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DEVELOPMENT AND VALIDATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY METHOD FOR VALSARTAN

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ABSTRACT
To develop an easy assay for the quantitation of valsartan the angiotensin II receptor antagonist. An isocratic separation was conducted by a reversed phase column (ODS) of a 150 mm in length x 4.6 mm with precolumn internal diameter and an average silica particle size of 5µm at room temperature. Mobile phase was a mixture of acetonitrile: potassium dihydrogen phosphate buffer solution (70: 30% v/v) at a flow rate of 0.8 mL/min and detected 273 nm. The response to 5–40 µg/ml valsartan was linear. The developed method was validated with respect to linearity, accuracy (recovery) and precision. This convenient method is suitable for determination of valsartan.

KEYWORDS: Valsartan, Losartan, HPLC, Validation.

1. INTRODUCTION

Valsartan ((*S*)-*N*-valeryl-*N*-[29-(1*H*-tetrazol-5-yl)biphenyl-4-yl)-methyl]-valine; Fig. 1 is an orally active angiotensin II receptor antagonist used as a drug for reduction of blood pressure. Valsartan is rapidly absorbed after oral administration but unfortunately suffers from low bioavailability of about 23% and the peak plasma concentration is achieved upon dosing in 2 to 4 hours with a half-life of 6 hours.[1-6]

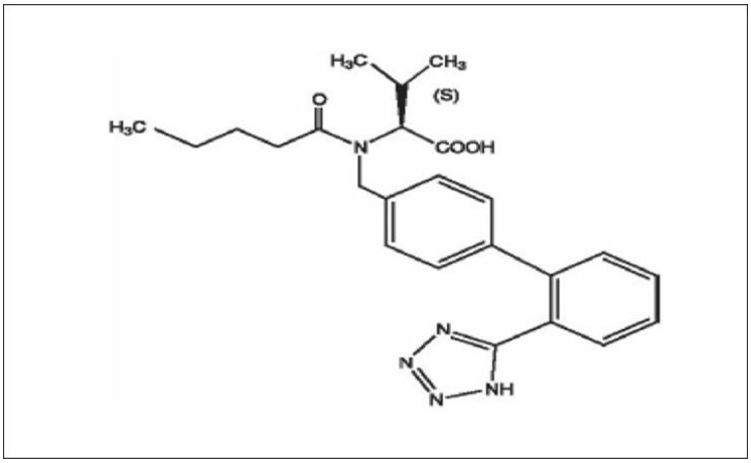

Fig. 1: Chemical structure of valsartan.

2. Experimental

2.1. Chemicals

Valsartan and losartan potassium were obtained as a gift form EIPICO Pharmaceutical Company (Cairo, Egypt). Potassium dihydrogen phosphate was procured from El-Nasr Pharmaceutical Chemicals Company (Cairo, Egypt). Acetonitrile and Methanol (HPLC-grade) were purchased from Fisher Scientific (Leicester, England).

2.2. Instrumentation

High performance liquid chromatographic system (Knauer Azura P6.1L, Germany) was utilized. The utilized system is a combination of variable single wavelength UV detector (UVD 2.1L) and sampling system (KNAUER, D-14163, Berlin). The control over this system is computerized using Knauer Clarity-Chrom software. Separation process was isocratic and it was

performed on 150 mm in length x 4.6 mm internal diameter reversed phase column (ODS) (Knauer Eurospher II 100-5, Germany). The average silica particle size of the column was 5µm. Bath sonicator from ultrasonic 57X, Clean America (NY, USA) was employed in this work. The pH was adjusted using pH meter fDigi-Sense®, Cole-Parmer Instrument Company, Niles, IL 60714 (USA). In addition, mixing was performed using vortex mixer from Maxi mix 11, thermolyne corporation (USA).

2.3 Preparation of mobile phase

Isocratic elution was used in the separation, the mobile phase was formed by mixing a pre-filtered 10 mM of potassium dihydrogen phosphate buffer solution (pH was adjusted to 3 with ortho-phosphoric acid) and acetonitrile (30:70). The prepared mobile phase was degassed by sonication. The flow rate was 0.8 ml/min and losartan was incorporated as internal standard, variable wavelength UV detector 273 nm was used.

2.4 Preparation of the standard stock solution

A concentrated drug solution of valsartan was done via dissolving 10 mg of the drug in 100 ml methanol to obtain a solution containing 100 µg/ml drug.

2.5 Preparation of the Standard working solutions

The stock solution was used to prepare drug solutions containing 5, 10, 20, 30, 40 µg/ml of valsartan for calibration standards.

2.6 Preparation of losartan as internal standard

A concentrated solution of losartan was fabricated by dissolving 40 mg of the drug in 100 ml methanol (HPLC grade), resulting in a solution containing 400 µg/ml losartan. 50 µl of losartan solution were transferred to five clean test tubes, then 1 ml from each working solution was added and vortexed.

2.7 Calculations

Peak area ratio between valsartan and losartan was calculated and plotted against the concentration.

2.8 Description of the assay

Calibration standards: Standard drug solutions were prepared to contain different concentrations of 5, 10, 20, 30, 40 µg/ml.

Samples preparation: After mixing the samples with the internal standard, samples were treated as describes under standard samples.

2.9 Assay validation

Validation of analytical procedures is completed to reveal that the technique is appropriate for its proposed use. Validation is performed aiming to show that the result(s) generated by a particular analytical procedure are reliable and accurate.[7] Validation of these assays was conducted in compliance with the requirements of the International Conference on Harmonization (ICH) guidelines (ICH, 1996). The validation parameters included Accuracy, Precision, Detection Limit, Quantitation Limit, Linearity and Range.

- ***Linearity and Range***

The analytical procedure linearity is its ability, throughout a given range, to achieve test results that are directly relative to the concentration of analyte in the sample.[7] The linearity is estimated from the correlation coefficient (R^2) of the regression line fitted to the calibration curve.

The Range of an analytical process is the intermission between the highest and lowest analyte concentration in the sample (counting these concentrations) for which it has been confirmed that the analytical procedure has a proper level of accuracy, precision and linearity.[7]

- ***Accuracy***

The accuracy reflects the nearness of the recorded concentrations to the theoretical concentrations.[7] The accuracy (expressed as % recovery) was thus assessed by comparing the nominal amount of the drug in the standards to the recovered amount.

- ***Precision***

Reflects the nearness of agreement (degree of scatter), between a sequence of measurements obtained for numerous samples of the identical homogeneous sample under the prescribed conditions.[7]

The precision is stated as Relative Standard Deviation (RSD). The mean and SD of different concentrations analyzed in different days (different concentrations injected every day for 3 days) were used to calculate the interday precision. To compute the intraday precision, different concentrations of the drug were injected three times within the same day. These were used to estimate the mean and the SD for each concentration.

The RSD (%) was obtained by dividing the standard deviation by the mean of each corresponding standard concentration and expressing the value as percentage. This RSD should be lower than 2% for the method to be precise in case of measuring *in vitro* samples and for compound analysis in pharmaceutical quality control.

- ***Detection limit***

The detection limit is the lowermost amount of analyte in a sample which can be detected, but not essentially quantitated, as an exact value.[7] It can be determined by signal/noise ratios of 3:1 or using the next equation:

$LOD=3.3*SD_{intercept}/slop$(ICH, 1996)

This equation was adopted in the current study.

- ***Quantitation limit***

The limit of quantitation is the lowermost amount of analyte in a sample which can be quantitatively determined with appropriate precision and accuracy.[7] It can be determined by signal/noise ratios of 10:1 or using the next equation:

LOQ=10*SD $_{intercept}$/slop (ICH, 1996)
This equation was used in our study.

3. RESULTS AND DISCUSSION

3.1 Chromatography

The mobile phase was optimized in an isocratic condition so as to obtain sharp symmetric peak with good separation from other peaks in the chromatogram. Fig. 2 shows representative chromatograms for valsartan.

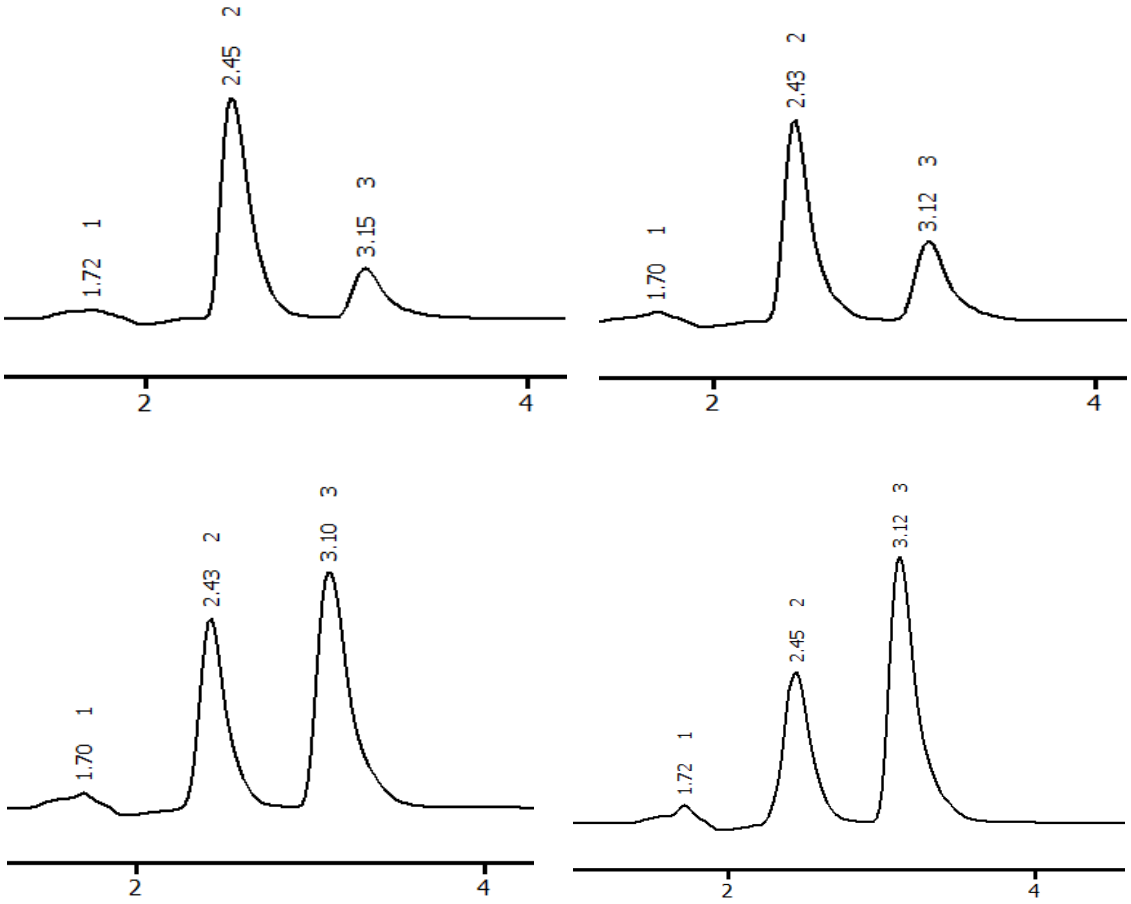

Fig. 2: Representative chromatograms of valsartan and losartan obtained after injecting different concentrations of drug solution (5, 10, 30 and 40 µg/ml).

The chromatograms reveal sharp symmetric peak for the drug with the retention time being recorded after 3.13 ± 0.02 minutes with internal standard losartan.

The constructed calibration curves are presented in Fig. 3 The mean slope and intercept for calibration curves of valsartan are shown in Table 1.

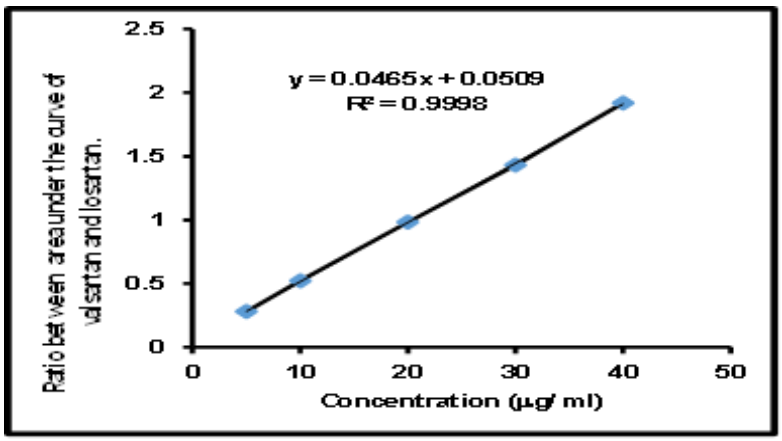

Fig. 3: The calibration curve of valsartan when using losartan as internal standard.

Table 1: Mean slope and intercept for different calibration curves of valsartan.

	Mean ± SD
Slope	0.0465± 0.009
Intercept	0.0509± 0.0017

3.2 Method validation

Linearity and Range

The calibration curve is a plot of peak area ratio between valsartan and losartan as a function of drug concentration (C).

This is shown in Fig 2. The calibration curve reflected good linearity of the assay in the concentrations range of (5-40 µg/ml) as indicated from the value of the correlation coefficient obtained after fitting the data to straight line equation (0.999). The linear regression of the calibration curve of valsartan produced an equation of

$y = (0.0465 \pm 0.009)\ x \pm (0.0509 \pm 0.0017)$

Where

Y is the peak area ratio

0.0465 is the slope

X is the drug concentration

0.0509 is the intercept

The correlation coefficient was always higher than 0.999 during the course of the validation

Accuracy

The accuracy was expressed as the closeness to the true value and is calculated as the percent recovery related to the nominal values. Tables 2 and 3 present the percentage of drug recovered relative to the nominal values. The calculated % recovery values were in the range of 99.5- 101.8% and 99.6-100.8% for the intraday and interday accuracy, respectively suggesting the accuracy of the assay.

Precision

The precision was measured as the Relative Standard Deviation (RSD) expressed as percentage over the concentration range of hydrochlorothiazide during the course of validation. This is presented in Tables 2 and 3 for the intraday and interday precision. For both intraday and interday tests, the results showed acceptable precision for all concentrations assayed. This is clearly indicated by the recorded lower values for the RSD which were in the range of 0.45-1.2% and 0.23-1.18% for the intraday and interday precision, respectively.

Limit of Detection and Limit of quantitation

The limit of detection of the drug was calculated to be 0.65µg/ml and the limit of quantitation was 1.98µg/ml.

Table 2: Intraday validation parameters of valsartan (n=3).

Nominal value (µg/ml)	Recovered concentration (µg/ml)	SD (µg/ml)	% RSD	%Recovery
5	4.98	0.022	0.45	99.6
10	10.18	0.121	1.19	101.8
20	20.05	0.198	0.99	100.3
30	29.69	0.289	0.97	98.9
40	40.19	0.211	0.52	100.5

Table 3: Inter-day validation parameters of valsartan (n=3).

Nominal value (µg/ml)	Recovered concentration (µg/ml)	SD (µg/ml)	% RSD	%Recovery
5	5.04	0.033	0.66	100.8
10	9.97	0.113	1.13	99.7
20	19.9	0.036	0.18	99.6
30	30.1	0.168	0.56	100.3
40	39.9	0.09	0.23	99.9

4. CONCLUSION

The present HPLC assay of valsartan offers advantage over those previously reported in term of convenience of using a commercially available internal standard losartan. The use of losartan as the internal standard indicates that the assay may be suitable for determination of the latter as well. The method has sufficient sensitivity for the studies of valsartan.

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