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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF GLIMEPIRIDE CIS ISOMER CONTENT IN BULK AND TABLET DOSAGE FORM USING ISOCRATIC NORMAL PHASE CHIRAL HPLC

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Abstract

A new, rapid, simple and selective isocratic normal phase chiral hplc method was developed for Glimepiride cis isomer content. Glimepiride is chemically 4-ethyl-3-methyl-N-[2-[4-[(4-ethylcyclohexyl) carbamoylsulfamoyl] phenyl]ethyl]-5-oxo-2H-pyrrole-1-carboxamide. Glimepiride and its *Cis-isomer* were resolved on immobilized cellulose tris(3,5-dichloro pheny carbamate) stationary phase, Chiralpak IC(250X4.6)mm,,5µm column using a mobilephase system containing of n-Hexane:EtOH (25:75 ,v/v) at 25°C Column oven temperature, Flow rate of 0.6 mL min^{-1} and detected on UV/VIS detector wavelength at 228 nm. The USP resolution between both the Glimepiride and its *Cis-isomer* was more than 2.0. The.Limit of detection (LOD) and Limit of Quantitation (LOQ) for Glimepiride Cis-isomer were 0.24 µg/mL and 0.80 µg/mL respectively. The sample solution and mobile phase were found to be stable for atleast 48Hrs.The final optimized developed method was validated as per International Conference on Hormonisation (ICH) guidelines in terms of Specificity, Limit of detection (LOD) Limit of Quantitation (LOQ), Pecision, Linearity, Accuracy ,Solution stability and Robustness. This method could be used to determination of Glimepiride Cis isomer content in bulk batch samples and also in tablet dosage forms.

Keywords: Glimeperide, Glimepiride Cis-isomer, Chiralpak IC, Method Validation.

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INTRODUCTION

Glimepiride is a chemical class of third generation sulfonyl urea which is more potent and had a longer duration of action, is used to treat typeII diabetes[1-5].Glimepiride (Figure1) is chemically 4-ethyl-3-methyl-N-[2-[4-[(4-methylcyclohexyl)carbamoylsulfamoyl]phenyl]ethyl]-5-oxo-2H-pyrrole-1-carboxamide.

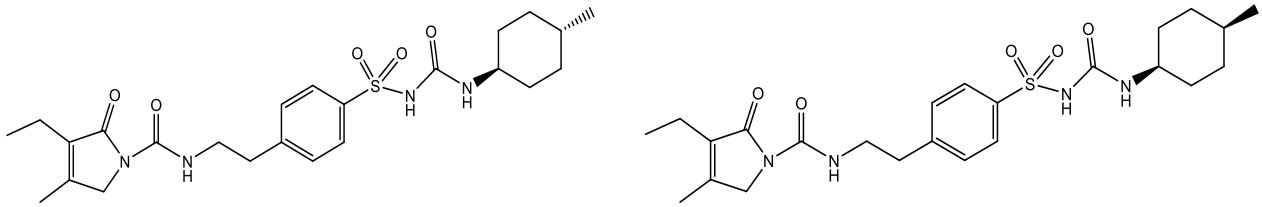

Fig.1: Chemical Structures of Glimepiride and its Cis isomer of Glimepiride

In USP (United States Pharmacopoeia) and EP(European pharmacopoeia) [6] there was a separate method described for the Glimeperide Cis-isomer content and limit was set to be not more than 0.8% Cis-isomer in Glimepiride drug substance.The relative retention time (RRT) in USP and EP method for Glimepiride and its Cis-isomer was 0.9,the column was diol and the mobile phase consisting of anhydrous acetic acid:IPA:n-Heptane(1:100:899 V/V/V). In literature,several analytical methods for Glimepiride Cis-isomer content and impurity quantification methods were reported [7-11] by reverse phase HPLC. The present method was developed in isocratic normal phase chiral HPLC.

The aim of the study was to develop a method to achieve a good base to base separation between Glimepiride and its Cis isomer which was facilitate to determine the Cis-isomer content in Glimepiride drugsubstance. The optimized method was validated as per International conference on Hormonisation(ICH) guidelines[12], hence this method could be useful for quality control of Glimepiride Cis-isomer content monitoring in drug substance as well as in tablet dosage forms.

MATERIALS AND METHOD

CHEMICALS AND REAGENTS

Samples of Glimepiride and its Cis-isomer were gift samples obtained from local pharmaceutical company. HPLC grade n-Hexane and Ethanol having purity 99.9% were purchased from MERCK, Mumbai,India.

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EQUIPMENT

High Performance Liquid Chromatography(HPLC) system was Agilent 1260 series,system equipped with quaternary pump ,auto sampler, degasser and photodiode array wavelength detector. Acquisition and processing of the chromatograms with Agilent installed EzchromeElite software. Semimicro balance was Sartorious with accuracy 0.001 mg.

PREPARATION OF STOCK SOLUTION

20 mg of Glimepiride drug substance was dissolved in mobilephase, sonicate it to dissolve completely and make up to 10 mL volumetric flask. 20 mg of Glimepiride Cis-isomer was dissolved in mobile phase, sonicate it to dissolve completely and make up to 10 mL volumetric flask.

METHOD DEVELOPMENT

Glimepiride and its Cis isomer were prepared by mixing equal volume of Glimepiride and its Cis isomer stock solution. This mixture solution was used for the development with different mobilephase composition of n-Hexane,n-Heptane,Ethanol,IPA(Isopropyl alcohol),DEA(Di ethylamine) and TEA(Tri ethylamine) , but system suitability parameters like USP resolution ,tailing factor and theoretical plates were not good enough when compared to final optimized chromatographic conditions.

OPTIMISED CHROMATOGRAPHIC CONDITION

The chromatographic column was Cellulose tris(3,5-dichloro pheny carbamate) stationary phase, Chiralpak IC(250X4.6)mm,,5µm (Diacel Chemical Industries,Tokyo,JAPAN) . The mobile phase consisting of n-Hexane:Ethanol (25:75,v/v).The flowrate of mobilephase was 0.6 $mLmin^{-1}$.The injection volume was 10 µL. The chromatographic peaks monitored wavelength at 228nm and the diluent was mobilephase.

METHOD VALIDATION

The Optimised final method was validated as per ICH guidelines Q2(B) interms of Specificity, Limit of detection (LOD), Limit of Quantitation (LOQ), Precision (Method precision and Intermediate precision), Linearity, Accuracy, Robustness, Solution stability and mobile phase stability.

SYSTEM SUITABILITY

Systemsuitability was evaluated by injecting the 0.8% spiked solution of Glimepiride Cis - isomer in to Glimepiride test solution since the specification limit of Cis-isomer as per

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compendial monographs was 0.8% and reporting of Glimepiride Cis-isomer content by using the below formula

$$\% \text{ Cis-isomer} = \frac{\text{Peak area of Cis isomer in sample}}{\text{Peak area of Cis isomer in 0.8\%Standard}} \text{ X } \frac{\text{Concentraion of Standard}}{\text{Concentraion of Sample}} \text{ X Potency of Cis-isomer}$$

The acceptance criterion for systemsuitabilty parameters USP resolution should be more than 2.0,Theoretical plates should be more than 4000 and Tailing factor should be less than 2.

SPECIFICITY

Injected individually Glimepiride solution, Glimepiride Cis-isomer solution , system suitability solution and Blank solution. It was observed that there was no interference peaks found from the blank to that of Glimepiride and its Cis-isomer retention peaks.

PRECISION

Method Precision/reproducibility was performed by six replicate injections (n=6) of 0.8% spiked solution of Cis-isomer in to Glimepiride solution were carried out. The acceptance criterion for method precision was the percentage relative standard deviation(%RSD) for Glimepiride Cis-isomer retention time was not more than 2% and the percentage relative standard deviation(%RSD) for Glimepiride Cis-isomer content was not more than 10% for six replicate preparations of 0.8% spiked solution.

Intermediate precision/repeatability was performed by different analyst and on different day by preparing six replicate injections(n=6) of of 0.8% spiked solution of Cis-isomer in to Glimepiride solution were carried out.The acceptance criterion for intermediate precision was the percentage relative standard deviation(%RSD) for Glimepiride Cis-isomer retention time was not more than 2% and the percentage relative standard deviation(%RSD) for Glimepiride Cis-isomer content was not more than 10% for six replicate preparations of 0.8% spiked solution,the percentage relative standard deviation(%RSD) for Glimepiride Cis-isomer content of twelve preparations(Repeatability and intermediate precision) was not more than 10%.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ) FOR GLIMEPIRIDE CIS-ISOMER

Limit of detection (LOD) and Limit of Quantitation (LOQ) for Glimepiride Cis-isomer were achieved by injecting a series of diluted solutions .LOD and LOQ values established by signal to noise ratio method. The acceptance criterion for LOD was signal to noise ratio 3 to 5 and for LOQ signal to noise ratio 10 to 15.

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PRECISION AT LIMIT OF QUANTITATION (LOQ)

Precision at Limit of Quantitation (LOQ) level was evaluated by injecting the LOQ solution in six times and calculated the percent Relative standard deviation(%RSD) of retention time and peak area. The acceptance criterion for precision at LOQ was the percentage relative standard deviation(%RSD) of Cis-isomer peak area was not more than 2% and for retention time was not more than 2% .

LINEARITY FOR GLIMEPIRIDE CIS-ISOMER

Linearity was established by preparing eight calibration points of Gimepiride Cis-isomer covering from LOQ (0.8 µg/mL) to 200%(32.4 µg/mL){0.80 µg/mL,3.24 µg/mL,8.10µg/mL,12.96 µg/mL,16.20 µg/mL,19.44 µg/ mL,24.30 µg/mL and 32.40 µg/mL} solutions were prepared in mobile phase from stock solution of Gimepiride Cis-isomer .Linearity calibration curve was obtained from Gimepiride Cis-isomer concentration versus peak area by using least squares method.The regression coefficient,slope and intercept was calculated. The acceptance criterion for linearity was the regression coefficient should not be less than 0.995.

ACCURACY

Accuracy was carried out by standard addition and recovery study were conducted and calculated against the amount of Gimepiride Cis-isomer presence in the sample to determine the accuracy of the optimized method.The accuracy/Recovery experiment carried out by preparing in triplicate preparations at LOQ,50%,100% and 150% of the specification level(0.8%). The acceptance criterion for the percentage recovery at LOQ,50%,100% and 150% should be with in the range of 90-110%.

ROBUSTNESS OF THE OPTIMIZED METHOD

To perform the robustness of the method ,experimental chromatographic parameters(Flow rate,Wavelength and mobile phase composition and wavelength) were altered to the spiked solution . For that flow rate was altered by 0.2 $mLmin^{-1}$ i.e from 0.4 to 0.8 $mLmin^{-1}$.The mobile phase composition was altered by ±10% i.e from 33% to 37% of Ethanol.Chromatographic peaks monitoring wavelength altered by ±3nm i.e from 225 to 231 nm. The robustness results were evaluated Gimepiride Cis-isomer content and resolution between Glimeperide and its Gimepiride Cis-isomer. The acceptance criterion for robustness was the absolute difference between the results obtained in Repeatability of Sample preparation-1 and those obtained by carrying out modifications in the method was not more than 10.0% of the specification level(0.8%).

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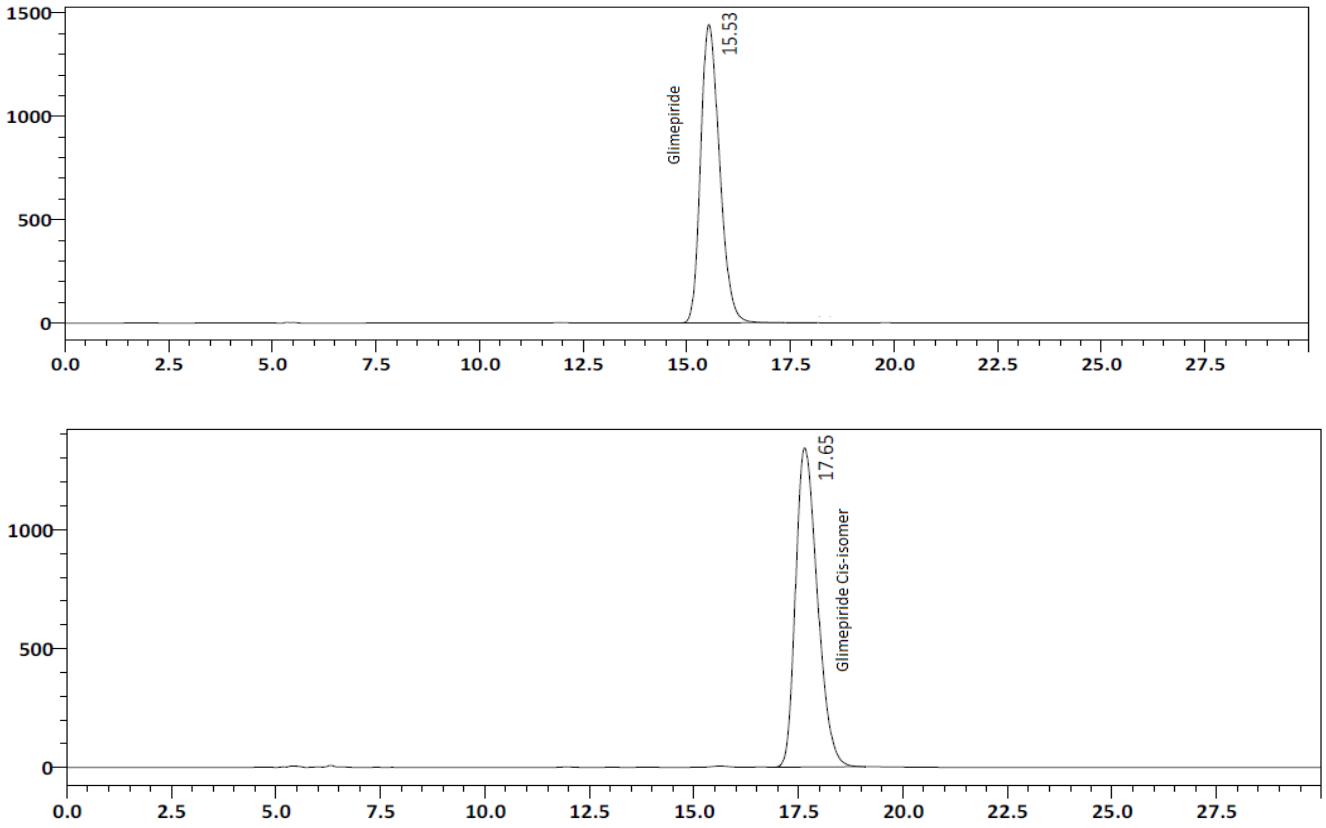
SOLUTION STABILITY

The solution stability of Glimeperide and its Gimepiride Cis-isomer in this method was performed on spiked solution at room temperature kept for around 48Hrs,which was carried out in intervels of 8Hrs,12Hrs ,16Hrs,24Hrs and 48Hrs and evaluated by the Gimepiride Cis-isomer content and resolution between two Glimeperide and its Gimepiride Cis-isomer ,it was evaluated by the percentage difference in Glimepiride Cis-isomer content with respect to initial Glimepiride Cis-isomer content in spiked sample solution and at different time intervals (8 Hrs,12 Hrs,24 Hrs and 48 Hrs) was should be less than 5%.

RESULTS AND DISCUSSION

SPECIFICITY

As per ICH guidelines Specificity was defined as" Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present".Specificity chromatograms were shown in the Figure2.



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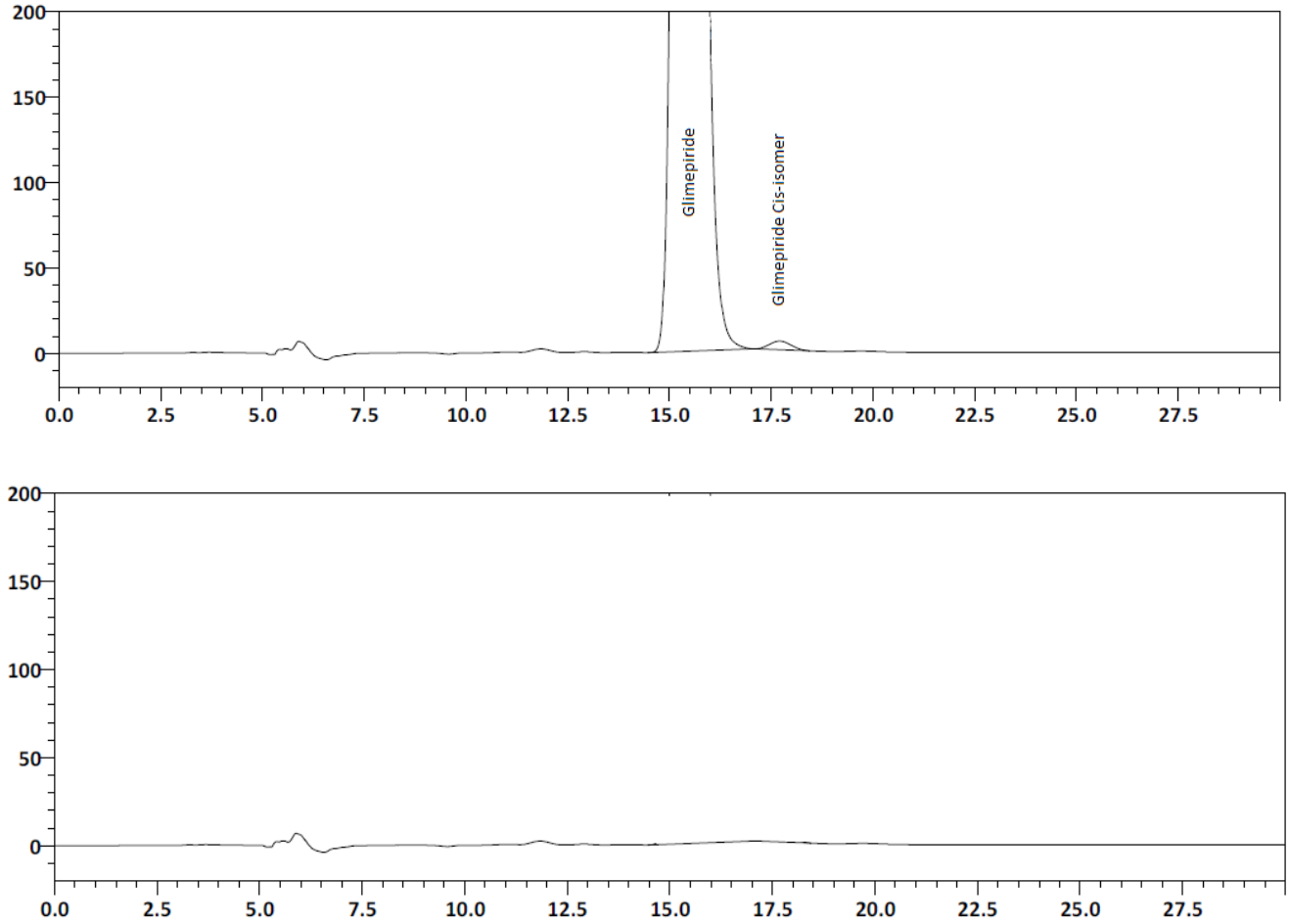

Fig. 2: (A)CHROMATOGRAM OF GLIMEPIRIDE (B)CHROMATOGRAM OF GLIMEPIRIDE CIS-ISOMER(C) ZOOMED CHROMATOGRAM OF 0.8% SPIKED GLIMEPIRIDE CIS-ISOMER IN GLIMEPIRIDE DRUG SUBSTANCE(D)BLANK CHROMATOGRAM.

SYSTEM SUITABILITY

The system suitability solution was prepared to obtained final concentration of Glimepiride 2mg/mL spiked with 0.8% of its Cis-isomer. The acceptance criterion for systemsuitabilty parameters USP resolution more than 2.0,Thereotical plates should be more than 4000 and Tailing factor should be less than 2.The results were summarized in the table 1

Table 1: Summary of System suitability results

Name of component	Retention Time(min)	Resolution	Thereotical plates	Tailing factor
Glimepiride	15.53	-	4125	1.29
Glimepiride Cis-isomer	17.65	2.27	4908	1.09

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METHOD PRECISION AND INTERMEDIATE PRECISION

As per ICH guidelines Precision was defined as" The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the optimised condions". In method precision experiment, the percentage relative standard deviation(%RSD) of the retention time was found to be 0.13% and the percentage relative standard deviation(%RSD) of the Cis-isomer content was found to be 0.05% for six replicate preparations of 0.8% spiked solution. In intermediate precision experiment, the percentage relative standard deviation (%RSD) of the retention time of Glimepiride Cis-isomer was found to be 0.13% ,the percentage relative standard deviation(%RSD) of the Cis-isomer content was found to be 0.16% for six replicate preparations of 0.8% spiked solution.by different analyst on different day and the percentage relative standard deviation(%RSD) of the Cis-isomer content was found to be 0.11% for twelve replicate preparations of 0.8% spiked solution.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ) FOR GLIMEPIRIDE CIS-ISOMER

As per ICH guidelines Limit of detection (LOD) was defines as" detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value" and Limit of Quantitation (LOQ) was defined as "The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy". LOD and LOQ were found to be 0.24 µg/mL and 0.81 µg/mL respectively by injecting the series of diluted solutions through signal to noise method, signal to noise for LOD and LOQ were found to 3.54 and 9.31 respectively. In precision at LOQ study the percentage relative standard deviation(%RSD) of the retention time was found to be 0.25% and peak area of the corresponding Glimeperide Cis-isomer was found to be 0.42% for six replicate injections.

LINEARITY FOR GLIMEPIRIDE CIS-ISOMER

As per ICH guidelines linearity was defined as" The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample". In linearity study,injecting concentration solutions from LOQ to 200% {0.8 µg/mL(LOQ), 3.24 µg/mL(20%), 8.10µg/mL(50%), 12.96 µg/mL(80%), 16.20µg/mL(100%), 19.44µg/mL(120%), 24.30µg/mL(150%) and 32.4µg/mL(200%). The calibration curve plotted against concentration versus peak area of (R)-enantiomer. The regression equation was found to

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be Y=209125x-810.13 and correlation coefficient (R^2) was 0.9998 which shows that method was linear.

Table 2: Summary of linearity results

S.No	% Level	Concentration(%) w.r.t. test sol'n concentration i.e., 2.0 mg/mL	Concentration (µg/mL)	Cis-isomer peak Area
1	LOQ	0.040	0.8	8701
2	20	0.162	3.24	33327
3	50	0.405	8.1	83349
4	80	0.648	12.96	133987
5	100	0.81	16.20	168218
6	120	0.972	19.44	203733
7	150	1.215	24.30	250302
8	200	1.620	32.40	339991

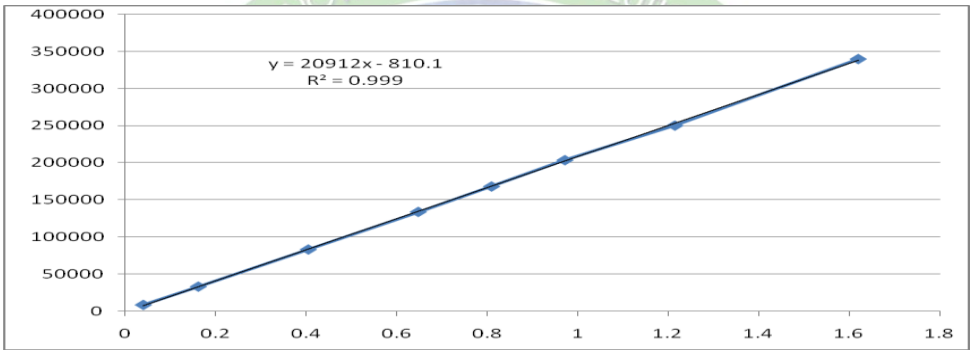

Fig.3: Linearity graph (Peak area vs Concentration)

ACCURACY:

As per ICH guidelines accuracy was defined as" The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found". In Accuracy experiment, recovery was detrmined by preparing three drug substance sample at LOQ ,50%, 100% and 150% of the specification level(0.8%).The percentage recoveries was ranging from 97.53 to 99.99%. The results were summarized in the table 3.

Table 3: Summarized accuracy results at LOQ,50%,100% and 150% of specification limit

Concentration (%)	Preparation	Amount added (%)	Amount Found (%)	%Recovery
LOQ	1	0.0404	0.0396	98.02
	2	0.0405	0.0395	97.53
	3	0.0404	0.0396	98.02
50%	1	0.4062	0.4036	99.35
	2	0.4069	0.4059	99.73
	3	0.4049	0.4033	99.61
100%	1	0.8058	0.8039	99.77

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	2	0.8098	0.8097	99.99
	3	0.8038	0.7998	99.51
150%	1	1.2056	1.1986	99.42
	2	1.2026	1.1925	99.16
	3	1.2056	1.1995	99.49

ROBUSTNESS

As per ICH guidelines robustness was defined as" the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage". In robustness experiment, flow rate was altered by 0.2 $mLmin^{-1}$ i.e from 0.6 to 1.0 $mLmin^{-1}$. The mobile phase composition altered by ±10% i.e from 72% to 78% of Ethanol. Chromatographic peaks monitoring wavelength altered by ±3nm i.e from 221 to 227 nm. The results were summarized in the table4.

Table 4: Summary of robustness results

Chromatographic condition	Changes	USP Resolution	Tailing factor	% Cis-isomer content	Absolute difference w.r.t unaltered condition
Flow rate ($mLmin^{-1}$)	0.4	2.107	1.14	0.78	0.01
	0.6	2.27	1.14	0.79	-
	0.8	2.010	1.14	0.81	0.02
% Mobile phase	n-Hexane:EtOH(22:78)	2.038	1.15	0.81	0.02
	n-Hexane:EtOH(25:75)	2.27	1.14	0.79	-
	n-Hexane:EtOH(28:72)	2.053	1.14	0.80	0.01
Wavelength (nm)	225	2.015	1.15	0.78	0.01
	228	2.27	1.14	0.79	-
	231	2.011	1.15	0.81	0.02

Solution stability

In solution stability study, 0.8% spiked solution was injected after 8 Hrs,12 Hrs,24 Hrs and 48 Hrs and it was evaluated that the percentage difference in Glimepiride Cis-isomer area with respect to initial for spiked sample solution and at different time intervals (8 Hrs,12 Hrs,24 Hrs and 48 Hrs) were in the range of 0.17 to 0.37% . The results were summarized in the table5.

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Table 5: Results of solution stability

Sample Name	Time Interval	Retention time of Cis-Isomer	Area of Cis-isomer	% difference w.r.t to initial
0.8% Spiked solution at room temperature	Initial	17.65	186772	-
	After 8 Hrs	17.73	186073	0.37
	After 12 Hrs	17.72	186947	-0.93
	After 24 Hrs	17.73	186436	0.17
	After 48 Hrs	17.72	186548	0.12

CONCLUSION

In conclusion, A new, rapid, simple and selective isocratic normal phase chiral hplc method was developed for Glimepiride cis isomer and validated as per ICH guidelines . The proposed method meets all acceptance criterion and can be used to determine the Glimeperide Cis-isomer content in bulk batch samples and also in tablet dosageform.

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