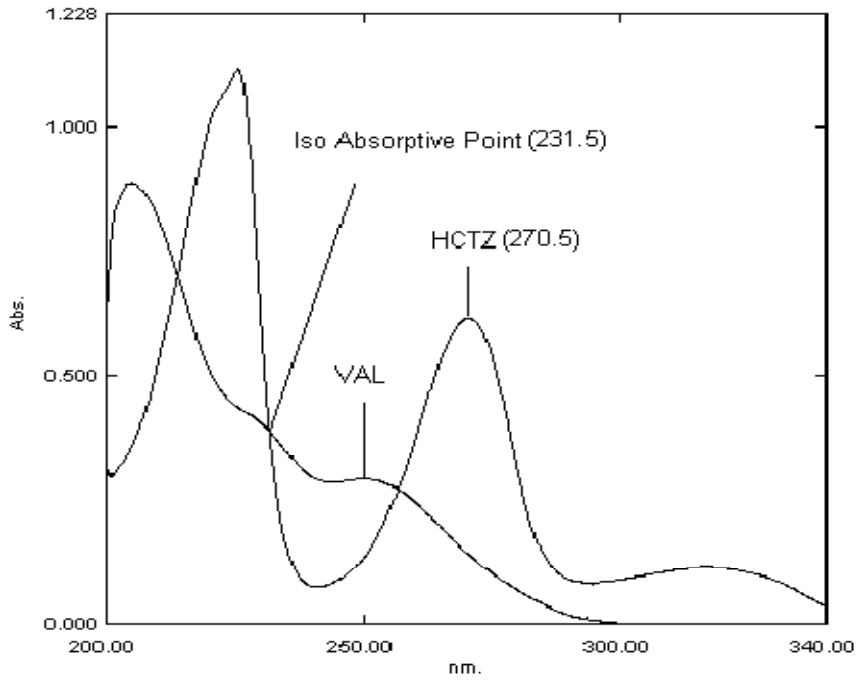


Figure: III Overlay spectra of VAL and HCTZ showing iso-absorptive point at 231.5

Determination of iso-absorptive point and wavelength of maximum absorbance

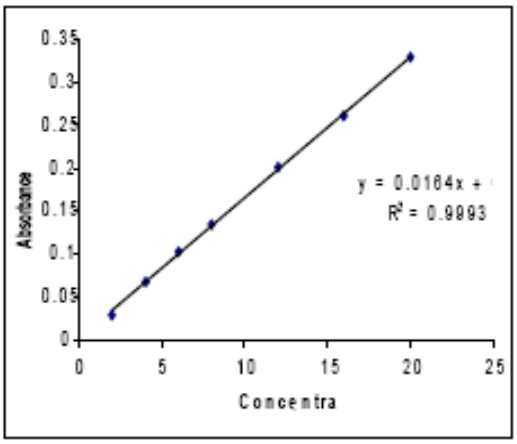
The working standard stock solutions of Valsartan and Hydrochlorothiazide were scanned in the range of 200 to 400 nm and the Iso- absorptive point was found at 231.5 nm. (Figure III)

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 dissolved in 40ml of 8M ammonium acetate solution with frequent shaking for 15minutes and final volume was made up with distilled water. The sample solution was then filtered through Whatman filter paper No.41 and first few ml were rejected. Ttransfer 1ml of solution into 10ml volumetric flask and dilute with distilled water . Then transfer 2 ml of solution into 10 ml volumetric flask and dilute to the mark with distilled water to get a final concentration 16 µg/ml of Valsartan and 2.5 µg/ml of Hydrochlorothiazide.

Calibration curve (Linearity)

A calibration curve was plotted over a concentration range of 2-20 mg/ml for both Valsartan and Hydrochlorothiazide. Accurately measured standard stock solution of Valsartan (0.2, 0.4, 0.6, 0.8, 1.2, 1.6, 2 ml) and standard stock solution of Hydrochlorothiazide (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 distilled water. The absorbance of each solution was measured at both the wavelength 231.5 nm and 270.5 nm. Calibration curves were constructed for Valsartan & Hydrochlorothiazide by plotting absorbance versus concentrations at both wavelengths. Each reading was average of three determinations.



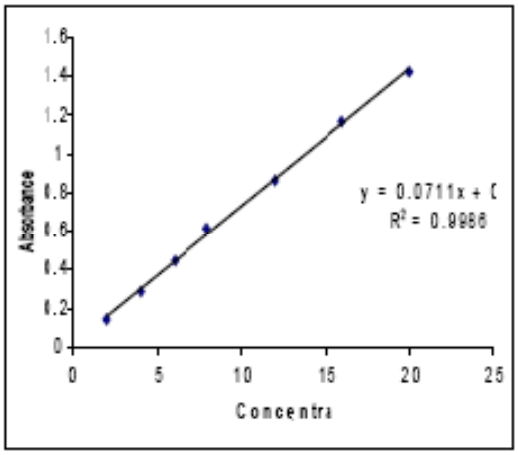


Figure: IV Calibration curve at 276.5 nm **Figure: V. Calibration curve of VAL at 270.5 nm**

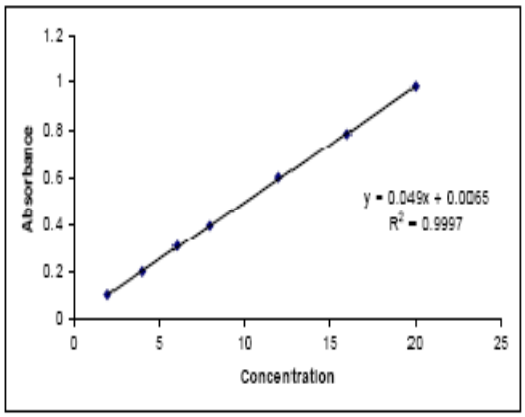


Figure: VI Calibration curve of HCTZ at 270.5 nm (Iso-absorptive point)

Estimation of Valsartan and Hydrochlorothiazide from pharmaceutical dosage form

The absorptivity coefficients of these two drugs were determined using calibration curve equation. The concentration of Valsartan and Hydrochlorothiazide 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; aX1 and aY1 are absorptivity of Valsartan and Hydrochlorothiazide respectively at 231.5 nm; aX2 and aY2 are absorptivity of VAL and HCTZ respectively at 270.5 nm; QM=A2/A1, QX= aX2/ aX1 and QY= aY2/ aY1.

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 Valsartan and Hydrochlorothiazide 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 Valsartan (10 µg/ml) and Hydrochlorothiazide (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 Valsartan and Hydrochlorothiazide (2 - 20 µg/ml) for five times at the same day. The Inter-day precision (C.V) was determined for standard solution of Valsartan and Hydrochlorothiazide (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 (Valsartan 16 µg/ml and Hydrochlorothiazide 2.5 µg/ml) with three different concentrations of standards (Valsartan 1,2,3 µg/ml and Hydrochlorothiazide 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 Valsartan and Hydrochlorothiazide were prepared in methanol 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 (l-max of Hydrochlorothiazide) and 231.5 nm (iso- absorptive point). It is evident from the spectra of Valsartan and Hydrochlorothiazide that these drugs obey the Lambert-beer's law at all the wavelength. Calibration curve of Valsartan and ydrochlorothiazide 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 Valsartan and Hydrochlorothiazide 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 Valsartan and Hydrochlorothiazide 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 Valsartan and Hydrochlorothiazide 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)	0.049	0.0164	0.0711
Intercept (c)	0.0065	0.0019	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)	0.81-1.72	0.62-1.71	0.63-1.92
Inter-day (n=5) (% CV)	0.56-1.82	0.71-1.69	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 ecofriendly. This method can be applied successfully for the estimation of Valsartan and Hydrochlorothiazide in bulk and in pharmaceutical formulations without interference and with good sensitivity.

REFERENCES

[1] R.K. Maheshwari, *The Indian Pharmacist.*,**2005**,4,63-68.
[2] R.K. Maheshwari, *Asian J.Chem.*,**2006**,18,393-396.
[3] R.K. Maheshwari, *The Pharma. Review.*,**2005**,3,123-125.
[4] R.K. Maheshwari, *Asian J.Chem.*,**2006**,18,640-644.
[5] R.K. Maheshwari, *Asian J.Chem.*,**2006**,18,1572-1574.

[6] R.K. Maheshwari, *Asian J.Chem.*,**2006**,18,3194-3197.
[7] R.K. Maheshwari, S.C.Chaturvedi, Jain N.K., *Indian Drugs*,**2005**,42,541-544.
[8] R.K. Maheshwari, S.C.Chaturvedi, Jain N.K., *Indian J. Pharm. Sci*., **2006**,68:195-198.
[9] R.K. Maheshwari, S.C. Chaturvedi, Jain N.K., *Indian J. Pharm. Sci*.,(In Press).
[10] R.K. Maheshwari, S.C.Chaturvedi, N.K. Jain, *Indian Drugs*.,**2006**,43,516-518.
[11] A. Markham, K.L. Goa, *Essential hypertension drugs*,**1997**,54:299-311.
[12] N. Daneshtalab, R.Z. Lewanczuk, F.J. Jamali, *Chromatogram*. B,**2002**,766,345-349.
[13] G. Carlucci, G. Palumbo, P. Mazzeo, M.G. Quaglia, *J. Pharm. Biomed Anal*.,**2000**,23,185-189.
[14] J.A. Schoenberger, *J. Hypertens*.,**1995**,13,43-47.
[15] L.E. Ramsay, W.W. Yeo. *J Hypertens*.,**1995**,13,(Suppl 1)
[16] J.B. McCrea, M.W. Lo, L. Tomasko, C.C. Lin, J.Y. Hsieh, N.L. Capra, et al. *J Clin Pharmacol*, **1995**,35,1200-1206.
[17] L.M. Ruilope, R.L. Simpson, J. Toh, K. E. Arcuri, A. I. Goldberg, C.S. Sweet. *Blood Press*, **1996**,5,32-40.
[18] P.R. Conlin, M. Elkins, C. Liss, A.J. Vrecenak, E. Barr, J. M. Edelman, *J. Hum. Hypertens*, **1998**,12,693-700.
[19] D. Del Castillo, J.M. Campistol, L. Guirado, L. Capdevilla, J.G. Martinez, P. Pereira, et al. *Kidney Int. Suppl*. **1998**,68,135-139.
[20] E. Satana, S. Altinay, N.G. Goger, S.A Ozkan, Z. Senturk, *J. Pharm. Biomed Anal*.,**2001**,25, 1009-13.
[21] DF Tian, XL Tian, T Tian, ZY Wang and FK Mo. *Ind. J. Pharm. Sci*. **2008**,70, 372-374.
[22] B.R. Kadam, S.B. Bari, **2007**,18, 260-269.