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Enantiomeric LC separation of valsartan on amylose based stationary phase

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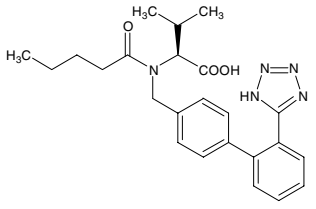
A simple, rapid and robust LC method was developed and validated for the enantiomeric separation of valsartan in bulk drug and formulation. The enantiomers of valsartan were resolved on a Chiralpak AD-H (amylose based stationary phase) column using a mobile phase consisting of n-hexane: 2-propanol: trifluoroacetic acid (85 : 15 : 0.2, v/v/v) at a flow rate of 1.0 mL/min. The resolution between the enantiomers was found to be not less than 3.2. The presence of trifluoroacetic acid in the mobile phase played an important role in enhancing chromatographic efficiency and resolution between the enantiomers. The calibration curve for the (R)-enantiomer showed excellent linearity over the concentration range of 600 ng/mL (LOQ) to 6000 ng/mL. The limit of detection and limit of quantification for the (R)-enantiomer were 200 and 600 ng/mL, respectively. The percentage recovery of the (R)-enantiomer ranged between 98.7 to 100.05 % in bulk drug samples of valsartan. The proposed method was found to be suitable and accurate for quantitative determination of (R)-enantiomer in bulk drug substance.

1. Introduction

Valsartan, (*S*)-*N*-(1-carboxy-2-methylprop-1yl)-*N*-pentanoyl-*N*-[2′-(1*H*-tetrazol-5-yl)-biphenyl-4ylmethyl] amine (Lars and Torp-Pederson 2005), is an angiotensin II antagonist for the treatment of essential hypertension (Corea et al. 1996) developed from a structurally new amino acid series (Buhlmayer et al. 1994). The (*R*)-enantiomer of valsartan failed to affect plasminogen activator I (PAI) activity induced by angiotensin-II in contrast to the (*S*)-enantiomer, indicating that the effect of the drug is stereospecific (Sironi et al. 2001). Owing to the pharmacological and toxicological difference between these enantiomers, it is quite important to develop an enantiospecific LC method for quality assurance of drugs.

Recent global advance in new regulatory guidelines for racemic or 'pure' pharmaceutical products necessitate development of rapid, sensitive and reproducible methods for quality control of optical antipodes present in drug substance.



A few HPLC methods were reported in the literature for the quantitative determination of valsartan in tablets (Tatar et al. 2002; Satana et al. 2001) human serum and human biological fluids (Macek et al. 2006; Nie et al. 2005). There is only one chiral high-performance liquid chromatography method for enantiomeric separation of valsartan by using a Chiral AGP (α-acid glycoprotein) column in the reversed phase mode (Francotte et al. 1996).

Polysaccharide-based stationary phases are quite popular with wide recognition for direct resolution of enantiomers. To the best of our knowledge, there were no validated LC methods for determination of the enantiomeric purity of valsartan in bulk drugs and formulations. In the present investigation, we report the development and validation of a normal-phase LC method using polysaccharide amylose based stationary phase (Chiralpak AD-H) column for determination of enantiomeric purity of valsartan in bulk drugs and in pharmaceutical dosage forms. We developed a rapid enantioselective and robust method with short analysis time and better resolution. The developed method was validated in view of linearity, accuracy, precision, LOD, LOQ and robustness.

2. Investigations and results

To develop the suitable chiral HPLC method for the separation of the enantiomers of valsartan, different mobile phases and stationary phases were employed. For this, different chiral columns were used namely Chiralcel OJ-H and Chiralpak AD-H. The enantiomeric separation for valsartan was not achieved by Chiralcel OJ-H using the mobile phase hexane : 2-propanol, 80 : 20. There was an indication of separation on Chiralpak AD-H column using the mobile phase n-hexane : 2-propanol (70 : 30, v/v) but the peak shape was broad. For further improvement in resolution, peak shape

and column efficiency, the peak modifier trifluoroacetic acid was used. Better separation was achieved on a Chiralpak AD-H column (resolution between enantiomers was found to be >3.0) using the mobile phase n-hexane : 2-propanol : trifluoroacetic acid (85 : 15 : 0.2, v/v/v) within an analysis time less than 7 min.

The system suitability was determined by injecting a racemic mixture containing equal quantity of the (R)-enantiomer and valsartan. The qualification criteria was resolution between two enantiomers, shown to be not less than 3.2 and the tailing factor should not exceed 1.5.

Method reproducibility was determined by measuring repeatability and intermediate precision (between-day precision) of retention times and peak areas for each enantiomer. In order to determine the repeatability of the method, replicate injections (n = 6) of a 1.0 mg/ml solution containing valsartan spiked with (R)-enantiomer (0.5%) were carried out. The intermediate precision was also evaluated over three days by performing six successive injections each day.

Linearity was assessed by preparing six calibration sample solutions of (R)-enantiomer with concentrations from 600 ng/mL (LOQ) to 6000 ng/mL (600, 1800, 2700, 3600, 4500 and 6000 ng/mL), prepared in mobile phase from (R)-enantiomer stock solution.

The regression curve was obtained by plotting peak area versus concentration, using the least squares method. The percentage relative standard deviation of the slope and Y-intercept of the calibration curve were calculated.

The S-valsartan bulk sample does not show the presence of (R)-enantiomer. Standard addition and recovery experiment were conducted to determine the accuracy of the present method for the quantification of (R)-enantiomer in bulk drug samples.

The study was carried out in triplicate at 0.4, 0.5 and 0.6% of the S-valsartan target analyte concentration. The recovery of (R)-enantiomer was calculated from the slope and Y-intercept of the calibration curve

Ten tablets of Diovan (80 mg valsartan) were finely ground using an agate mortar and pestle. The ground material, which was equivalent to 500 mg of the active pharmaceutical ingredient (valsartan), was extracted into methanol in 100 ml volumetric flask by vortex mixing followed by ultrasonication. The resultant mixture was filtered through a 0.45-μm-membrane filter. The filtrate was used as stock solution for preparing the accuracy test solutions. Filtrate (10 ml) was taken in a 100 ml volumetric flask and made up to the volume with the mobile phase. This solution corresponds to an analyte concentration of 1.0 mg/ml.

Limit of detection and limit of quantification of (R)-enantiomer (LOD & LOQ) were achieved by injecting a series of dilute solutions of (R)-enantiomer (ICH 1995).The precision of the developed enantioselective method for (R)-enantiomer at limit of quantification was checked by analyzing six test solutions prepared at LOQ level and calculating the percentage relative standard deviation of area.

The flow rate of the mobile phase was 1.0 mL/min. To study the effect of flow rate on resolution of enantiomers, it was changed from 0.8 to 1.2 mL/min while the other mobile phase components were held constant. The effect of 2-propanol content on resolution was studied by varying from −1 to +1% and the effect of trifluoroacetic acid concentration on resolution was studied by varying from −0.1 to +0.1% while the other mobile phase components were held constant. The effect of column temperature on resolution was studied at 25 °C and 35 °C instead of 30 °C while other mobile phase components were held constant.

Stability of valsartan in solution at analyte concentration was studied by keeping the solution in tightly capped volumetric flasks at room temperature on laboratory bench for 2 days. Content of (R)-enantiomer was checked at 6 h intervals during the study period.

3. Discussion

The mechanism of chiral separation methods is the interaction of chiral stationary phase (CSP) with analytes to form short-lived, transient diastereomeric complexes. The complexes are formed as a result of hydrogen bonding, dipole-dipole interactions, pi bonding, electrostatic interactions, and inclusion complexation (Okamoto and Kaida 1994; Okamoto and Yashima 1998). As discussed earlier, enantiomers of valsartan could not be separated on Chiralcel OJ-H (Tris-4-methyl benzoate ester derivative of cellulose). The chiral stationary phase (CSP) that gave the best resolution was Chiralpak AD-H (3,5-tris-dimethylphenylcarbamate derivative of amylose coated on silica gel). The separation of valsartan enantiomers on chiralpak AD-H was due to the interaction between the polar group of analytes and the polar carbamate group on the CSP. The carbamate group on the CSP interacts with the NH group of analytes through hydrogen bonding, the oxygen atom of valsartan form dipole-dipole interaction between CSP. The π−π interaction occurred between phenylcarbamate and the valsartan aromatic ring, steric fit which are stabilized by insertion of aromatic portion of valsartan in to chiral grooves (asymmetric centers). Amylose forms a helical structure and possesses more defined grooves (asymmetric centers) different from cellulose derivatives. These polysaccharides contain a large number of chiraly active sites and thus a relative high probability of interaction with the solute leading to separation of two enantiomers. Peak tailing may result from silanol effect. A small amount of trifluoroacetic acid acidic as modifier in the mobile phase when analytes contain acidic functions reduces peak tailing by masking the residual silanol group of the chiral stationary phase.

A representative chromatogram of valsartan enantiomers is shown in Fig. a, showing an excellent resolution ($R_s = 3.9$)

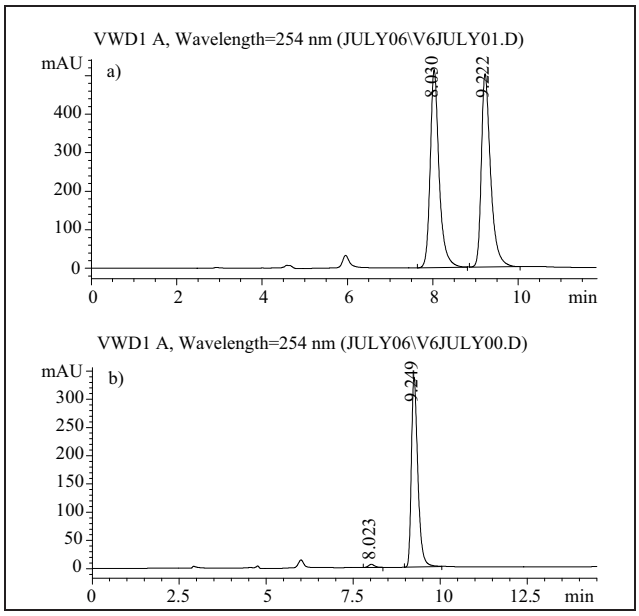


Fig.: a) Enantiomeric resolution of valsartan on Chiralpak AD-H column. Mobile phase consisted of n-hexane: 2-propanol: TFA (85 : 15 : 0.2, v/v/v), flow rate 1.0 mL/min, UV-254 nm; column temperature 30 °C. b) Typical HPLC chromatogram of (S)-valsartan bulk sample (1.0 mg/mL) spiked with (R)-enantiomer (0.5%)

Table 1: System suitability report

Enantiomers	R_t (min)	α	Rs	N	T
(R)-Enantiomer	8.0	1.14	3.9	6848	1.1
(S)-Enantiomer	9.22	–	–	5840	1.2

n = 3, R_t – retention time, α – enantioselectivity, Rs – USP resolution, N – number of theoretical plates (USP tangent method) and T – USP tailing factor

between the two enantiomers, and a symmetric peak shape with tailing 1.2 was obtained. In the optimized method, the typical retention times of (R)-enantiomer and valsartan were about 8.0 and 9.2 min, respectively. The system suitability of the chiral liquid chromatographic method on Chiralpak AD-H is presented in Table 1.

In the precision study, the percentage relative standard deviation (R.S.D.) was less than 0.7% and 0.9% for the retention times of the (R)-enantiomer and valsartan respectively. Peak area (R.S.D.) was 0.8% for valsartan and 1.2% for (R)-enantiomer (Table 2). In the intermediate precision study, the results showed that R.S.D. values were in the same order of magnitude than those obtained for repeatability (Table 2).

The limit of detection (LOD) and limit of quantification (LOQ) concentration were estimated to be 200 and 600 ng/mL for the (R)-enantiomer, when signal-to-noise ratios of 3 and 10 were used as the criteria. The method precision for (R)-enantiomer at limit of quantification was less than 1.9% R.S.D. (Table 2).

The described method was linear in the range of 600–6000 ng/mL for (R)-enantiomer in valsartan. The calibration curve was drawn by plotting the peak area of (R)-enantiomer versus its corresponding concentration with a correlation coefficient of 0.999. The equation of the calibration curve for (R)-enantiomer was $Y = 18967\ x - 9710.7$. The percentage relative standard deviation of the slope and Y-intercept of the calibration curve were 1.5 and 2.8, respectively (Table 2).

Recovery (Tables 3, 4) was calculated from slope and Y-intercept of the calibration curve, obtained in linearity study and percentage recovery ranged from 98.7 to 100.5% (Table 3). A HPLC chromatogram of spiked (R)-enantiomer at 0.5% level in valsartan sample is shown in Fig. b.

Table 2: Validation results of the developed liquid chromatographic method

Validation parameter	Results
Repeatability (n = 6, % R.S.D.)	
Retention time (R-enantiomer)	0.7
Retention time (S-enantiomer)	0.9
Peak area (R-enantiomer)	1.4
Peak area (S-enantiomer)	0.8
Intermediate precision (n = 9, % R.S.D.)	
Retention time (R-enantiomer)	0.6
Retention time (S-enantiomer)	0.7
Peak area (R-enantiomer)	1.2
Peak area (S-enantiomer)	1.5
LOD-LOQ (R-enantiomer)	
Limit of detection (ng/mL)	200
Limit of detection (ng/mL)	600
Precision at LOQ (% R.S.D.)	1.9
Linearity (R-Enantiomer)	
Calibration range (ng/ml)	600–6000
Calibration points	6
Correlation coefficient	0.999
Slope (% R.S.D.)	1.4
Intercept (% R.S.D.)	2.8

Table 3: Recovery results of (R)-enantiomer in bulk sample

Amount spiked (ng)	Amount found	Recovery (%)	% RSD
4005	3976	99.3	1.5
5002	5055	100.05	1.3
6004	5899	98.7	1.8

Table 4: Recovery results of (R)-enantiomer in commercial formulations

Amount spiked (ng)	Amount found	Recovery (%)	% RSD
4005	3956	98.7	1.9
5002	4950	98.9	1.7
6004	5805	96.8	1.8

The resolution between valsartan and (R)-enantiomer was greater than 3.0 and enantioselectivity (α) was better under all separation conditions tested, demonstrating sufficient robustness. As flow rate of mobile phase and column temperature was increased the resolution decreased to (2.78) and enantioselectivity was not affected (1.13). While percentage of 2-propanol in mobile phase was increased the resolution decreased (2.7) and selectivity also decreased to 1.15, while a higher percentage of trifluoro acetic acid in the mobile phase increased resolution (3.0) and selectivity (1.12).

The % R.S.D. of valsartan content during solution stability and mobile phase stability experiments was within 1.5%. Hence valsartan sample solution and mobile phase were stable for at least 48 h.

A simple, rapid and accurate chiral HPLC method has been developed and validated for the enantiomeric separation of valsartan. The method was completely validated with respect to accuracy, precision, linearity, LOD, LOQ and robustness as per ICH guidelines. The developed method can be conveniently used by the quality control department for the quantitative determination of chiral impurity (R-enantiomer) in the bulk drug substance.

4. Experimental

4.1. Chemicals and equipment

Samples of (R)-enantiomer and valsartan were obtained from Hetro labs limited (Hydrabad, India). Valsartan tablets (Diovan 80 mg) were purchased from Novartis Pharmaceutical limited (Mumbai, India). HPLC grade n-hexane was purchased from Qualigens Fine chemical (Mumbai, India). HPLC grade 2-propanol and trifluoroacetic acid (TFA) were purchased from Merck Ltd. (Mumbai, India).

HPLC system used was an Agilent Technology (1100 series, Germany) system equipped with auto sampler, quaternary pump, degasser and a UV Detector. The out putsignal was monitored and processed using Agilent Chemstation software.

4.2. Sample preparation

The stock solution of (R)-enantiomer and valsartan (5.0 mg/mL) was prepared by dissolving the appropriate amount of substance in methanol. For quantification of (R)-enantiomer in valsartan, a solution of 1.0 mg/mL concentration was used.

4.3. Chromatographic conditions

The chromatographic column used was 250 × 4.6 mm ChiralPak AD-H (Daicel Chemical Industries, Ltd., Tokyo, Japan) packed with 5 mm particles. The mobile phase was n-hexane: 2-propanol : trifluoroacetic acid (85 : 15 : 0.2, v/v/v). The flow rate of the mobile phase was 1.0 mL/min. The column temperature was maintained at 30 °C and the eluent was monitored at a wavelength of 254 nm. The injection volume was 10 µL. Cellulose based chiral stationary phase Chiralcel OJ-H (Diacel Chemical Industries, Ltd., Tokyo, Japan) was employed during the method development.

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