



BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF VALSARTAN IN RABBIT PLASMA

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ABSTRACT

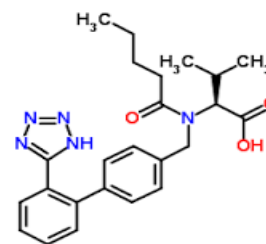
Objective: To develop a liquid–liquid extraction based reversed-phase high performance liquid chromatography method for estimation of valsartan in rabbit plasma. **Methods:** Chromatographic separation was carried out using ODS Thermosil C₁₈ (250mm×4.6mm) 5µm, with mobile phase composed of ammonium acetate buffer (20mM) and acetonitrile in 60:40 v/v ratio, pH 6.5. The analyte was monitored with UV detector at 265nm. The developed method was validated with respect to specificity, linearity, accuracy, precision, LOD, LOQ and ruggedness. **Results:** The peak area ratio of valsartan to that of internal standard was used for the quantification of samples. The flow rate was 1.0 ml/min and the eluent was monitored at 265 nm. Calibration curves were linear in the concentration range of 10-200 ng/mL. The LOD and LOQ of present method were found to be 1.8 ng/mL and 5.4 ng/mL respectively. Extraction recoveries of drug from rabbit plasma were found to be >90 %. **Conclusion:** A simple, specific, reproducible and sensitive RP-HPLC method was developed and validated for the estimation of valsartan in rabbit plasma.

Keywords: RP-HPLC method, Valsartan, liquid–liquid extraction, rabbit plasma.

INTRODUCTION

Valsartan is an angiotensin-receptor blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. Valsartan lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II. Unlike angiotensin-converting enzyme (ACE) inhibitors, ARBs do not have the adverse effect of dry cough. Valsartan may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of heart failure,

systolic dysfunction, myocardial infarction and coronary artery disease ^[1].



Structure of Valsartan

MATERIALS AND METHODS

Reagents and Materials: Valsartan and Losartan were obtained as gift samples from Dr. Reddy's

Laboratories Ltd., Hyderabad. All other reagents were purchased from Standard Reagents Pvt. Ltd., Hyderabad. HPLC Grade water (0.45 µm membrane filtered) was produced in the laboratory.

Chromatographic instrumentation: The analysis was carried out on a Shimadzu corporation Kyoto Japan LC-2010AT HPLC pump, SPD-20A UV detector, Rheodyne injector equipped with low pressure quaternary gradient pump along with dual wavelength UV detector, column oven, auto sampler and LC solution 1.2 SP1 software. ODS Thermosil C₁₈ (250×4.6mm), 5µ column was used for drug separation. The analyte was monitored with UV detector at 265 nm. A glass vacuum-filtration apparatus (fitted with 0.22 µ filter) was used to filter mobile phase. Ultrasonic bath was used to remove dissolved gases and entrapped air in mobile phase. A model Genie-2 Spinix vortex mixer, a cold centrifugation (Sigma, Germany) were employed during sample processing and TurboVap LV Evaporator (Caliper Life Sciences, Hopkinton, MA, USA) for evaporation purpose after extraction^[2, 3].

Chromatographic conditions: Chromatographic separation was carried out using ODS Thermosil C₁₈ (250×4.6mm) 5µ, with mobile phase composed of ammonium acetate buffer (20mM) and acetonitrile in 60:40 v/v ratio, pH 6.5. The analyte was monitored with UV detector at 265nm (Table 1).

Preparation of standard solutions: All standard stock solution and working standard solutions were prepared in volumetric flask. 100 mg of valsartan was dissolved in mobile phase to give standard stock solution of 100 mg/mL. Losartan potassium was used as an internal standard (IS). The working standard solutions were prepared by further serial dilution from the stock solutions with mobile phase mixture. Calibration standards of 10, 20, 50, 80,100, 120, 150 and 200 ng/mL in control rabbit plasma samples were prepared by mixing with 20 µL of working stock solutions of Valsartan^[4].

Sample preparation: Frozen plasma samples were obtained from National Institute of Nutrition, Hyderabad, thawed and allowed to reach room temperature. An aliquot of 200 µL of rabbit plasma sample was mixed with 10 µL of internal standard solution. Subsequently, sample was extracted with 1.5 ml of tertiary-butyl methyl ether (TBME). The mixture was vortexed for 15 min and centrifuged at 3,000 rpm for 10 min. After centrifugation, the organic phase was transferred to glass tubes and evaporated to dryness using TurboVap LV Evaporator at 50⁰C under stream of nitrogen for 5

min. The residue remaining after drying was reconstituted with 150 µL of mobile phase mixture and vortexed for 1 min then, a volume of 20 µL was injected into the HPLC system^[5, 6].

Validation of method^[7]: Validation of the developed method was carried out as per ICH guidelines for specificity, linearity, accuracy, precision, LOD, LOQ and ruggedness.

Specificity: The specificity of method was proved by processing and analyzing blanks prepared from six independent lots of control plasma along with six extracted LOQ-QC samples. The method is selective if there is no interfering peak present at the retention time of the drug or IS. If there is any interfering peak present at the retention time of drug then its response should be less than 20% of mean response of six extracted LOQ-QC samples.

Linearity: A calibration curve is the relationship between instrument response and known concentrations of the analyte. The linearity of developed method was evaluated with a total of three calibration curves over the concentration range of 10-200 ng/mL by plotting the peak response (area) ratio of valsartan to Losartan potassium (y) versus concentration of valsartan in plasma (x) (Fig 4, Table 2).

Accuracy and precision: The different concentrations including lower and upper limits of each sample (LOQ, LQC, MQC and HQC) in 12 determinations were analyzed on the same day and on three different days in order to determine the intra-day and inter-day accuracy and precision. Accuracy studies of the method was determined by spiking the known amount of analyte to sample solution, with plasma and the percentage mean recovery was found to be 94.05 %, 94.42 % , 95.00 % and 98.68 % for 20 %, 50 %, 100 %, 150 % of Losartan respectively. Precision of the method and system were found to be within limits. % RSD of internal standard and standard was found to be 0.7, 0.8 respectively and the ratio of standard and internal standard is 0.73.

Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection (LOD) is defined as the lowest quantity of substance which gives a peak area of three times the baseline noise. The equation $2H/h$ was used to calculate the ratio of signal size to that of noise. Where, H is the height of the peak in a chromatogram obtained with the prescribed reference solution and h is the noise in blank chromatogram. The limit of quantification (LOQ) was defined as the lowest concentration with

ratio of signal-to-noise more than 5 with accuracy of 80-120% and precision of 20% to its nominal value. Limit of detection was found to be 1.8 ng/mL and limit of quantification was found to be 5.4 ng/mL for Valsartan.

Ruggedness: The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts. % RSD Assay values between two analysts were not greater than 2.0 %.

CONCLUSION

A simple, specific, reproducible and sensitive RP-HPLC method was developed for the estimation of Valsartan in rabbit plasma. The method was validated over concentration range of 10-200 ng/mL with correlation coefficient of 0.998 and was found to offer good accuracy and precision. The exclusive advantage of the method is the small processing volumes used for extraction without jeopardizing the sensitivity. This method can be used to estimate the concentration of Valsartan in rabbit plasma in routine analysis.

Table 1: Summary of Chromatographic conditions

S. No	Parameters	Description
1.	Column	ODS Thermosil C ₁₈ (250×4.6mm) 5μ
2	Mobile Phase	Ammonium acetate: Acetonitrile , 60:40 v/v
3	pH	6.5
4	Flow rate	1.0 mL/min
5	Column & sample temperature	Room temperature (20-25°C)
6	Detection Wavelength	265 nm
7	Detector	UV
8	Injection Volume	20 μL
9	Retention time	Valsartan – 2.6 min
10	Run time	6 minutes

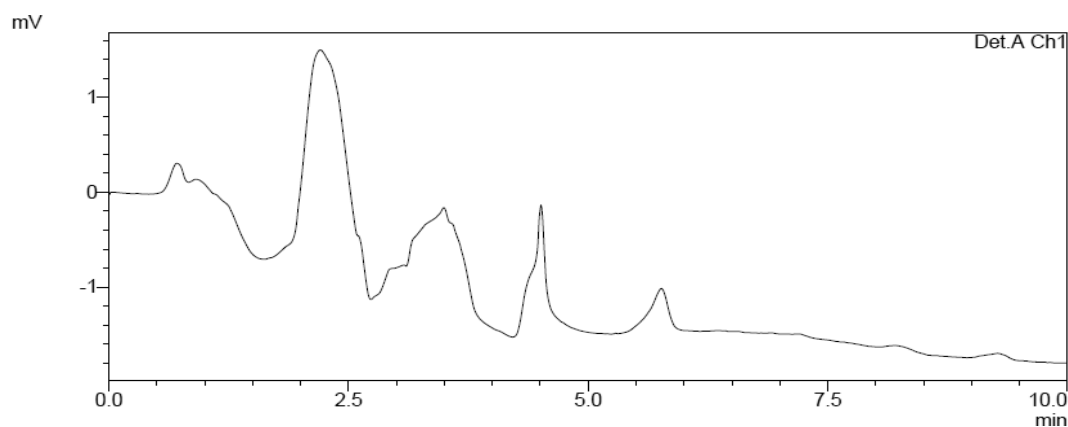


Fig 1: Typical chromatogram of blank

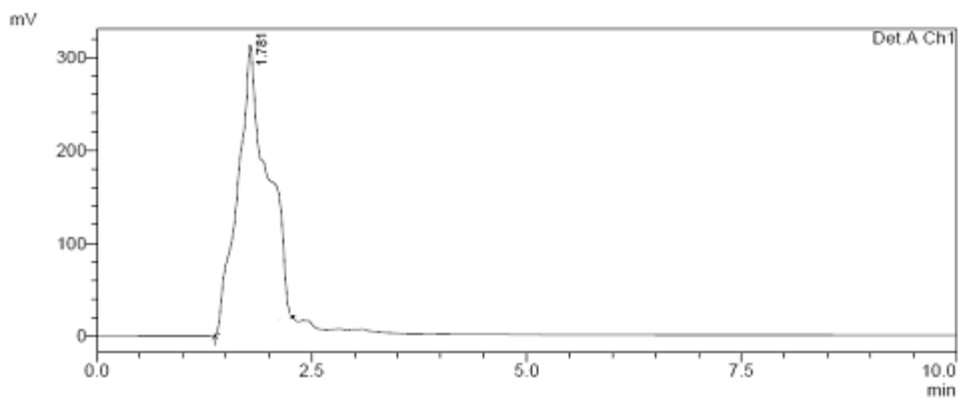


Fig 2: Typical chromatogram of plasma

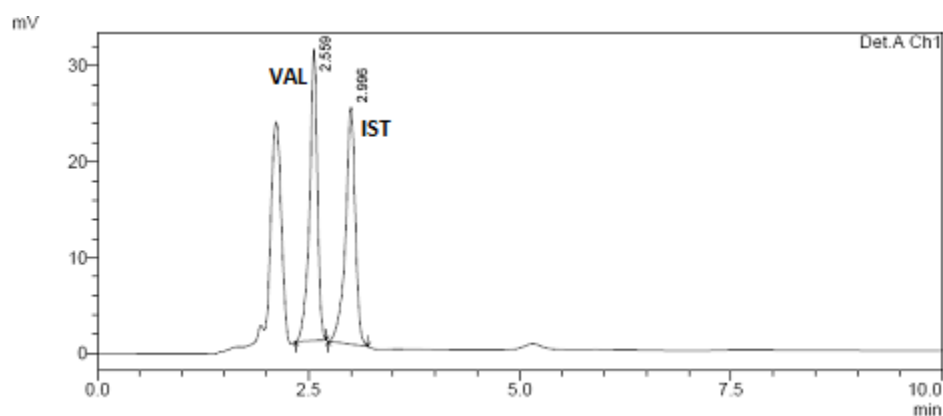


Fig 3: Typical chromatogram of Valsartan with internal standard

Table 2: Calibration curve of Valsartan

Concentration (ng/mL)	Area
10.0	5672
20.0	13727
50.0	99280
80.0	181031
100.0	242821
120.0	295266
150.0	378652
200.0	525109

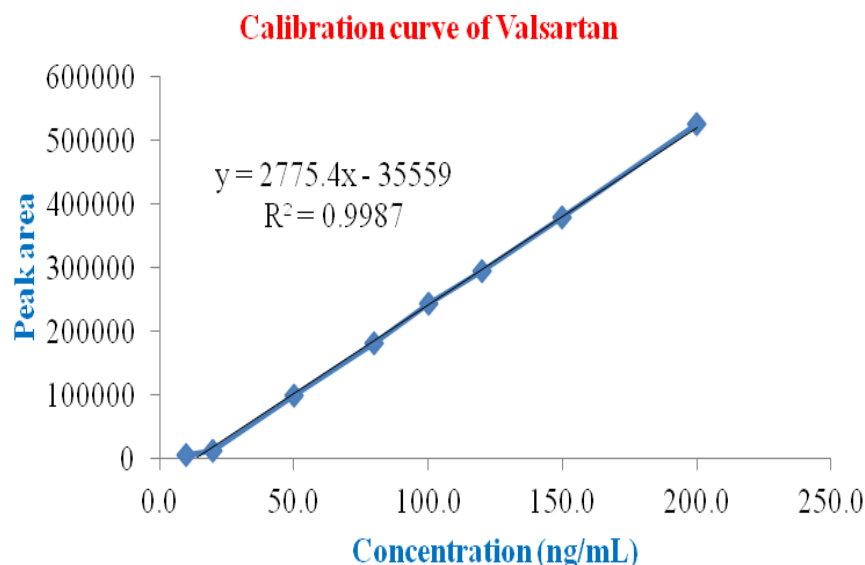


Fig 4: Calibration curve of Valsartan

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