IJPSR (2014), Vol. 5, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 September, 2013; received in revised form, 09 December, 2013; accepted, 15 January, 2014; published 01 February, 2014

DEVELOPMENT AND VALIDATION OF NEW RP-HPLC ASSAY METHOD FOR VALSARTAN IN PURE AND IN FORMULATIONS

G. Sivasankara Rao*[1], T. Naga Raju T [2], P.V.V.S. Ramarao [2], S.V.M. Vardhan [3] and D. Ramachandran [1]

Department of Chemistry, Acharya Nagarjuna University [1], Guntur, Andhra Pradesh, India
Department of Chemistry [2], Department of Biochemistry [3], K.U. Dr. M.R.A.R. P.G. Centre, Nuzvid, Andhra Pradesh, India

Keywords:

Valsartan, RP-HPLC,Validation

Correspondence to Author:

G. Sivasankara Rao

Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

E-mail: svm2032@gmail.com

ABSTRACT: An isocratic, reversed phase HPLC method was developed, using an Inertsil ODS C_8 (100 x 4.6) mm,5u column and the mobile phase containing an homogenous mixture of buffer and acetonitrile (65:35,v/v). The flow rate of the mobile phase was maintained at 1.0ml/min and the detection was carried out at wavelength 250 nm. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, selectivity, ruggedness to prove the stability indicating ability of the method.

INTRODUCTION: Valsartan [1-3], N-[p-(o-1H-Tetrazol-5-ylphenyl) benzyl]-N-valeryl-L-valine1 (**Fig. 1**) is an angiotensin II receptor antagonist, used in the treatment of hypertension. Few HPLC methods [4-8] have been reported for the estimation of valsartan either in single or combined form in biological fluids and tablet forms.

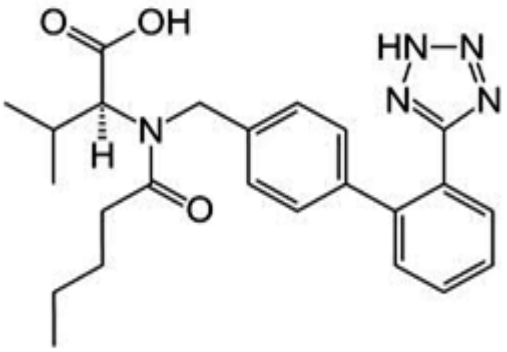

FIG. 1: CHEMICAL STRUCTURE OF VALSARTAN

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(2).596-99

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(2).596-99

Accordingly, the objective of this study was to develop and validate the new RP-HPLC assay method for valsartan in pure and pharmaceutical formulations as per ICH guidelines (2006). This paper presents a new RP-HPLC assay method for valsartan in pure and pharmaceutical formulations.

EXPERIMENTAL:

Material and reagents: Valsartan drug was made available from Merck Ltd. India (purity 99.8). Potassium dihydrogen phosphate, dipotasssium hydrogen phosphates were obtained from Qualigens Fine Chemicals, India Ltd. Orthophosphoric acid and Acetonitrile were obtained from Rankem laboratories, India. All chemicals and reagent were used as HPLC grades, Milli-Q-Water was used throughout the experiment.

Chromatographic Conditions: The present HPLC analysis was performed on Waters Alliance® HPLC System equipped with 2695 separation module, Auto sampler and UV-Vis detector. The chromatographic column of 100mm length and

internal diameter of 4.6 mm filled with Inertsil C_8 stationary phase with particle size of 5µ was used. The instrumental settings were a flow of 1.0 ml/min, the injection volume was 20µl.The output signal was monitored and integrated using Waters (Alliance) Empower software.

Mobile Phase: The mobile phase containing 1.2gm potassium dihydrogen phosphate and 0.25gms of dipotasssium hydrogen phosphate in 1000ml water filter and mixed. Adjust the buffer to pH 6.5 with dilute orthophosphoric acid. Prepare an homogenous mixture of buffer and acetonitrile (65:35,v/v)filtered through a 0.45µm nylon filter and degassed.

Preparation of standard stock solution preparation: Weigh and transfer 100 mg of valsartan working standard into 100ml volumetric flask add 60ml of mobile phase and sonicate to dissolve and dilute to volume with diluent. (Stock solution)

Working Standard preparation: Transfer aliquots of the above standard stock solution into series of 100ml volumetric flasks and dilute to volume with mobile phase to obtain concentration range of 25-125µg/ml.

Pharmaceutical preparation: For analysis of commercial twenty tablets was weighed and finely powdered. An accurately weighed quantity of tablet powder equivalent to 100mg of valsartan was transferred into 100ml volumetric flask add 60ml of diluent, sonicate to dissolve for 10mins and dilute to volume with diluent(mobile phase). The solution was then filtered through 0.45µ filter. From this aliquots of this solution were transferred and diluted to a series of 100ml volumetric flasks and the volume in each flask was made up to the mark with distilled water to give concentrations(25-125µg/ml) that obey within the linearity range.

RESULTS AND DISCUSSION: The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Selectivity: Stress studies were performed for valsartan bulk drug to provide the stability indicating property and selectivity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (0.5N Hydrochloric acid) **Fig. 2**, alkali (0.025N NaOH) **Fig. 3**, heat (60^{o}C**) Fig. 4** to evaluate the ability of the proposed method to separate valsartan from its degraded products.

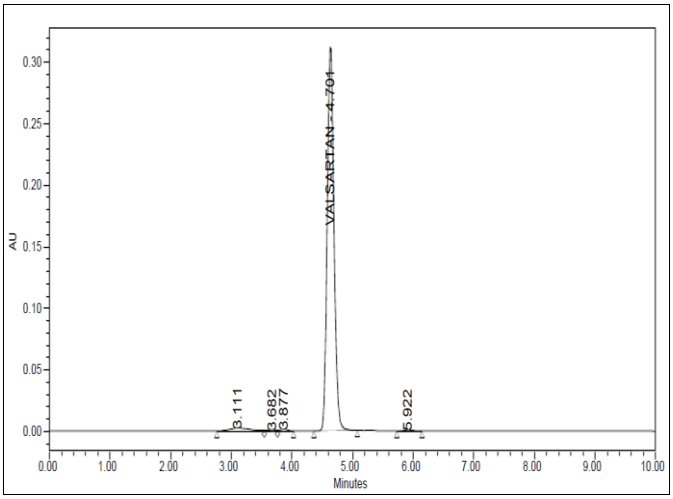

FIG. 2: VALIDATION CHROMATOGRAM FOR SPECIFICITY IN 0. 5N HCl

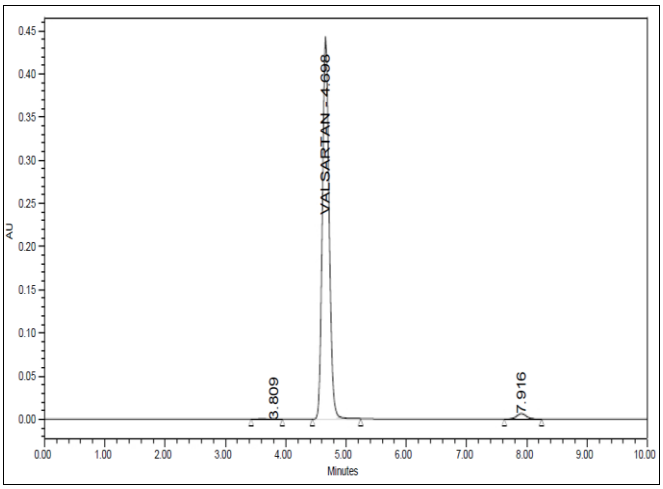

FIG. 3: VALIDATION CHROMATOGRAM FOR SPECIFICITY IN 0.025N NaOH

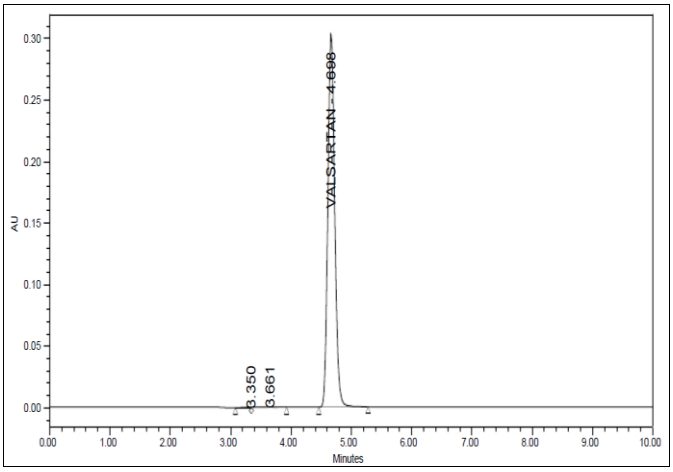

FIG. 4: VALIDATION CHROMATOGRAM FOR SPECIFICITY IN HEAT AT 60^{o}C

Linearity and Calibration: Linearity test solutions for the method were prepared from valsartan stock solutions at six concentrations levels from tested from 25% to 150% of the targeted level of the assay concentration valsartan. Standard solutions containing 25-125µg/ml of valsartan in each linearity level were prepared and these linearity solutions were injected in triplicate into the HPLC system. A calibration graph **Fig. 5** was obtained by plotting peak area verses the concentration data was treated by least-squares linear regression analysis. It was found that the calibration graph represented Fig. 5 was found to be linear in the mentioned concentrations the slopes and correlation coefficients are shown in **Table 1.**

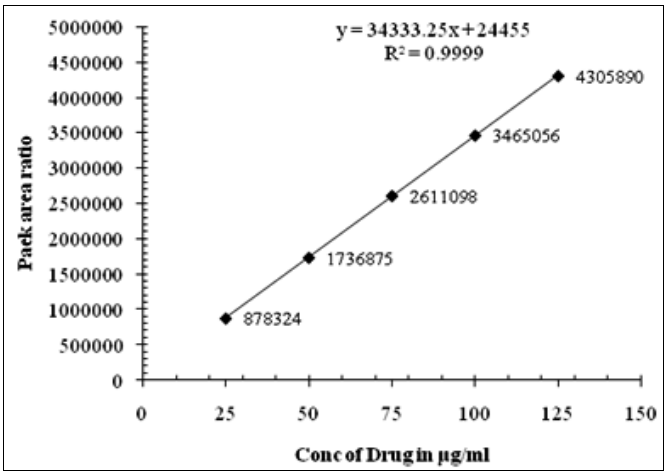

FIG. 5: LINEARITY GRAPH OF VALSARTAN

Sensitivity: The LOD and LOQ for valsartan were found to be 0.0066µg/ml and 0.022µg/ml, respectively (**Table 1**).

TABLE 1: CALIBRATION RESULTS DATA

Regression Parameters	Results
Regression equation; Slope (b)	34333.52
Intercept (a)	24455
Correlation coefficient	0.9999
Standard deviation on intercept(S_a)	0.0195
Standard deviation on slope (S_b)	0.00117
Standard error on estimation(S_e)	0.0186
Limits of Detection (LOD)[µg/ml]	0.0066
Limits of Quantification [LOQ)[µg/ml]	0.022

Accuracy: Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80%, 100% and 120%.

The recovery samples were prepared as aforementioned procedure. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for valsartan ranged from 98.91% to 100.62% (**Table 2**).

TABLE 2: RESULTS OF ACCURACY BY THE PROPOSED METHOD

Accuracy levels in %	Amount of sample (formulation) added in ppm	Amount recovered	%Recovery
50	50	50.31	100.62
100	100	99.56	99.56
150	150	148.36	98.91

*****Average of six determinations

Precision: The precision of the method was studied by determining the concentrations of the valsartan drug in the formulation for six times. The results of the precision study (**Table 3**) indicate the reliability of the method (RSD %< 2).

TABLE 3: RESULTS OF PRECISION BY THE PROPOSED METHOD

S. No.	Retention time	Peak area
1	4.705	3468046
2	4.701	3502147
3	4.706	3494760
4	4.709	3498032
5	4.702	3513548
6	4.705	3510035
Avg*	4.705	3497761
Std Dev	0.003	16193.7
% RSD	0.061	0.46

Ruggedness: A 0.02µl aliquot (50µg/ml) was injected to study the ruggedness of valsartan by two different analytical chemists (Analyst-1and Analyst -2) and the results were recorded in **Table 4** and are in the acceptable range for valsartan. The results showed the % R.S.D. was less than 2% respectively.

ASSAY OF IMATINIB IN FORMULATION: The assay for the dosage forms of valsartan was established by injecting solution of sample formulation [discussed in the experimental part] with the present chromatographic condition developed so as to obtain concentration in the range of linearity previously determined.

All determinations were carried out in six replicates and it was found to be more accurate and reliable. The average drug content was found to be 99.93% of the labelled claim. The results were shown in **Table 5**.

TABLE 4: RESULTS OF RUGGEDNESS BY THE PROPOSED METHOD

Ruggedness	Analyst -1		Analyst-2	
No. of Injections	**Retention Time**	**Area**	**Retention Time**	**Area**
Injection-1	4.701	3502147	4.699	3471326
Injection-2	4.706	3494760	4.699	3473064
Injection-3	4.709	3498032	4.704	3470369
Injection-4	4.702	3513548	4.710	3469846
Injection-5	4.705	3510035	4.705	3468055
Injection-6	4.705	3468046	4.706	3472438
Avg*	4.705	3497761	4.704	3470850
Std Dev	0.003	16193.7	0.004	1827.6
% RSD	0.061	0.46	0.091	0.053

*****Average of six determinations

TABLE 5: AMOUNT OF VALSARTAN IN DOSAGE FORMULATIONS

Formulation (mg)	Labeled Amount (mg)	Recovered Amount (mg)*	%Recovery
DIOVAN	80	79.95	99.93

*****Average of six determinations

CONCLUSIONS: The method developed for quantitative assay of valsartan is rapid, precise, accurate and selective. The proposed method was completely validated in accordance to ICH guidelines (2006). The stability-indicating studies proved that the selected drug valsartan was stable for acid, base and thermal degradation conditions revealing that the developed RP-HPLC method is selective and stability-indicating. The values of slopes and intercepts of the calibration graphs indicated the high reproducibility of the proposed method It can therefore be concluded that the developed method presented in this paper can be conveniently used for the assay determination of valsartan in bulk drug and pharmaceutical dosage forms.

ACKNOWLEDGEMENTS: The authors are grateful to the Merck pharmaceutical (Mumbai, India) for gift samples valsartan, Director, Bio Lee Life sciences Pvt Ltd, Hyderabad and Chairman(BOS-Chemistry), Acharya Nagarjuna University, Guntur-522210 (AP), India for providing necessary laboratory facility for this research work.

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How to cite this article:
Rao GS, Naga Raju TT, Ramarao PVVS., Vardhan SVM and Ramachandran D: Development and validation of new RP-HPLC assay method for Valsartan in pure and in formulations. *Int J Pharm Sci Res* 2014; 5(2): 596-99.doi: 10.13040/IJPSR.0975-8232.5(2).596-99