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Spectrophotometric Determination of Valsartan using *p*-Chloranilic Acid as π-Acceptor in Pure and in Dosage Forms

S. M. Mallegowda, H. N. Deepakumari and H. D. Revanasiddappa*
Department of Chemistry, University of Mysore, Manasagangotri, Mysore-570 006, India.

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ABSTRACT

The aim of this work was to develop a simple, sensitive and extraction free spectrophotometric method for the quantitative estimation of valsartan in both pure and in pharmaceutical preparations. The developed method is based on the charge transfer complexation reaction between valsartan (VRT) as n- electron donor and *p*-chloranilic acid (*p*-CA) as π-acceptor. VRT reacts with *p*-CA in methanol to produce a bright pink colored complex with a maximum absorption at 530 nm. Beer's law was obeyed in the concentration range of 5-50 µg/mL. The linear regression equation of the calibration graph is A = 0.0081+0.0092C with a regression coefficient (r) of 0.9976 (n = 7). The molar absorptivity is calculated to be 2.06×10^3 L mol^{-1} cm^{-1} and the Sandell sensitivity is 0.1025 µg cm^{-2}. The limits of detection (LOD) and quantitation (LOQ) values are calculated according to ICH guidelines. The method developed is successfully applied to the determination of VRT in dosage forms.

INTRODUCTION

Chemically valsartan (VRT) is (S)-3-methyl-2-(N-{[2'-(2H -1 ,2 ,3 ,4-tetrazol-5-yl) biphenyl -4-yl] methyl} pentanamido) butanoic acid (Fig. 1) has an empirical formula of $C_{24}H_{29}N_5O_3$ is a potent angiotensin receptor π antagonist with particularly high affinity for the type I (AT_1) angiotensin receptor. By blocking the action of angiotensin, valsartan dilates blood vessels and reduces blood pressure (Marks, 2007).

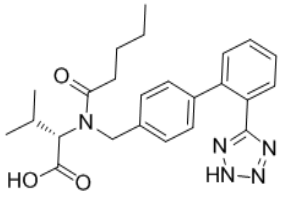


Fig. 1: Structure of valsartan.

Several analytical methods have been reported for the determination of VRT in combination with other anti- hypertensive agents in pharmaceutical formulations. Very few methods have been appeared in the literature for the determination of valsartan individually, those include high performance liquid chromatography (Zarghi *et al.,* 2008, Li *et al.,* 2000, Gonzales *et al.,* 2000, Francotte *et al.,*1996, Parambi *et al.,* 2011), RP-HPLC (Vinzuda *et al.,* 2010, Manoranjani *et al.,* 2011, Patnaik *et al.,* 2011), UV- and second derivative-spectrophotometric and LC method (Tatar *et al.,* 2002), HPLC with UV Detection (Piao *et al.,*2008) and uv-spectrophotometry (Gupta *et al.,* 2010) and spectrofluorimetric determination of losartan and valsartan in human urine (Gagigal *et al.,* 2001) and simultaneous determination of valsartan and hydrochlorothiazide in tablets by first-derivative ultraviolet spectrophotometry and LC [Satana *et al.,* 2001]. The VRT is not yet appeared in any pharmacopoeia either alone or in combinations with other drugs.

The purpose of the present work is the development of a simple spectrophotometric method for the determination of VRT in bulk and in pharmaceutical formulations through the formation of charge transfer complexation reaction between valsartan (VRT) as n- electron donor and *p*-chloranilic acid (*p*-CA) as π-acceptor.

* *Corresponding Author*
Dr. H. D. Revanasiddappa,
Email: hdrevanasiddappa@yahoo.com
Tel: +0821 2419669

MATERIALS AND METHODS

Apparatus

Absorbance measurements for spectrophotometric analysis were performed using a Systronics Model 166 digital spectrophotometer provided with 1-cm matched quartz cells.

Reagents and standards

Analytical reagent grade chemicals and reagents were used.

p-chloranilic acid (0.05 %, w/v)

It was freshly prepared by dissolving 0.05 g *p*-chloranilic acid (Rolex, Mumbai, India) in 100 mL acetone.

Standard VRT solution

The pure grade VRT, certified to be 99.99% was received from Cipla India Ltd., Mumbai, India, as a gift sample and was used as such. A stock standard solution equivalent to 100 µg/mL of VRT was prepared by dissolving 10 mg of the pure drug in 100 mL methanol.

Pharmaceutical formulations of VRT such as VALZAAR [Torrent], DIOVAN [Novartis Pharma] were purchased from local markets.

Procedure for calibration graph

Accurately measured aliquots of standard solution of VRT (100µg/mL) corresponding to 0.5, 1.0, 1.5, 2.0, ……..5.0 mL were transferred into a series of 10 mL calibrated flasks using a micro burette. To this solution was added 3.5 mL 0.05 % *p*-CA, then shake well and the contents were diluted to the mark with methanol and mixed well. The absorbance of the bright pink colored complex was measured at 530 nm after 5 min against the reagent blank prepared similarly, but without drug content.

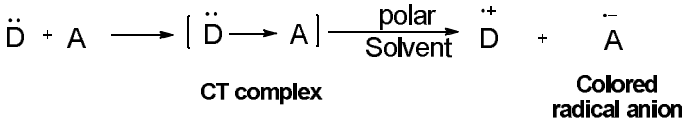
Procedure for dosage forms

In order to determine the contents of VRT in commercial dosage forms (label claim: 40 and 80 mg tablet), the contents of ten tablets were weighed accurately and ground into a fine powder. An amount of powder equivalent to 10 mg of VRT was accurately weighed and transferred into two separate 100mL calibrated flasks and 30 mL of methanol was added. The content was shaken for about 30 min; the volume was diluted to the mark with methanol and mixed well and filtered using a Whatman no.41 filter paper. The filtrate containing VRT at a concentration 100 µg/mL was subjected to analysis by the procedure described above.

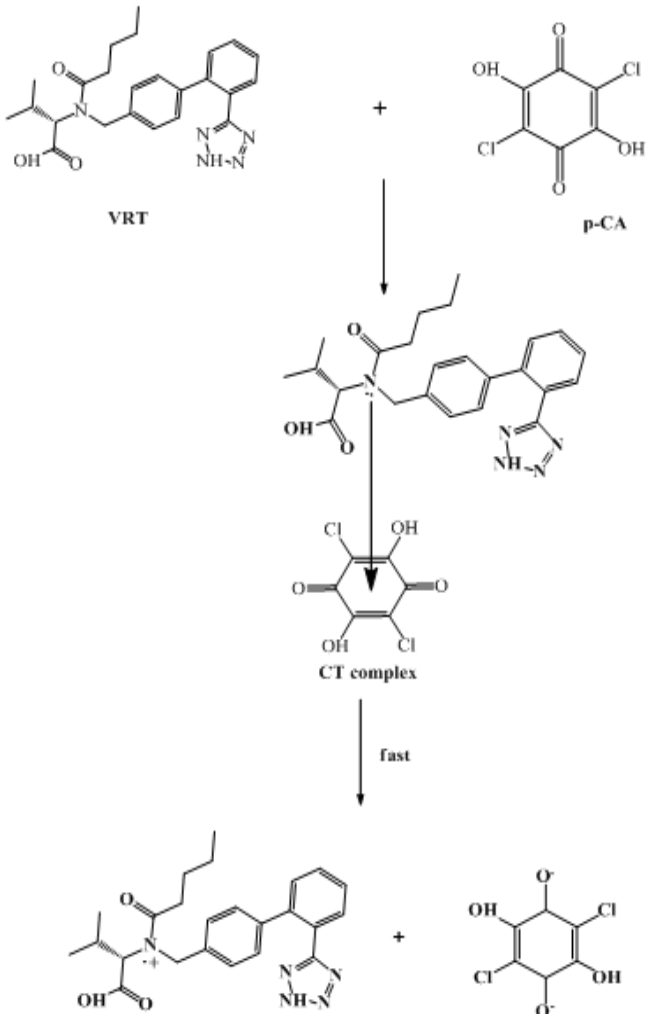
RESULTS AND DISCUSSION

Chemistry of the method

The method developed involves charge-transfer complex formation between the basic nitrogenous VRT as n-donor and *p*-chloranilic acid (*p*-CA) in polar solvent as π-acceptor. The formed charge transfer (C-T) complex was subsequently dissociated into radical anions, which are colored species. The absorbance of the colored complex was measured at 530 nm, and it was observed as shown in the following equation:



Thus, *p*-CA was used as reagent in the proposed method for the estimation of VRT. The possible reaction pathway for VRT - *p*-CA complex is shown in scheme1.



Scheme. 1: Proposed reaction scheme.

The influence of various factors on the formation of charge-transfer complex *viz.*, reagent concentration, reaction time and stability of the colored complex were studied and maintained throughout the experiment to determine the quantity of VRT in bulk and in dosage forms. Highest absorbance values were obtained with 3.5 mL of 0.05 % *p*-CA, which remained unaffected by further addition of *p*-CA. Thus, 3.5 mL of the reagent was used for the determination in the developed method. It was observed that the formed complex was stabilized within 5 min in the

developed method. The color of the C-T complex remained stable at room temperature (27 ± 3 ºC) for a period of 2 h.

Method validation

The developed method was validated in terms of linearity and sensitivity, limit of detection (LOD) and limit of quantitation (LOQ), precision, accuracy, selectivity and recovery following the ICH guidelines **(**International Conference On Harmonization, guidelines).

Linearity, sensitivity, limits of detection and quantification

A calibration graph was constructed using a standard solution of VRT at respective wavelength and concentration of VRT in the ranges is given in Table 1. Under the optimum experimental conditions, a linear relationship existed between the absorbance and concentration of the drugs. The regression analysis of the calibration curve using the method of least-squares was made to calculate the slope (b), intercept (a) and correlation co-efficient (r) values are presented in Table 1. The optical characteristics such as absorption maxima, Beer's law limit, molar absorptivity and Sandell's sensitivity value of the method are also given in Table 1.

Table. 1: Analytical and regression parameters of the proposed methods.

Parameter	Method
λ_{max} nm	530
Beer's law range (µg/mL)	5.0 – 50
Molar absorptivity (ε), (L $mol^{-1}cm^{-1}$)	2.06 x 10^3
Sandell sensitivity (µg cm^{-2})	0.1025
Intercept (a)	0.0081
Slope (b)	0.0092
Correlation coefficient (r)	0.9976
S_a	0.0170
S_b	0.0004
LOQ (µg/mL)	1.1169
LOD (µg/mL)	0.3686

*$y=a+bx$, where c is the concentration of VRT in µg/mL and y is the absorbance at the respective λ_{max}., S_a is the standard deviation of the intercept, S_b is the standard deviation of the slope.

Intra and inter-day precision and accuracy

The accuracy of an analytical method expresses the closeness between the proposed method and reference method. Further, the accuracy and precision (intra-day) of the proposed method were evaluated by replicate analysis (n=5) of calibration standards at three different concentration levels in the same day. Precision and accuracy of inter-day were measured by performing the calibration standards at cited three concentrations on five consecutive days. Both precision and accuracy were based on the calculated percent relative standard deviation (RSD, %) and percent relative error (RE, %) values, respectively for a. b. thedeveloped method were found to be satisfactory. The analytical results obtained from this investigation are summarized in Table 2.

Application to analysis of pharmaceutical samples

The validity of the proposed method was ascertained by the statistical comparison of the results obtained by a reference method (Patnaik *et al.*, 2011) with the proposed method by applying Student's t-test for accuracy and F- test for precision in some commercial formulations.

The results were compared with those of the reported method. Statistical analysis of the results using the Student's-t and F-tests revealed no significant difference between the reported method at the 95% confidence level with respect to accuracy and precision (Table 3).

Table. 3: Results of determination of VRT in tablets and statistical comparison with the reference method.

Tablet brand Name	Nominal amount mg per tablet	Found*(% of nominal amount ± SD)	
		Reference method	Proposed method
VALZAAR[a]	40	100.88±0.48	99.62±0.704 t = 1.68, F = 2.15
DIOVAN[b]	80	100.80 ±0.46	100.38±0.854 t = 0.51, F = 3.45

Marketed by: a. [Torrent], b. [Novartis Pharma]
*Mean value of five determinations
Tabulated t and F-values at 95 % confidence level are 2.77 and 6.39, respectively.

Recovery study

To test the applicability of the proposed method, recovery experiments were carried out by standard addition method. In this study, pre-analyzed tablet powder was spiked with pure drug at three different concentrations and the total was found by the proposed method. Each determination was repeated three times. The recovery of the pure drug added was quantitative and revealed that co-formulated substances did not interfere in the determination. The results of recovery study are compiled in Table 4.

Table. 4: Results of recovery experiments *via* the standard addition technique.

Tablet brand name	VRT mg per tablet	Pure VRT added, µg/mL	Total found µg/mL	Pure VRT recovered* % ± SD
VALZAAR[a]	40	20	20.02	100.22 ± 0.77
		30	29.83	99.16 ± 0.88
		40	39.85	99.49 ±0.46
DIOVAN[b]	80	20	19.91	99.13±0.77
		30	30.20	100.98±0.85
		40	40.31	101.03±0.95

* Mean value of three determinations.
Marketed by: a. [Torrent], b. [Novartis Pharma]

Table. 2: Evaluation of intra-day and inter-day accuracy and precision results.

	VRT taken, µg/mL	intra-day[a]			inter-day[b]		
		VRT found[c], µg/mL	Precision[d]	Accuracy [e]	VRT found[c], µg/mL	Precision[d]	Accuracy [e]
Method	10	9.93±0.10	1.02	0.66	9.97±0.25	2.50	0.21
	20	19.97±0.06	0.30	0.17	19.84±0.13	0.63	0.81
	40	40.07±0.33	0.83	0.18	39.79±0.29	0.74	0.52

Mean value of five determinations, b. Mean value of five determinations, c. Mean value of three determinations, d. Relative standard deviation (%), e. Bias%: (found-taken/taken)×100.

CONCLUSION

The proposed method is simple and sensitive and in addition, the method has wider linear dynamic range with good accuracy and precision which could be applied for the determination of valsartan in bulk drug and dosage forms. From the calculated t- and F-values at the 95 % confidence level, it is clear that, the results obtained by the proposed method are in good agreement with those obtained by the reference method. The small values of R.E and R.S.D. indicate the reliability, accuracy and precision of suggested procedure. The results obtained in Table 3 are considered to be of high accuracy and can be successfully applied to the routine assay of valsartan in pharmaceutical formulations.

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