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Der Pharma Chemica, 2014, 6(3):234-239
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

A validated chiral HPLC method for the enantiomeric purity of alogliptin benzoate

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ABSTRACT

An isocratic chiral stationary phase high-performance liquid chromatographic (CSP-HPLC) method has been developed and validated for the quantitation of (S)-isomer in Alogliptin Benzoate. Separation was achieved with a Lux cellulose 2 (250×4.6mm, 5µm) column. The ratio of ethanol and diethyl amine in the mobile phase were optimized to obtain the best separation. UV detection was performed at 230 nm. The described method is linear over a range of LOQ – 1.5 µg/mL of (S)-isomer. The mean recovery of (S)-isomer was found to be in the range of 100–102%. The method is simple, rapid, accurate, selective and precise, useful in the quality control of bulk manufacturing.

Keywords: Chiral Separation, Alogliptin, (S)-isomer, Enantiomeric purity, Validation.

INTRODUCTION

Alogliptin is an orally administered anti-diabetic drug in the DPP-4 inhibitor class. Molecular Formula $C_{18}H_{21}N_5O_2$ and IUPAC name 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-3,4- dihydropyrimidin-1(2H)- yl}methyl) benzonitrile.

Separation of enantiomers has become very important in analytical chemistry, especially in the pharmaceutical and biological fields, because some stereoisomers of racemic drugs have quite different pharmacokinetic properties and different pharmacological or toxicological effects [1-2]. This is one of the most vital reasons why the regulatory authorities insist more on stringent investigation for evaluating the safety and the effectiveness of drugs containing chiral centers. Most of the pharmaceutical industries are now concentrating towards the study of the therapeutic effect of pure enantiomers of the existing drug molecules. Enantiomeric separations have acquired importance in all the stages of drug development and the commercialization process. A control and accurate quantification of undesired enantiomers in Active Pharmaceutical Ingredient is essential [3], therefore, the development of new methods for efficient chiral separations mainly based on HPLC, capillary electrophoresis (CE) or gas chromatography (GC) is more than necessary. Among the chromatographic methods so far developed, HPLC methods based on chiral stationary phases are widely employed for the assays of drug isomers in pharmaceutical preparations and biological fluids.

To the best of our knowledge no chiral HPLC method is reported in the literature for the enantiomeric separation of alogliptin isomers. Therefore, the aim of this study is to develop a chiral HPLC method for the determination of enantiomeric purity by accurate quantification of un-required (S)-isomer of alogliptin and validate as per the ICH guideline [4].

MATERIALS AND METHODS

Chemicals and reagents

Samples of alogliptin benzoate and (S)-isomer (Fig. 1) were synthesized at Dissymmetrix laboratory, Hyderabad, India. HPLC grade ethanol is obtained from Brampton, Ontario L6T 3Y4 (Canada). Analytical grade diethyl amine (DEA) is purchased from Merck (India). LC grade water was deionized with Milli-Q Elix and then filtered using Milli-Q gradient, Millipore water purification system (USA).

Equipment

The HPLC system consisted of quaternary gradient pump, auto sampler, column oven and a variable wavelength detector. The output signal was monitored and integrated using EZ-Chrom Elite Chromatography Data Software (1200 series HPLC, Agilent, USA).

Preparation of standard solutions

Dissolved an accurately weighed quantity of (S)-isomer working standard in minimum quantity of ethanol and made up with mobile phase to obtain a solution having known concentration of about 0.001 mg/mL and injected in to the system. Dissolved an accurately weighed quantity of alogliptin benzoate sample in minimum quantity of ethanol and made up with mobile phase to obtain a solution having known concentration of about 0.2 mg/mL and injected in to the system.

Chromatographic conditions

A Lux Cellulose-2 chiral stationary phase analytical column (250 mm × 4.6 mm, 5µm packing) (Phenomenex India Pvt Ltd) was used. A mixture of ethanol and DEA in the ratio of 100:0.5 (v/v) was used as mobile phase. It was filtered through a 0.45µm-nylon membrane using a Millipore vacuum filtration system. The mobile phase was pumped through the column at a flow rate of 1.0 mL/min. The sample injection volume was 20µL. The detector was set to a wavelength of 230 nm.

RESULTS AND DISCUSSION

Method Development

To achieve separation between enantiomers of Alogliptin benzoate, chiral stationary phases (CSPs) containing cellulose and amylose derivatives were evaluated with suitable mobile phase compositions. The chiral discrimination of enantiomers occurs when they bind with the stationary phase forming transient diastereomeric complexes. The most important interactions between the analyte and the CSP are hydrogen bonding, dipole–dipole interactions, and pi–pi interactions, together with the rigid structure (Cellulose-based CSP) or helical structure (Amylose-based CSP) of the chiral polymer bound to the support. Various combinations of n-hexane: 2-propanol and n-hexane: ethanol was used as the mobile phase in our initial efforts in the normal-phase separation. These trials were made initially in the absence of DEA and then by adding DEA to the mobile phase. Attempts to separate the enantiomers on cellulose carbamate derivertized columns in normal-phase proved futile. Even with the use of Amylose ester derivertized columns the enantiomers could not be separated.

The enantiomers could be separated only on Cellulose carbamate derivertized CSP (Lux Cellulose 2, 250mm length, 4.6 mm internal diameter and 5µm particle size) with mobile phase comprising Ethanol and DEA or *n*-hexane, ethanol and DEA. Various experiments were conducted, to select the best combination of stationary and mobile phase that could give optimum resolution and selectivity for the enantiomers. No separation was achieved on Lux Amylose 2, Daicel Chiralpak AD 3 columns. Very good separation was achieved on Lux Cellulose 2 with resolution of 4.55. In the case of CSPs with carbamate derivatives, binding of solute to the CSPs is achieved through the interactions between the solutes and the polar carbamate groups on CSPs. Solutes can bind to the carbamate groups on the CSPs forming transient diastereomers through hydrogen bonding using the CO and NH groups and also through dipole–dipole interaction using the CO moiety. Alogliptin benzoate has NH and CO (carbonyl) functional groups and these could well be contributing to the interactions with the carbamate groups on CSP, resulting in separation. The aromatic ring on the solute could provide additional stabilizing effect to the solute–CSP complex as reported by Wainer et al. The use of ethanol in mobile phase provided better selectivity and resolution than 2-propanol. A comparison of the System suitability results obtained using ethanol and 2-propanol clearly indicate ethanol is the solvent of choice. The addition of DEA up to 0.1% (by volume) to the mobile phase resulted in improved peak shapes, better resolution and shorter run times. The effect of ethanol concentration, DEA concentration, temperature and flow rate on resolution (*RS*), retention time (t_R) and selectivity (α) were examined and the most optimum conditions were found to be a mobile phase consisting of ethanol: diethyl amine (100.0:0.5, v/v/v) at flow rate of 1.0 mL/min with the column maintained at ambient temperature. The method validation was carried out on the same. The enantiomeric separation of alogliptin benzoate on Chiralpak AD-3, Amylose-2 and Lux

cellulose 2 columns is shown in Fig. 2.

Table 1: Accuracy results for (S)-isomer.

(S)-isomer spike level (%, m/m)	Added (µg) (n=3)	Recovered (µg)	% Recovery	Mean Recovery	%RSD	95% Confidence interval
0.25	25.06	25.10	100.2	100	0.20	100 ± 0.2
		25.05	100.0			
		25.16	100.4			
0.50	50.12	51.21	102.2	102	0.15	102 ± 0.2
		51.05	101.9			
		51.14	102.0			
0.75	75.18	75.85	100.9	101	0.21	101 ± 0.2
		75.94	101.0			
		76.18	101.3			

Table 2: Precision results for (S)-isomer.

Repeatability	
Mean of (S)-isomer content (%, *m/m*) *(n=6)*	0.153
Standard deviation (SD)	0.005
%RSD	3.4
Intermediate Precision	
Analyst-1/Day-1	
Mean of (S)-isomer content (%, *m/m*) *(n=6)*	0.152
Standard deviation (SD)	0.004
%RSD	2.7
Analyst-2	
Mean of (S)-isomer content (%, *m/m*) *(n=6)*	0.148
Standard deviation (SD)	0.004
%RSD	2.8
Overall %RSD *(n=12)*	2.8
Day-2	
Mean of (S)-isomer content (%, *m/m*) *(n=6)*	0.152
Standard deviation (SD)	0.004
%RSD	2.7
Overall %RSD *(n=12)*	2.6

Quantification of (S)-isomer

Known concentration of standard solution (0.001mg/mL) was used for the quantification of (S)-isomer in alogliptin benzoate sample (0.2 mg/mL). Not more than 0.50%*m/m* of (S)-isomer is found in alogliptin benzoate.

Method Validation

Specificity

Alogliptin benzoate and (S)-isomer were injected separately to confirm the retention times. System suitability solution was then injected. (S)-isomer and alogliptin benzoate peaks are eluted at 11.55 minutes and 15.43 minutes respectively (relative retention 0.75). The resolution between the peaks was found to be 4.55. The asymmetry for (S)-isomer and alogliptin peaks are 1.1 and 1.2 respectively.

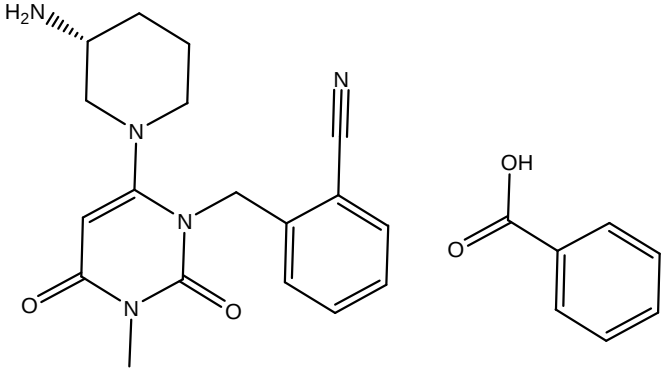
Linearity

Standard solutions at ten different concentration levels ranging from 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 0.75, 1.00, 1.25 and 1.50µg/mL were prepared (0.025 – 0.75% of analyte concentration of 0.2 mg/mL). Each sample solution was injected in triplicate. The mean responses recorded were plotted against concentration. The correlation coefficient for (S)-isomer was found to be 0.9998, which indicated good linearity. The calibration equation for (S)-isomer was found to be $y = 106716x + 1302$.

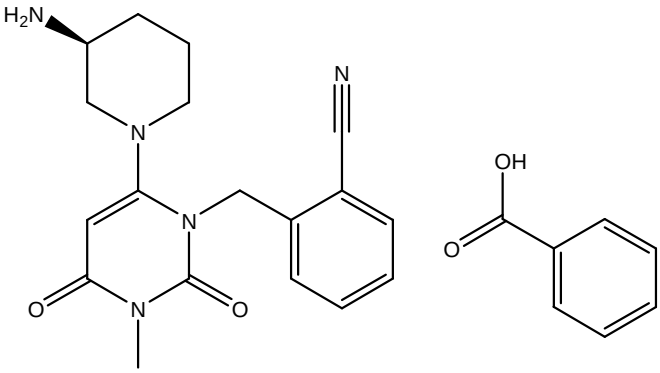
Accuracy

Alogliptin benzoate sample was spiked with (S)-isomer at 0.25, 0.50 and 0.75% of analyte concentration of 0.2 mg/mL. Each spiked solution was prepared in triplicate and injected. The mean recoveries, recovery percentage and %RSD were calculated. The mean recoveries of (S)-isomer at each spike solution with 95% confidence interval are found to be 100±0.2%, 102±0.2% and 101±0.2% respectively. Accuracy results are shown in Table 1. The acceptance criteria for recovery at each level are between 80 and 120% as per in-house validation protocol.

Fig. 1 Structural formula for Alogliptin benzoate (R) and (S)-isomer



Alogliptin Benzoate (R)_isomer



Alogliptin benzoate (S)_ isomer

Precision

Repeatability was demonstrated by injecting six individual spiked test preparations of alogliptin benzoate (0.2mg/mL). Intermediate precision was demonstrated by analyzing same preparations of alogliptin benzoate by two different analysts on two different days. Intra-day variations of (S)-isomer content in alogliptin benzoate are expressed in terms of %RSD values. The values calculated were found to be 3.4% for repeatability, 2.8% and 2.7% for intermediate precision. Repeatability and intermediate precision results are shown in Table 2.

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantification for (S)-isomer was calculated from the linearity data using residual standard deviation of the response and slope of the calibration curve. A typical S/N ratio of 2-3 and 9-10 are generally considered to be acceptable for LOD and LOQ respectively. LOD and LOQ values are found to be 0.0106µg/mL and 0.0354µg/mL respectively.

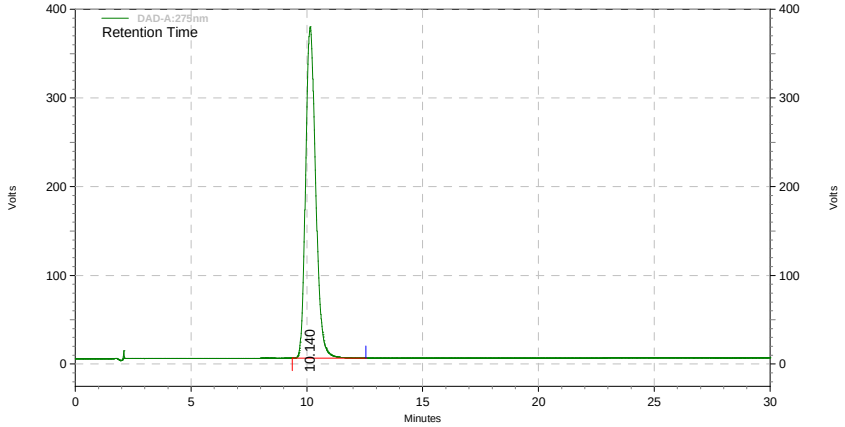
Robustness

In order to demonstrate the robustness of the method, chromatographic conditions were deliberately altered and the resolution was checked between (S)-isomer and alogliptin peaks. To study the effect of variation of flow rate on the resolution, 0.1 units of flow were changed from 1.0 mL min^{-1} (i.e. 0.9 and 1.1 mL min^{-1}). The effect of column temperature on resolution was studied at 20 and 35°C instead of 25°C. In all the above varied conditions, the composition of the mobile phase was held constant as those of the initial condition.

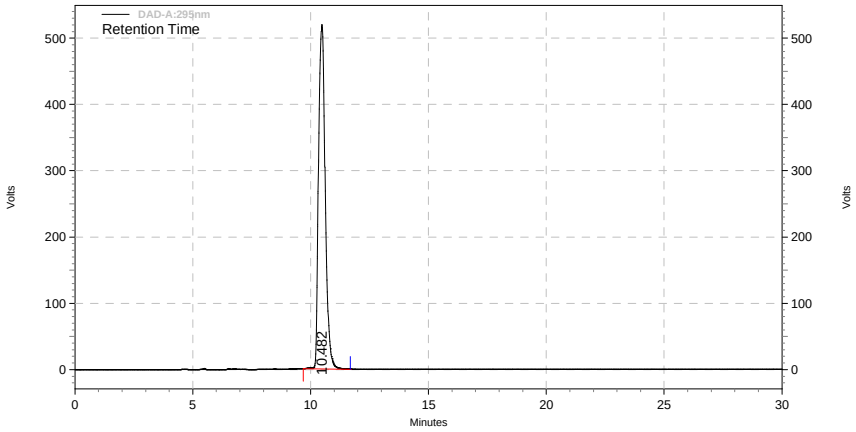
In all the deliberate varied chromatographic conditions carried out (flow rate and temperature) the resolution between (S)-isomer and alogliptin was greater than 3.0, illustrating the good robustness of the method.

Fig. 2: Typical chromatograms of enantiomeric separation of racemic alogliptin on Lux Amylose 2, Chiralpak AD 3, Lux Cellulose 2 columns; mobile phase composed of ethanol: DEA (100:0.5, v/v); flow rate 1.0 mL/min; UV-230 nm

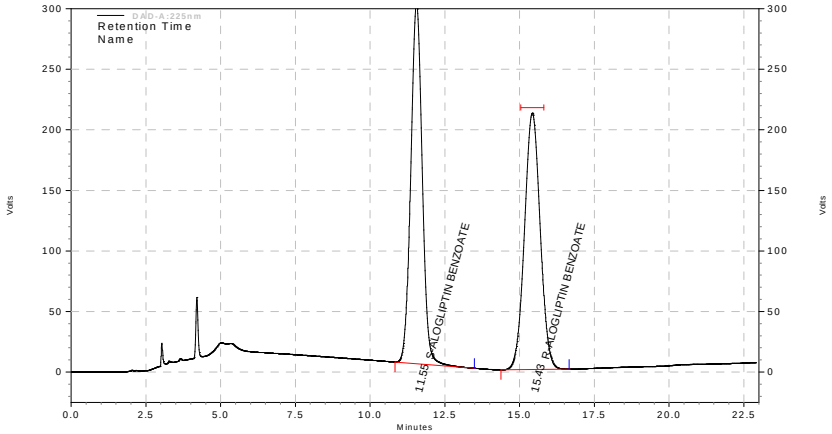
Column: Chiral Amylose-2



Column: Chiralpak AD-3



Column: Lux Cellulose-2



Batch Analysis

The (S)-isomer content in three different batch samples of alogliptin benzoate was determined and found to be less than 0.5% *m/m*. Other related substances were evaluated by a reverse phase HPLC method, known and unknown impurities (any other impurity) are less than 0.10% and total impurities are less than 1.0% (excluding (S)-isomer content by chiral stationary phase HPLC).

Stability in solution

Standard solutions of (S)- Alogliptin Benzoate and (R)- Alogliptin Benzoate were prepared in the mobile phase at analyte concentration. Each standard solution was analyzed immediately after preparation (Fig. 4a and b) and divided into two parts. One part was stored at 2–8 ◦C in a refrigerator and the other at bench top in tightly capped volumetric flasks. The stored solutions of each isomer were reanalyzed after 24 h. No change in either the chemical or enantiomeric purity was observed. The area obtained for each isomer after 24 h did not show any significant change compared with the area of initial analysis. This indicates that both isomers were stable in the mobile phase for at least 24 h when stored either at 2–8 ◦C or at bench top.

CONCLUSION

A chiral HPLC method for the separation of Alogliptin Benzoate enantiomers was developed and validated. The chiral separation was achieved in cellulose carbamate derivertized column (Lux Cellulose-2). This method is simple, accurate and has provided good linearity, precision and reproducibility. The results of analysis obtained with this HPLC method and a validated CE method are comparable. The practical applicability of this method was tested by analyzing various batches of the bulk drug and formulations of Alogliptin Benzoate.

Acknowledgements

The authors wish to thank the management of GITAM University & Dissymmetrix Private Limited for supporting this work.

REFERENCES

[1] 1. FDA policy statement for the Development of New Stereoisomeric Drugs, Washington DC (**1992**).
[2] 2. E.J. Ariens, E.W. Wuins, *Clin. Pharmacol. Ther.*, **42**, 361 (**1987**)
[3] 3. G. Sridhar, R. Pramod Kumar, M.K. Srinivasu, Sangaraju Sivaiah, K.B.Chandrasekhar, B.M. Rao, *Analytical Chemistry: An Indian Journal*, **6**, 35 (**2007**)
[4] 4. ICH Q2(R1), Guidelines on Validation of Analytical Procedures: Text and methodology, (**2005**).