Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF VALSARTAN IN BULK BY RP-HPLC METHOD

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ABSTRACT

A simple, highly specific, stable and more economical RP-HPLC method has been developed for quantification of Valsartan from bulk using a mobile phase consisting mixture of methanol and phosphate buffer (pH 2.5) in the ratio of 75:25 (v/v) at the flow rate of 1.2 ml/min. A Phenomenex C18 (250×4.6mm, 5µ particle size) column was used as stationary phase. The retention time for Valsartan was 5.1min. Linearity was observed in the concentration range of 20 to100 µg/ml, with good linearity response greater than 0.995. The mean % recovery obtained is 99.5%. The results of validation suggest that the developed RP-HPLC method could be employed successfully for the estimation of Valsartan in routine analytical work.

Keywords: Valsartan, RP-HPLC, Validation.

INTRODUCTION

Valsartan[1] (VLS) is (*S*)-3-methyl-2-[N-({4-[2-(2*H*-1, 2, 3, 4–tetrazol-5-yl) phenyl] phenyl} methyl) pentanamido] butanoic acid. The chemical structure of VLS is shown in figure 1.

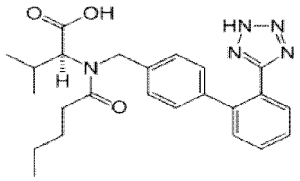

Figure 1: Chemical Structure of Valsartan

Valsartan appears as white to practically white fine powder. It is soluble in ethanol and methanol, and slightly soluble in water.

Valsartan, a specific angiotensin II antagonist, is used alone or with other antihypertensive agents to treat hypertension. VLS competes with angiotensin II for binding at the AT_1 receptor subtype. As angiotensin II is a vasoconstrictor which also stimulates the synthesis and release of aldosterone, blockage of its effects results in a decrease in systemic vascular resistance. Literature survey reveals a few HPLC[2, 3] and Spectrophotometric[4] methods for determination of Valsartan in bulk, tablet dosage forms and in rat plasma.

MATERIALS AND METHODS

Instrument

High performance liquid chromatographic system (HPLC) (Shimadzu) equipped with two LC 20AD liquid pumps, Rheodyne injector, pH meter and analytical balance.

Chemicals

Valsartan has been obtained as a gift sample from Dr Reddy's Laboratories Private Limited (Hyderabad, India), methanol of HPLC grade, potassium dihydrogen orthophosphate of Merck grade, orthophosphoric acid and Milli-Q water.

Preparation of Standard Stock Solution

10mg of VLS was accurately weighed, transferred into 10ml of clean dry volumetric flask and dissolved in methanol, the volume was made up to the mark with methanol to give 1000ppm.

Method

The analysis was performed by using Column of C18 (250×4.6mm, 5µ) with a flow rate of 1.2ml/min. The mobile phase consists of methanol and phosphate buffer, pH 2.5, in the ratio of 75:25, the injection volume was 20µL and the detection was at 250 nm using U.V. detector.

Linearity

Appropriate aliquots of standard stock solution were taken in different 10 ml volumetric flasks and diluted up to mark with mobile phase to obtain final concentration of 10ppm, 20ppm, 30ppm, 40ppm and 50ppm of VLS respectively. The solutions were injected using a 20µg/ml fixed loop system and chromatograms were recorded.

Precision

The precision of the analytical method is determined by assaying sufficient number of samples and relative standard deviation is calculated.

Method

Preparation of Standard Stock Solution

Accurately weigh 10mg of VLS and transferred into 10ml volumetric flask and dissolved in methanol and volumes were made up to the mark with diluent. 1ml of above

solution is diluted to 10 ml with diluent to obtain the concentration of 100µg/ml of VLS.

Preparation of Working Standard Solution

From the standard stock solution aliquots of 1, 2, 3, 4,5ml were transferred it into a five different 10ml volumetric flasks. The volumes were made up to the mark with the diluent to obtain the concentration of 10, 20, 30, 40, 50µg/ml of VLS.

20µl of various working standard solution was injected and obtained chromatograms were recorded.

Accuracy

Accuracy was found out by recovery study[5] using standard addition method. It was conducted by three replicate measurements at three different concentrations as low, medium, high quality control samples.

Robustness

The robustness of the analytical method is determined by analysis of aliquots from homogenous lots by varying different physical parameters, but still within the specified parameters of the assay. For example change parameters like flow rate, mobile phase ratio and detection wavelength.

Method

20µl of working standard solution were injected in different chromatographic conditions and chromatograms were recorded.

Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantified by the analytical method. The detection limit is usually expressed as the concentration of analyte (parts per million).

It is determined based on the standard deviation (σ) of response and the slope (S). The detection limit may be expressed as

$$DL = 3.3\ \sigma/S$$

Limit of Quantification

The quantitation limit of an analyte procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

It is determined based on standard deviation (σ) of the response and the slope (S).

Quantitation limit may be expressed as

$$QL = 10\ \sigma/S$$

RESULTS AND DISCUSSION

Optimized chromatographic conditions

Different conditions were employed to develop a rapid and economic method for estimation of VLS. The optimized conditions were found by using phenomenex C18 (250×4.6mm, 5µ) with a flow rate of 1.2ml/min. the mobile phase consisted of methanol and phosphate buffer, pH 2.5 (75: 25) with the detection carried out at 250nm. The retention time was found to be 5.16min (figure 2).

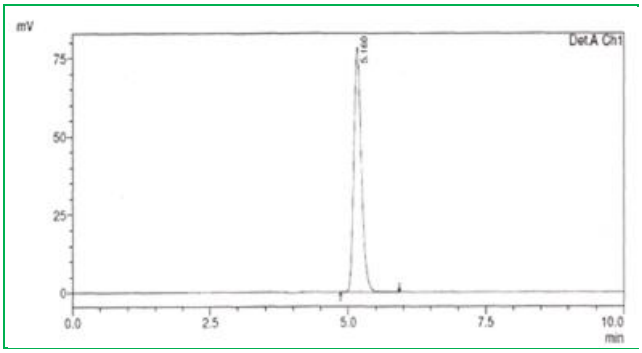

Figure 2: Chromatogram for Valsartan

METHOD VALIDATION

Calibration Curve for Valsartan

Calibration curve was drawn by plotting average peak area versus concentration as shown in figure 3. The linearity range was found to be 20-100µg/ml. The regression equation for Valsartan was found to be y =28023x + 39129 and correlation co-efficient, r^2 = 0.9953. The linearity data of Valsartan is shown in Table-1. The limits for detection and quantification are also shown in the same table.

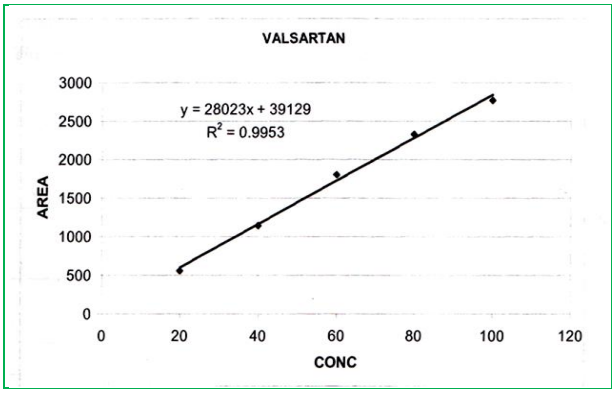

Figure 2: Linearity curve of Valsartan

Table 1: Linearity data for Valsartan

Concentration (µg/ml)	Peak Area
20	559296
40	1145744
60	1801392
80	2323373
100	2772803
Correlation coefficient	0.9953
Slope (m)	28023
Intercept(c)	39129
LOD	$3*10^{-4}$µg
LOQ	0.002µg

The % relative standard deviation was found to be 0.77 (table 2) which suggests that the method is precise.

Table 2: Precision of proposed HPLC method

Preparation	Retention time	Area obtained
Preparation-1	5.232	1572312
Preparation-2	5.223	1535785
Preparation-3	5.198	1544413
Preparation-4	5.187	1598823
Preparation-5	5.175	1600229
Average	5.203	1570312
Standard Deviation	0.040305	19740.3
% RSD	0.77	1.25

The results for accuracy studies, performed by measuring recovery are shown in table 3. The results show a % recovery of 99.1- 99.8% suggesting that the method developed is very accurate.

Table 3: Accuracy studies

Recovery level	% Recovery
80% accuracy	
Preparation -1	99.1
Preparation -2	99.7
Preparation -3	99.6
Average	99.4
100% accuracy	
Preparation -1	99.3
Preparation -2	99.8
Preparation -3	99.5
Average	99.5
120% accuracy	
Preparation -1	99.6
Preparation -2	99.3
Preparation -3	99.6
Average	99.5

The data when flow rate is changed is given in table-4. From the results, it can be seen that the method is robust.

Table 4: Robustness (flow rate)

S. No.	Flow rate 1.0ml/minute		Flow rate 1.2ml/minute		Flow rate 1.4ml/minute	
	Retention time	Area obtained	Retention time	Area obtained	Retention time	Area obtained
1.	6.445	2757.435	5.121	2231.471	3.937	1811.801
2.	6.392	2742.214	5.139	2275.800	3.941	1824.134
3.	6.378	2754.428	5.128	2289.725	4.112	1818.132
4.	6.395	2752.128	5.123	2205.994	4.124	1814.187
5.	6.394	2751.357	5.127	2212.383	4.130	1821.143
SD	0.0256	5.7105	0.0069	37.7452	0.0145	5.0103
RSD	0.004	0.00207	0.0013	0.01682	0.0035	0.0027
%RSD	0.4	0.2	0.1	1.6	0.3	0.2

CONCLUSION

The results suggest that a suitable, easy, less time consuming validated method has been developed for Valsartan. The RP-HPLC procedure was optimized with a view to develop accurate and stable assay method with the pure drug. A C18 phenomenex, 250*4.6mm column in isocratic mode, with mobile phase methanol: buffer (75:25) was used. The flow rate was 1.2ml/min and identical components were measured with U.V.Detector at 250nm. Linearity was assessed by plotting concentration vs. Area which is shown in Fig: 2 with the linearity in the range of 20-100µg/ml with correlation coefficient of 0.995 with good linearity response. The % recovery was found to be within limits of the acceptance criteria with mean recovery of 99.5%. Robustness, LOD and LOQ were determined and results are given. The results of the validation suggested that the developed RP-HPLC method could be employed successfully for the estimation of VLS.

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