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A VALIDATED CHIRAL HPLC METHOD FOR THE ENANTIOMERIC PURITY OF ANAGLIPTIN

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ABSTRACT

An isocratic chiral phase high-performance liquid chromatographic method has been developed and validated to quantitation the (R)-isomer in Anagliptin. Separation was achieved in a Lux Cellulose-3 (250×4.6mm, 5µm) column. The ratio of hexane, ethanol and diethyl amine in the mobile phase were optimized to obtain the best separation. UV detection was performed at 254 nm. The described method is linear over a range of LOQ – 1.4 µg/mL of (R)-isomer. The mean recovery of (R)-isomer was found to be in the range of 100–102%. The method is simple, accurate, selective and precise. The method can be used in the quality control of bulk manufacturing.

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INTRODUCTION

Separation of enantiomers for racemic drugs in the pharmaceutical and biological fields has become very important in analytical chemistry [1]. The enantiomers of racemic drugs have relatively different pharmacokinetic properties and toxicological effects [2]. This is one of the important reasons why the regulatory authorities insist more on stringent investigation for evaluating the safety and the effectiveness of drugs containing chiral centers. Enantiomeric separations have acquired importance in all the stages of drug development and the commercialization process. The physical and chemical properties of the two enantiomers are same, so the development of methods for quantitative analysis of chiral compounds is extremely challenging and is very difficult [3]. Therefore, the development of new methods for efficient chiral separations on HPLC, capillary electrophoresis (CE) or gas chromatography (GC) is very important. Among the chromatographic methods HPLC methods based on chiral stationary phases are widely used for the assays of drug isomers in pharmaceutical compounds and other biological compounds [4].

EXPERMENTAL

Materials

Samples of Anagliptin and (R)-isomer were synthesized at Stereokem Laboratory, Hyderabad India. HPLC grade ethanol is obtained from Fisher scientific UK, n-Hexane (HPLC grade) was purchased from Merck (Mumbai, India). 2-Propanol was purchased from Merck chemical (Mumbai, India). Analytical grade Diethyl amine (DEA) is purchased from Merck (India).

Equipment

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

Chromatographic conditions

The analysis and validation was performed on Lux Cellulose-3 column (250mm×4.6mm, 5µ) Phenomenex India Pvt Ltd. The mobile phase was prepared by Hexane, Ethanol, and diethyl amine in the ratio of 80:20:0.1 (v/v). The flow rate is 1.0 mL/min, and the column was maintained at ambient temperature. The injection volume was 10 µL and the detector wavelength was tuned at 254 nm. The column was washed with a mixture of *n*-hexane and 2- propanol (90:10, v/v) at the end of each day at a flow rate of 0.5 mL/min for 1 hour.

Preparation of standard solutions

Accurately weighed quantity of (R)-isomer working standard (0.1 mg/mL) is dissolved in a 100 ml volumetric flask with minimum quantity diluent and made up to mark with diluent, and the solution injected in to the system. Accurately weighed quantity of Anagliptin sample (mg/mL) is dissolved in a 10 ml volumetric flask minimum quantity diluent and made up to mark with diluent. The solution injected in to the system.

RESULTS AND DISCUSSION

Method development

The method development for the separation enantiomers of Anagliptin were tried on cellulose and amylose derivatives columns with a suitable mobile phase compositions. The chiral seperation 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: ethanol, *n*-hexane: IPA was used as the mobile phase in 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. Finally the separation is achieved on cellulose carbamate derivatized column.

The separation of enantiomers were achieved only on Cellulose carbamate derivatized CSP (Lux Cellulose-3 250mm length, 4.6 mm internal diameter and 5µ particle size) with mobile phase comprising *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, Lux Cellulose 4 and Daicel chiralpak AD 3 columns. The good separation was achieved on Lux Cellulose 3 with good resolution. Anagliptin has NH and CO (carbonyl) functional groups and these could be contributing to the interactions with the carbamate groups on CSP, resulting in separation. The aromatic ring on the analyte will provide additional stabilizing effect to the analyte–CSP complex as reported by Wainer *et al*. The use of ethanol in mobile phase provides better selectivity and resolution. 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 (*R*S), retention time (tR) and selectivity (α) were examined and the most suitable conditions were found with mobile phase consisting of Hexane, ethanol and diethyl amine in the ratio of (80:20:0.1 v/v/v) at flow rate of 1.0 mL/min with the column temperature maintained ambient.

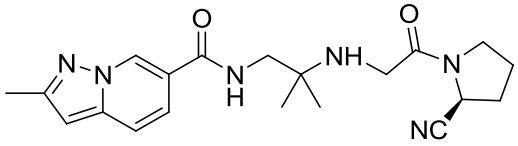

Fig. 1 Structure of (S)-Isomer:

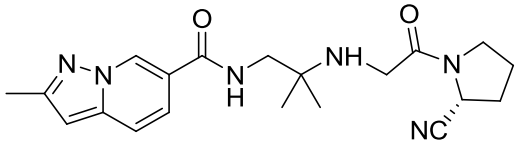

Fig. 2 Structure of (R) Isomer:

Quantification of (R) isomer

The known concentration of standard solution (0.001 mg/ml) is used for the quantification of (R)-isomer in Anagliptin (mg/ml).Not more than 0.5% m/m of (R)-isomer is detected in Anagliptin.

Method Validation

The LC method developed has been validated for the quantification of (R)-isomer in Anagliptin using the following parameters. Standard solution is used for the quantification of (R)-isomer.

Specificity

Anagliptin and Anagliptin (R)-isomer are injected separately to confirm the retention times. System suitability solution was injected. (R)-isomer and Anagliptin are eluted at 9.11 and 11.17 minutes respectively. The resolution between the peaks is found to be more than 3.00. The asymmetry for (R)-isomer and Anagliptin are 1.03 and 1.02.

Linearity and range

The linearity of the method was studied for the concentration covering a range of 0.10, 0.20, 0.30, 0.40, 0.50, 0.75, 1.00, 1.25, 1.50 µg/mL (solutions were prepared from 0.025-0.75% of analyte concentration 0.1 mg/mL). Each solution was injected in triplicate. The mean responses recorded were plotted against concentration. The correlation coefficient for (R)-isomer was found to be 0.9999.

Accuracy

The accuracy of the method was evaluated by spiking the standard drug with known concentration of the unwanted isomer ((R)-isomer) at 0.25, 0.50 and 0.75% of analyte concentration of 0.1 mg/mL. The recoveries were calculated from the slope and the intercept method from the calibration curve of (R) - Anagliptin standard. The recoveries ranged from 100±2% to 102±2% (Table 1).

Precision (repeatability and reproducibility)

The method repeatability was evaluated by calculating the area of the unwanted isomer in spiked samples for six replicate injections was expressed in terms of RSD. The reproducibility is obtained by the analysis performed on three consecutive days, six times each day. The precision studies for (R) - Anagliptin were performed at the limit of quantification (LOQ) and at 150% of analyte concentration. The results were precise for estimation of the unwanted isomer (Table 2).

Limit of detection (LOD) and limit of quantitation (LOQ)

The limits of detection(LOD) and limit of quantitation (LOQ) of Anagliptin enantiomers were estimated by obtaining the detector signal for the peaks and by performing serial dilution of a solution of known concentration. The LOD and LOQ were found to be 0.09 µg/mL and 0.25 µg/mL respectively. A typical peak signal to noise ratios must be about 2–3 at LOD level and 9–10 at LOQ level.

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in the chromatographic conditions, i.e. change in flow rate by 0.1 mL/min and change in the ratio of mobile phase (2% absolute).The method was demonstrated to be robust over an acceptable working range of its HPLC operational parameters and results are shown in Table 3.

Table 1: Accuracy data.

(R)-isomer spiked level (%m/mg)	Added (µ)(n=3	Amount recovered (µ g)	% Recovery	Mean Recovery
		25.51	102.8	
0.25	25.07	25.65	102.1	102.4
		25.67	102.2	
		50.55	100.5	
0.50	50.03	50.47	100.2	100.4
		50.25	100.4	
		75.15	101.7	
0.75	75.04	75.05	100.6	101.1
		75.85	101.1	

Table 2.

Repeatability	
(R)-isomer content(%,m/m)(n=6)	:
Mean of	0.023
Standard deviation (SD)	0.003
%RSD	1.3
Intermediate precession	
Analyst-1 Day-1:	
(R)-isomer content(%,m/m)(n=6)	:
Mean	0.023
Standard deviation (SD)	0.003
%RSD	1.29
Analyst-2	
(R)-isomer content(%,m/m)(n=6)	:
Mean	0.023
Standard deviation (SD)	0.003
%RSD	1.29
Over all %RSD(n=12)	1.3
Intermediate precession	
Day-2	
(R)-isomer content(%,m/m)(n=6)	:
Mean of	0.022
Standard deviation (SD)	0.003
%RSD	1.3
Over all %RSD(n=12)	1.7

Table 3.

Flow rate(mL/min)	Effect of change in flow rate				
	Compound	**RT**	**RR**	**Resolution**	**Asymmetry**
0.9	(R)-isomer	10.15	0.85	0.0	1.04
	Anagliptin	13.30	1.00	3.15	1.03
1.0	(R)-isomer	9.11	0.84	0.0	1.03
	Anagliptin	11.17	1.00	3.00	1.02
1.1	(R)-isomer	8.12	0.83	0.0	1.03
	Anagliptin	10.97	1.00	2.85	1.02
Composition(v/v/v/v)	Effect of change in mobile phase composition				
Hexane:Ethanol:DEA	Compound	RT	RR	Resolution	Asymmetry
78:22:0.1	(R)-isomer	10.06	0.77	0.0	1.03
	Anagliptin	12.12	1.00	2.06	1.02
80:20:0.1	(R)-isomer	9.11	0.84	0.0	1.03
	Anagliptin	11.17	1.00	3.00	1.02
82:18:0.1	(R)-isomer	9.95	0.78	0.0	1.03
	Anagliptin	13.10	1.00	3.15	1.02

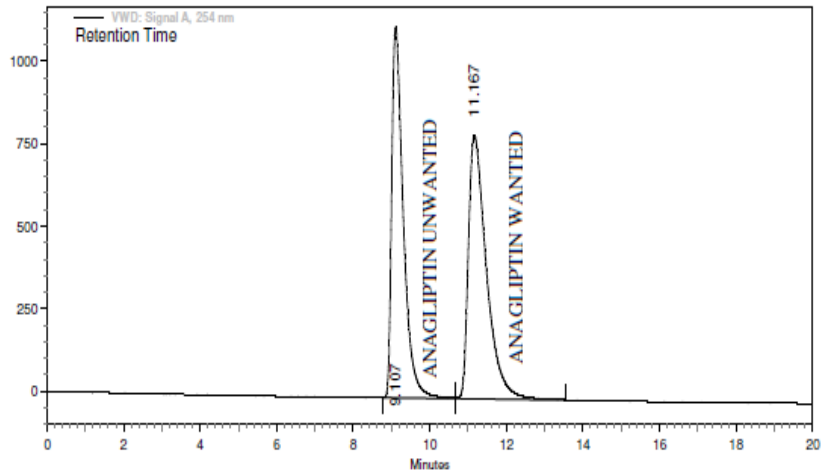

Fig a: Typical Chromatogram of enantiomeric separation of racemic Anagliptin Benzoate.

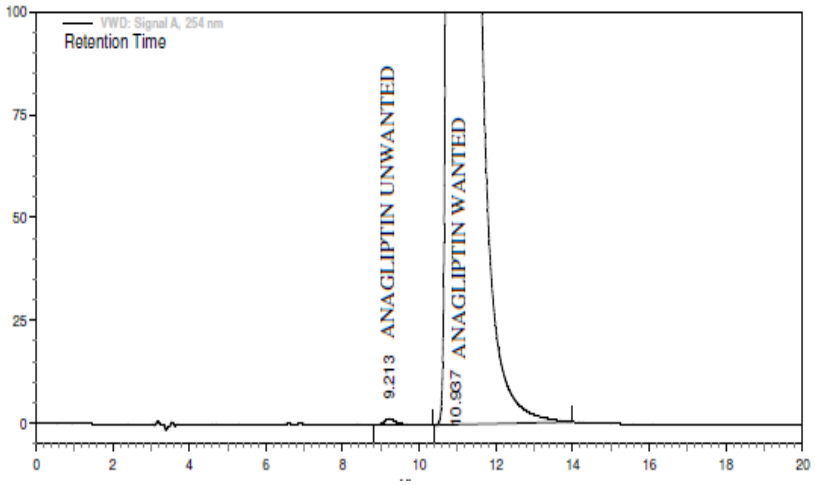

Fig b: Typical Chromatogram of enantiomeric separation of Standard Anagliptin Benzoate.

Batch analysis

The (R)-isomer content in three batch samples of Anagliptin is determined and found to be less than 0.5% m/m. Related substance purity is evaluated by reverse phase HPLC method, the known and unknown impurities are less than 0.1% and total mpurities are less than 1.0% (excluding (R)-isomer content).The chromatogram showing racemic sample and real sample with (R)-isomer (0.1%) are shown in Fig a & b.

Stability in solution

The Standard solutions of (*S*) - Anagliptin and (*R*) - Anagliptin were prepared in the diluent at analyte concentration. The standard solutions are 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 hours 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 hours when stored either at 2–8 ˚C or at bench top.

CONCLUSION

A chiral HPLC method for the separation of Anagliptin enantiomers was developed and validated. The chiral separation was achieved in cellulose carbamate derivatized column (Lux Cellulose-3, Phenomenex). 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 Anagliptin.

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