Available Online at

http://www.ijcpa.in

January-March 2017

DOI : http://dx.doi.org/10.21276/ijcpa

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Research Article | Volume-4 | Issue-2 | Article ID: 1158

DEVELOPMENT AND VALIDATION OF ENANTIOSELECTIVE ANALYSIS OF ANAGLIPTIN BY USING CHIRAL LUX CELLULOSE - 3 COLUMN

[1]Nagamalleswari Gampa*, [2]Naga Thirumalesh Chevala, [1]Pracheet Pinnamaneni, [1]Arulsamy Elphine Prabahar, [1]Venkata Suresh Ponnuru, [2]Rama Rao Nadendla

[1]Department of Pharmaceutical Analysis, [2]Department of Pharmaceutics,
Chalapathi Institute of Pharmaceutical Sciences, Acharya Nagarjuna University, Lam, Guntur, Andhra Pradesh, India.

Corresponding Author:** Email: **malleswari.naga2011@gmail.com

Received: 10 November 2016 / Revised: 2 December 2016 / Accepted: 1 February 2017 / Available online: 31 March 2017

ABSTRACT

The current research concern about the development of stereo specific normal phase high performance liquid chromatographic method for the separation and estimation of enantiopurity of anagliptin (AGT) by using Lux cellulose- 3 [cellulose tris (4- benzoate)] chiral column, n- Hexane, ethanol and triethylamine (TEA) (80:20:0.5 v/v/v) solvent ratios were used as mobile phase at flow rate of 1ml/min in isocratic mode. Both AGT- (R) (desired form) and AGT- (S) (undesired form) detected at 254 nm with retention time of 9.11 min and at 8.09 min respectively, and having correlation coefficient (R^2) of 0.999. Inter day and intraday precision of the developed method was < 1.0 % RSD and % mean recovery was found to be 99.074. Enantioselective method was validated for linearity, precision, accuracy, limit of detection & quantification, solution stability, robustness and ruggedness, developed method can be used for the separation and estimation of enantiopuirty of AGT- (R) in recemate mixture.

***Keyword**: Anagliptin, enantiopurity, enantiomer, HPLC.*

1. INTRODUCTION

AGT is the novel dipeptidly dipeptidase- 4 inhibitor (DDP-4) which is prescribed for the treatment of type- 2 diabetes mellitus. AGT helps in improving the blood glucose levels by stimulating the endogenous insulin secretion by inhibiting glucagon and reduction in the gastric empting time by increasing the production of incretin hormones [1].AGT is available in 2 stereospecific forms R and S forms, which were mirror images of each other. Among the 2 stereospecific forms AGT- (R) is pharmacological active. Chiral analysis is the technique which is used for quantification, separation of recemate mixtures and their precursors or metabolites which is necessary for the development of pharmaceutically active compounds[2-4]. By using HPLC with chiral stationary phase, enantiomers separation and enantiopurity can be estimated[2]. One of the technique used for the separation of enantiomers is, auxiliary chiral reagent is coupled with the enantiomers which are to be separated. Auxiliary chiral reagent converts them into diastereomers thereby enantiopurity can be estimated by achiral technique. Chiral chromatography will not modify enantiomeric form of molecule which are to be modified

hence this method can be consider as thermodynamically enantioselective method[3]. Achiral technique can be used for the enantiopurity of molecule by using a selective chiral mobile phase or chiral mobile phase additives. Combination of chiral selector and mobile phase as well as chiral stationary phases can also be used[3].Estimation of enantiopurity of a drug depends on the composition, pH and temperature of the mobile phase. Among different types of chiral stationary phases cellulose and amylose are the two common types used for the chiral analysis because of their wide range of application in the pharmaceutical industry[4,5]. Present research deals with development and validation of anagliptin enantiomers.

2. MATERIALS AND METHODS

2.1 Reagents

Anagliptin (AGT) enantiomers purchased from MEC (Medchem Express), Ethanol, n- hexane, triethylamine (TEA), acetonitrile (ACN), isopropyl alcohol (IPA) of HPLC grade were procured from Merck life science Pvt. Ltd, Mumbai.

2.2 Instrumentation

Quantitative normal phase high performance liquid chromatography (NP-HPLC) was performed on gradient HPLC (Agilent, 1200 infinity LC) with UV detector. Column used for the chiral analysis was Lux Cellulose- 3 (250mm × 4.6mm, 5µ) Phenomenex India Pvt Ltd. ATX-224, analytical electronic balance Mfg. By Shimadzu and Titrasys- 352 pH meter Mfg. by Systronics were used.

2.3 Chromatographic conditions

Method development and validation was performed by using Lux Cellulose- 3 (250mm × 4.6mm, 5µ) column. Initially HPLC was flushed for 6h with IPA at flow rate of 0.5ml/min, after the completion of flushing, column was subjected for washing with mixture of n- hexane and 2- propanol (90:10 v/v) for 30min.Different mobile phase ratios were tried for the separation of recemate mixture with resolution, finally mobile phase ratios were optimized. Initial trails results were shown in Table: 1 and Fig. I, II and III. The mobile phase used for the analysis is composed of n – Hexane, ethanol and triethylamine (80:20:0.5 v/v/v) which is filtered through nylon 0.45 micron 47mm membrane filter and then degassed by using bath sonicator for 10min.initially column was subjected for the saturation with mobile phase at 0.9ml/min flow rate, back pressure was maintained at 100 - 140bar. Run time was adjusted to 15min and absorbance was recorded at 254nm.

2.4 Preparation of standard samples

About 10 mg of the bulk drug AGT- (R) and AGT- (S) was weighed separately and transferred into two 10 ml volumetric flasks. Mobile phase was added to dissolve the drug. The volume was made up to the mark with the mobile phase.

2.5 Method validation

Developed enantioselective method was validated with reference to ICH guidelines[6].

2.6 Linearity

Calibration curve was constructed by preparing 3 sets of AGT- (R) standard solutions in mobile phase at concentration range of 2 to 10µg/ml. 20µL standard solutions were injected into the column and peak area and retention time were recorded. Calibration curve was constructed by plotting concentration against peak area. Slop and regression were calculated from the plot.

2.7 Inter-day and intra- day precision and accuracy

Mean concentration (6 µg/ml) was selected from the standard concentrations and 6 replicates of standard concentration (n=6) were injected. Intraday and inter day precision were calculated by performing six successive runs by different analysts. %RSD's were calculated for the precision and accuracy, respectively.

2.8 Sensitivity

LOQ and LOD were calculated from the slope of regression equation. **3.3σ/S** and **10σ/S** were used for calculating LOD and LOQ respectively. Where σ is the SD of *y*- intercept of regression line and S is the slope of calibration curve.

2.9 Solution stability

Solution stability was tested by keeping the standard solutions in refrigerator for 72h and enantiopurity was estimated at regular intervals of time throughout the study period.

2.10 Robustness

Robustness was determined based on the peak resolution of the recemate mixture by purposefully altering the chromatographic conditions. It was determined for the parameters such as change in flow rate (increase and decrease of 0.2ml from 0.7 to 1.1 ml/min), change in wavelength (increase and decrease of 2nm from 252 to 256nm), change in mobile phase composition (increment of 5ml of solvent 75:25:0.5 to 85:15:0.5), change in column lot, and change in instrument Mfg. (Agilent, 1200 infinity LC was replaced by Shimadzu LC- 20AT), enantiopurity and resolution between the R and S forms in recemate were determined.

2.11 Ruggedness

Ruggedness of the method was evaluated by performing intraday and inter day precision by using mean standard concentration.

3. RESULTS AND DISCUSSION

3.1 Method validation

Method validation was performed with reference to the ICH guideline. AGT recemate were separated to the baseline successfully by using cellulose- 3 column, n – Hexane, ethanol and triethylamine (80:20:0.5 v/v) as mobile phase. AGT- (S) eluted faster than AGT- (R) and their retention times were found to be 8.09min and 9.11minrespectively, were shown in the Fig. IV. Method was validated for various parameters which were represented in the Table: VII, the enantiomers of AGT- (R) were linear in the range of 2-10µg/ml. Inter day and intra-day precision was evaluated, % RSD was found to <1%. % RSD for repeatability and reproducibility for six successive determinations were found to be less than 1%, LOQ and LOD for AGT- (R) was found to be 0.247 and 0.0818 respectively. The results confined that the method is accurate and precise. From the linearity data of AGT- (R) with the linear increase in the concentration, increase in the peak area was observed (Table VI).

3.2 Linearity

Calibration curve was constructed and curve revealed a linearity range from 2µg/ml to 10µg/ml for AGT- (R) with correlation coefficient (R^2) of 0.999 (Fig. V).

3.3 Sensitivity

Developed method was found to be sensitive, LOQ was determined for the AGT developed method and it was found to 0.247 µg/ml.

3.4 Precision and accuracy

The recoveries were calculated from the slop and intercept of the calibration curve for the standard. Accuracy data for the AGT- (R) was shown in the Table: III. Inter- day and intraday precision were performed and shown in the Table: IV and V. % RSD for inter day and intraday precision were found to be 0.889338 and 0.418411 respectively.

3.5 Solution stability

The stability of the standard solution was determined and there was no significant change in the enantioselectivity and peak area after 72h[7].

3.6 Robustness

Robustness of the method was observed by changing the flow rate, detection wavelength and mobile phase ratios. It was found that there is no significant change in peak resolution and retention time. % RSD is <1 which indicates there is no significant change. Robustness data was shown in the Table: VI.

3.7 Ruggedness

Ruggedness of the method was determined by inter changing the analysts and it was found that there is no significant change in the separation and enantiopurity of AGT.

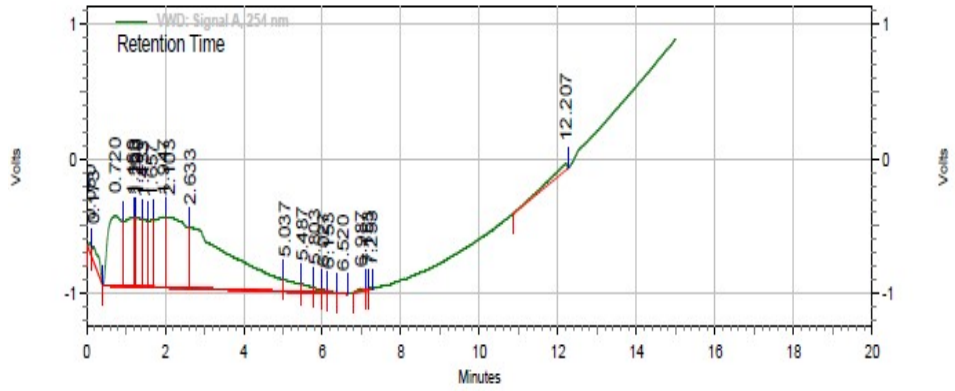

Figure-I: Chromatogram of Anagliptin Standard solution

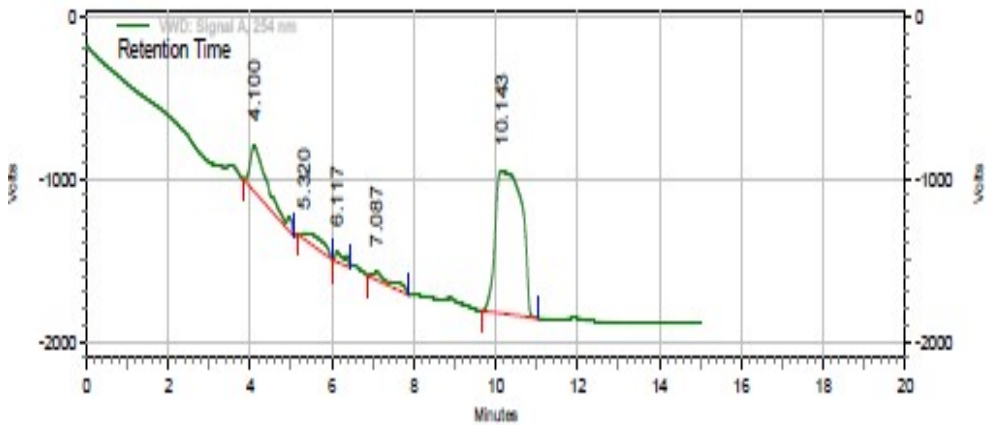

Figure-II: Chromatogram of Anagliptin Standard solution

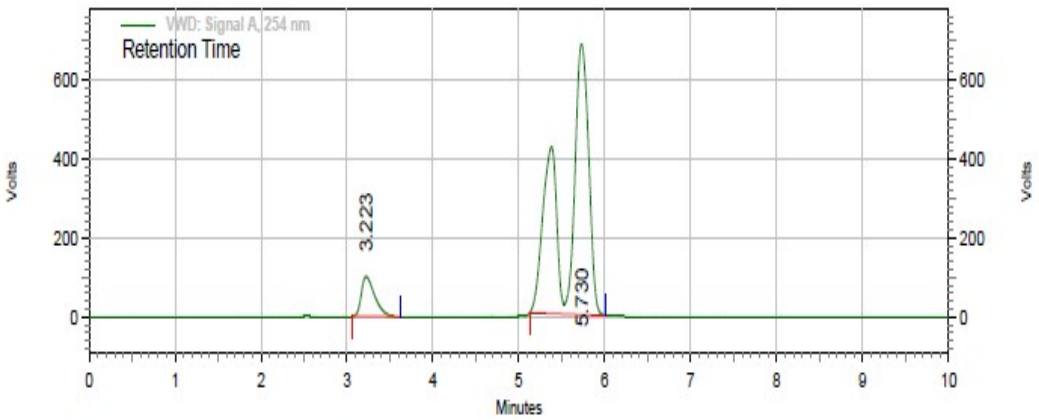

Figure-III: Chromatogram of Anagliptin Standard solution

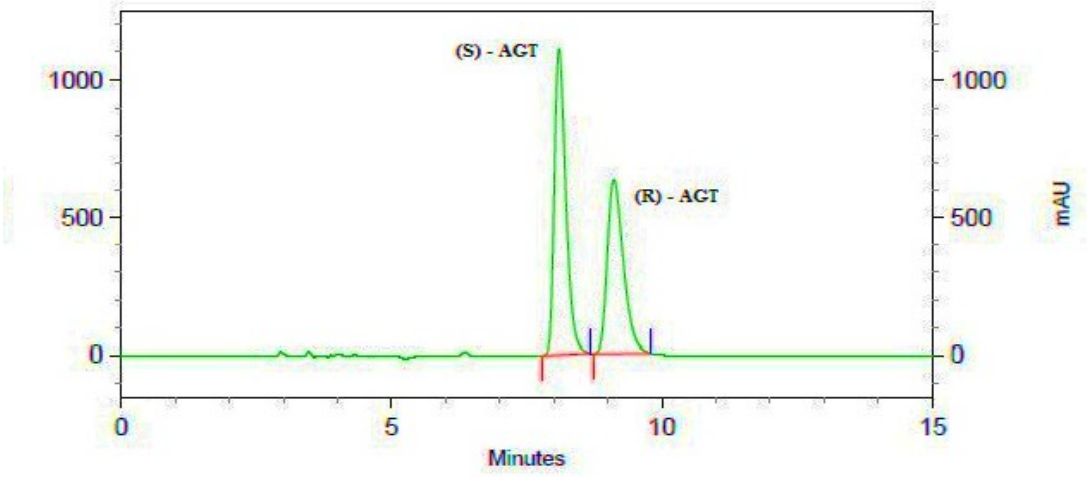

Figure-IV Chromatogram of AGT Standard solution of recemate mixture (10µm/ml)

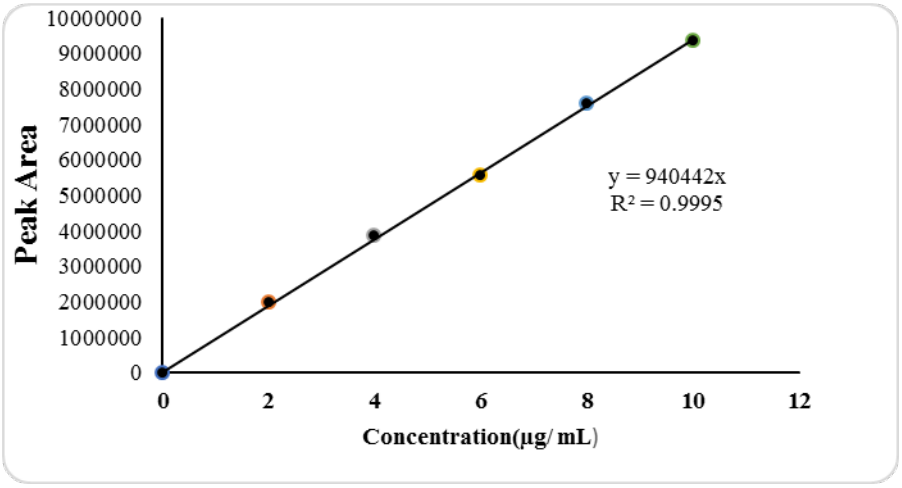

Figure-V Standard Graph of AGT – (R)

Table-I: Initial trails (for optimization of mobile phase ratios)

S. No	Mobile Phase composition	Detection wavelength	Flow rate	Column	Observation
1	n-hexane: methanol (20:80)	254nm	0.7ml/min	Lux Cellulose-3	No peaks
2	n-hexane: ethanol (40:60)	254nm	0.7ml/min	Lux Cellulose-3	Separation of peaks (resolution <1)
3	n-hexane: ethanol (80:20)	254nm	0.9ml/min	Lux Cellulose-3	Separation of peaks (resolution >1)

Table-II: Linearity for AGT – (R)

S. No.	Concentration (µg/ml)	Peak Area
1	2	1957218
2	4	3866342
3	6	5573529
4	8	7575362
5	10	9347344

Table-III: Accuracy data for AGT- (R)

Nominal Concentration	% Accuracy	Final Concentration	Trails	Amount recovered (µg/ml)	% Recovery
4	80%	7.2	t1	7.4	102.77
			t2	7.1	98.611
			t3	6.9	95.833
			Avg		**99.074**
4	100%	8	t1	8.3	103.75
			t2	8.1	101.25
			t3	7.8	97.5
			Avg		**100.833**
4	120%	8.8	t1	8.9	101.136
			t2	8.7	98.863
			t3	8.6	97.727
			Avg		**99.242**

Table-IV: Precision (Inter day) of AGT - (R)

Concentration(µg/ml)	Trail	Peak Area
6	t1	5573529
6	t2	5521492
6	t3	5603982
6	t4	5493921
6	t5	5619352
6	t6	5593427
SD		**49514.94**
%RSD		**0.889338**

Table-V: Precision (Intraday) of AGT - (R)

Concentration(µg/ml)	Trail	Peak Area
6	t1	5573529
6	t2	5599568
6	t3	5535290
6	t4	5573984
6	t5	5560282
6	t6	5593687
SD		**23316.91**
%RSD		**0.418411**

Table-VI: Robustness of AGT - (R)

Parameters	USP resolution between AGT - (S)and AGT-(R)	% RSD
Flow rate		
0.9	1.985	0.390
1.0	2.165	0.184
1.1	2.324	0.324
Mobile phase ratios (n- Hexane: Ethanol: TEA)		
75:25:0.3	1.964	0.509
80:20:0.5	2.163	0.232
85:15:0.7	2.355	0.370

Table-VII: Validation parameters of AGT - (R)

S. No	Parameters	AGT - (R)
1	Linearity	2-10µg/ml
2	Precision	
	Intra - day (n= 6) (% RSD)	0.856
	Inter - day (n= 6) (% CV)	0.921
3	Specificity	Specific pass
4	LOQ	0.247
5	LOD	0.0818

4. CONCLUSION

A simple enantioselective method was developed and validated for enantio puritic estimation of AGT. The developed method was found to be robust and rugged with acceptable linearity range, sensitivity, precision and accuracy and this method can be used effectively for the separation and quantitative estimation.

5. ACKNOWLEDGEMENT

The authors are thankful to University of Grant Commission (UGC), New Delhi, India for providing financial assistance; and Chalapathi Institute of Pharmaceutical Sciences, Department of Pharmaceutical Analysis, Guntur (India) for providing required facilities for carrying out research smoothly.

REFERENCES

1. Abe M. Anagliptin in The Treatment of Type 2 Diabetes: Safety, Efficacy, and Patient Acceptability, 2015;163–71.
2. Ndorbor T, Wang Y, Huaijing D, Zhizhang D, Kolawole JA, Hua H. Chromatography Chromatographic and Molecular Simulation Study on The Chiral Recognition of Atracurium Besylate Positional Isomers on Cellulose Tri- 3, 5-Dimethylphenycarbamate (CDMPC) Column and Its Recognition Mechanism, 2013;4(3):4–11.
3. Choi HMS, Dong DJ, Lee W. Validation and Application of a Simple Reverse Phase HPLC Method For In Vitro Dissolution Studies of Memantine Hydrochloride Tablet. J Pharm Investig, 2015.
4. Kang HJJ, Cho JPC. Simultaneous Analysis of Ibuprofen and Pamabrom by HPLC. J Pharm Investig, 2015;45(6):555–60.
5. Kraml CM, Zhou D, Byrne N, Mcconnell O. Enhanced Chromatographic Resolution of Amine Enantiomers as Carbobenzyloxy Derivatives in High-Performance Liquid Chromatography and Supercritical Fluid Chromatography, 2005;1100:108–15.
6. Conference, International et al. 2005. “ICH Harmonized Tripartite Guideline Validation of Analytical Procedures:” 1994(November 1996)
7. Ester CA--, Sushmitha P, Narasu ML, Suryanarayana M V. A Validated Chiral LC Method For The Enantiomeric Separation of Ethyl-Methyl, 2012;5(1):5–9.