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Article

A Validated LC Method for the Determination of the Enantiomeric Purity of Aliskiren Hemifumarate in Bulk Drug Samples

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High-performance liquid chromatography enantioseparation of aliskiren hemifumarate was accomplished on an immobilized-type Chiralpak IC chiral stationary phase under both polar organic and reversed-phase modes. A simple analytical method was developed and validated using a mixture of acetonitrile–*n*-butylamine 100:0.1 (v/v/) as a mobile phase with a flow rate maintained at 1.0 mL/min. Ultraviolet detection was carried out at 228 nm. Resolution between the two enantiomers was greater than 3.0. This method was capable of detecting the R-isomer to a level of 0.2 μg/mL. The method was validated as per International Conference on Harmonization guidelines and found to be robust. The method is very useful for routine evaluation of the quality of aliskiren hemifumarate in bulk drug manufacturing units.

Introduction

Aliskiren (2(S),4(S),5(S),7(S)-N-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy2,7 diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl] octanamide hemifumarate) contains a new chemical entity, which belongs to the pharmacotherapeutic group of renin inhibitors. Aliskiren is used for the treatment of essential hypertension. Essential hypertension has been identified as a major risk factor for cardiovascular diseases. Several therapeutic choices are currently available to lower blood pressure (BP), including diuretics, β-blockers (BB), angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARB) and calcium channel blockers (CCB). Some of the currently used antihypertensive drugs intervene at different points of the renin-angiotensin system (RAS). Aliskiren exhibits a new mode of action compared with other drugs acting on the RAS. It selectively inhibits human renin, the enzyme responsible for the conversion of AGT to Ang I; therefore, the final production of the potent vasoconstrictor Ang II (increasing arterial tone, adrenal aldosterone secretion, renal sodium reabsorption, sympathetic neurotransmission and cellular growth) is inhibited by blocking the renin system at its very origin.

Aliskiren hemifumarate has four chiral centers, but is obtained as a single diastereoisomer, all S-configured. Development of an analytical method for the quantitative determination of stereoisomers in drug substances with chiral centers is an important and challenging task during drug synthesis and formulation research in the pharmaceutical industry. It is highly essential to monitor and control other isomers to meet stringent quality requirements. Although many analytical techniques like gas chromatography (GC), capillary electrophoresis (CE) and liquid chromatography (LC) can be employed for this purpose, LC with a chiral stationary phase (CSP) is the most widely used technique (1–4). To the best of our knowledge, no chiral high-performance liquid chromatography (HPLC) methods have been reported for the enantio separation of aliskiren. In this study, a simple and robust chiral HPLC method is developed and validated as per International Conference on Harmonization (ICH) guidelines.

Experimental

Chemicals

(*S*)- and (*R*)-isomers of aliskiren (Figure 1) were received from Dr. Reddy's Laboratories (Hyderabad, India). LC-grade acetonitrile purchased from Merck (Darmstadt, Germany) and *n*-butylamine was purchased from Loba Chemie (India). The purity of the (*S*)- and (*R*)-isomer impurities used in this study was greater than 98%.

Equipment

The LC system used for method development and method validation was a Waters 2695 binary pump plus autosampler and a 2996 photodiode array detector. The output signal was monitored and processed using Empower software on a Pentium computer (Digital Equipment Co.).

Chromatographic conditions

The chromatographic column was a Chiralpak-IC (250 × 4.6 mm) with 5-μm particles. The mobile phase consisted of 0.1% *n*-butylamine in acetonitrile (v/v). The flow rate of the mobile phase was 1.0 mL/min. The column temperature was maintained at 35°C and the detection was monitored at a wavelength of 228 nm. The injection volume was 20 μL. A mixture of acetonitrile and water in the ratio of 9:1 (v/v) was used as diluent.

Preparation of solutions

Stock solutions of both aliskiren enantiomers were prepared at 2-mg/mL concentrations by dissolving an appropriate amount in the diluent.

Results and Discussion

Method development

Several methods have been reported in the literature for the quantification of aliskiren (5–7), but no methods have reported

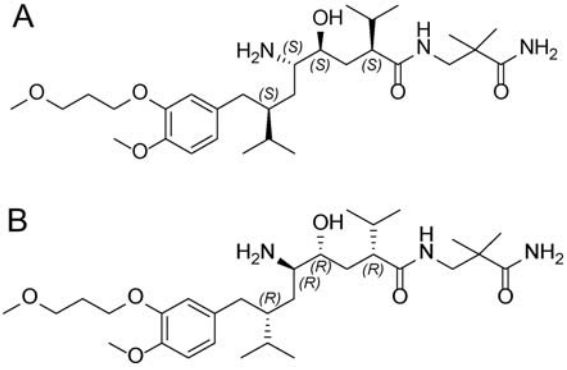

Figure 1. Chemical structures of aliskiren enantiomers: aliskiren, chemical name (2(*S*), 4(*S*),5(*S*), 7(*S*)- *N*- (2- carbamoyl-2- methylpropyl)-5-amino- 4-hydroxy2,7diisopropyl-[4-methoxy-3-(3-methoxypropoxy)phenyl] octanamide (A); aliskiren enantiomer, chemical name, (2(*R*),4(*R*),5(*R*),7(*R*)-*N*-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy2, 7 diisopropyl -8-[4-methoxy-3-(3-methoxypropoxy)phenyl] octanamide (B).

Table I
Chiral Selectivity Results on Various CSPs

Column	Mobile phase	Retention time	Resolution
Chiralpak AD-H	80:20 *n*-hexane–isopropyl alcohol; 1 mL/min	3.9 and 3.9	No resolution
Chiralpak OD-H	80:20 *n*-hexane–isopropyl alcohol; 1 mL/min	3.4 and 3.4 min	No resolution
Chiralpak -OJ	80:20 *n*-hexane–isopropyl alcohol; 1 mL/min	3.2 and 3.2 min	No resolution
Chiralpak -IA	80:20:0.1 *n*-hexane–sopropyl alcohol–diethyl amine; 1 mL/min	17.09 and 18.45	Less than 1.0
Chiralpak -IB	80:20:0.1 *n*-hexane–isopropyl alcohol–diethyl amine; 1 mL/min	4.32 and 4.32	No resolution
Chiralpak -IC	80:20:0.1 *n*-hexane–isopropyl alcohol–diethyl amine; 1 mL/min	4.75 and 4.75	No resolution
Cyclobond	90:10 0.1% acetic acid–acetonitrile, pH 4.0 with ammonia solution.	2.25 and 2.25	No resolution
Chiral AGP	95:5 0.01M ammonium acetate–methanol, pH 4.5 with acetic acid	5.7 and 6.06	Less than 1.0
Chiralpak IA	95:5:0.1 *n*-hexane–ethanoln-butylamine	16.32 and 17.77	1.54
Chiralpak-IC (reversed-phase mode)	100:0.1 acetonitrile–*n*-butylamine	14.53 and 11.81	3.23

for the separation and quantification of aliskiren enantiomers by HPLC. Initial method development experiments were screened on Chiralpak-AD-H, Chiralpak-OD-H, Chiralpak-OJ, Chiral-AGP, Cyclobond, Chiralpak-IA, Chiralpak-IB and Chiralpak-IC with mobile phases consisting of a mixture of *n*-hexane, isoprpyl alcohol and ethanol in different proportions; the results are shown in Table I Owing to the basic nature of the molecule, 0.1% diethylamine was also used for the better peak shape and selectivity. *n*-Hexane, isopropyl alcohol, ethanol and diethylamine were purchased from Merck. Separation was achieved on Chiralpak-IA and Chiralpak-IC columns, although the resolution was poor.

To further optimize the chromatographic conditions, the Chiralpak-IC column was used because it had higher selectivity than the other columns screened under this study. Chiralpak IC is the latest column in the immobilized polysaccharide category of columns. Its chiral selector *tris*(3,5-dichlorophenylcarbamate) was immobilized on silica gel and hence compatible with all kinds of solvents. The procedure of anchoring the semi-synthetic polymer to the silica matrix offers high chemical stability without any drawbacks in terms of enantioselectivity and efficiency. The immobilized polysaccharide-derived CSPs have been used under reversed-phase conditions for the resolution of many classes of chiral substances (8–9).

In the first part of our study, the resolving ability of the Chiralpak IC CSP toward the aliskiren isomer was investigated in the polar organic mode using pure methanol, ethanol and acetonitrile with a 0.1% addition of diethylamine (DEA). The best resolution was achieved by using the acetonitrile–DEA 100:0.1 (v/v) eluent, which had a resolution factor (Rs) value greater than 3.0. Although the resolution was found to be good, the peak shape of both isomers was very broad. To improve the peak shape, further experiments were conducted by using additives like ethylenediamine (EDA), ethanolamine (EtNA) and *n*-butylamine (*n*-BUA). The best peak shape and sensitivity were achieved with a mobile phase consisting of *n*-BUA as an additive. The final mobile phase conditions were optimized as acetonitrile–*n*-BUA 100:0.1 (v/v), at which the resolution among the two enantiomers was greater than 3.0 (Figure 2). A typical HPLC chromatogram of aliskiren bulk sample spiked with (R)-isomer is shown in Figure 3.

Limits of detection and quantification

The limit of detection (LOD) represents the analyte concentration that yields a signal-to-noise ratio (S/N) of three. The LOD found for (R)-aliskiren was found to be 0.2 μg/mL. The limit of quantification (LOQ) represents the analyte concentration that yields an S/N of ten. The LOQ found for (R)-aliskiren was 0.6 μg/mL.

Linearity

Concentrations of (R)-aliskiren test solutions ranged from 0.6 and 4.5 μg/mL and were prepared by diluting the stock solution. A calibration curve was constructed by plotting (R)-aliskiren peak areas versus concentration. The slope, intercept and correlation coefficient were determined from linear least-squares regression analysis. The results revealed an excellent correlation between the peak area and concentration of the analyte.

Precision

The repeatability of the method was evaluated by the determination of peak area relative standard deviation (RSD) of (R)-aliskiren in spiked samples after six replicate injections. Peak area RSD values were obtained for analyses performed six times daily over three consecutive days. The precision studies for (R)-aliskiren were performed at the levels of 0.6 μg/mL (LOQ) and 4.5 μg/mL, respectively. The results are summarized in Table II and indicated that suitable precision was involved in the (R)-aliskiren determination.

Accuracy

The accuracy of the method was demonstrated at four different concentration levels in triplicate. The analyses were carried out

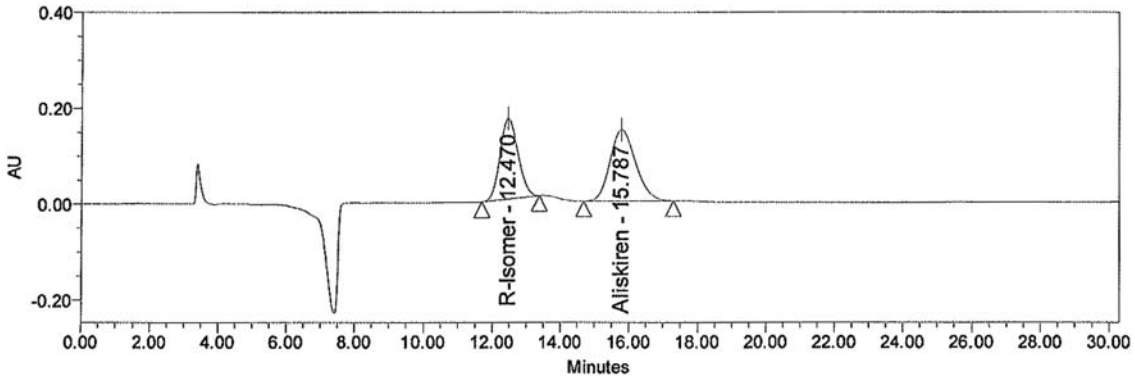

Figure 2. Enantiomeric separation of racemic aliskiren.

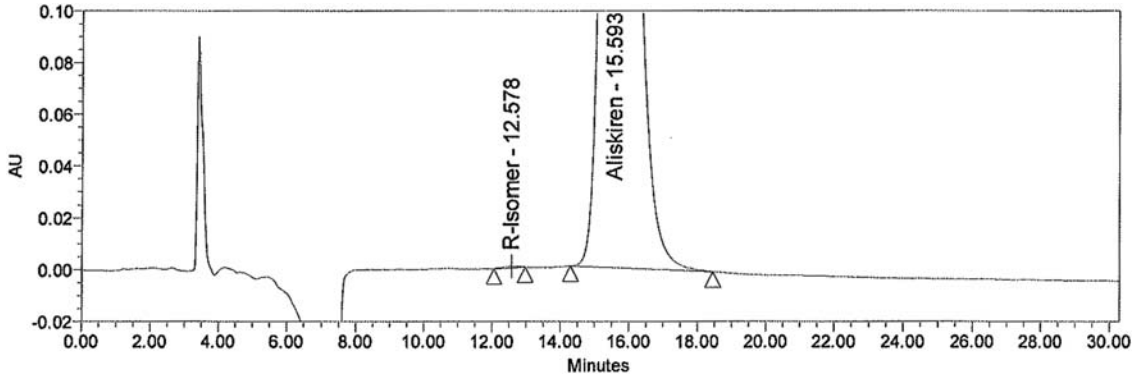

Figure 3. Typical HPLC chromatogram of aliskiren bulk sample spiked with (*R*)-isomer.

Table II
Precision for (*R*)-Aliskiren*

Repeatability at LOQ (0.2 μg/mL) level	
Retention time	0.07
Peak area	2.05
Repeatability at 4.5 μg/mL level	
Retention time	0.06
Peak area	1.2
Intra-day precision at 4.5 μg/mL level	
Retention time	0.08
Peak area	1.1
Inter-day precision at 4.5 μg/mL level	
Retention time	0.1
Peak area	1.4

*$n = 6$.

at 50, 75, 100 and 150% of the 0.15% specification limit with a target concentration of 2 mg/mL. The percentage recoveries were between 96 and 102%.

Robustness

The robustness of the method was studied by varying a number of method parameters. The experimental conditions were purposely altered to determine the impact on chromatographic resolution between (*S*)- aliskiren and (*R*)-aliskiren. The effect of flow rate on resolution was studied by varying the flow rate by ± 0.2 mL/min. In addition, the *n*-BUA percentage in the mobile phase was also varied to determine the robustness of the method. In both experiments the resolution was found to be above 3.0, which illustrates the robustness of the method (Table III).

Table III
Results from Robustness Study

Condition	Variation	Rs
Flow rate	0.8 mL/min	3.4
	1.2 mL/min	3.3
n-butylamine (%)	0.08	3.4
	0.12	3.2

Conclusion

An isocratic chiral liquid chromatographic method was developed for the enantiomeric separation of (*R*)-aliskiren from (*S*)-aliskiren. The method was validated following ICH guidelines and found to be precise, accurate, specific and robust for the determination of (*R*)-aliskiren. Hence, this method can be used in quality control laboratories for the routine determination of (*R*)-aliskiren in aliskiren bulk drug samples.

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