DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC METHOD FOR ESTIMATION OF GLIMEPIRIDE.

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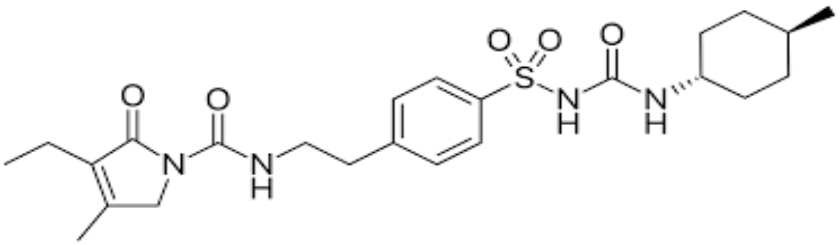
ABSTRACT

Glimepiride is a pharmacologically active substance which belongs to class of oral hypoglycemic. This second generation sulfonylurea derivative is used to treat diabetes mellitus type 2.Chromatographic (HPTLC) method has been developed and validated for estimation of Glimepiride in standard and tablet dosage form. The method employed TLC aluminum plates precoated with silica gel 60 F 254 as the stationary phase. The mobile phase used was mixture of Toluene:Chloroform: Ethanol 4:4:1 v/v/v. Band detection was carried out at 234 nm. Standard was prepared as 1mg/ml in Methanol. Sample was prepared as 10mg/ml. The correlation coefficient was found to be 0.9948 and the recovery was obtained at 99.62%

INTRODUCTION

Glimepiride (1-[4-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)-ethyl] phenyl] sulfonyl]-3-trans-(4-methylcyclohexyl) urea) belongs to class of second generation sulfonylurea derivative mainly treats diabetes mellitus of type 2.[1-2] Glimepiride has molecular formula C24H34N4O5S. Its molecular weight is 490.62. It has melting point 207°C and pka 4.99. It is soluble in Methanol, Insoluble in water and solubility in DMSO (3mg/ml) and DMF (10mg/ml) respectively.[3-4] It acts as an insulin secretagogue. It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors. Its gastrointestinal absorption is complete with no interference from meals. Significant absorption can occur within one hour and distribution throughout the body is 99.5% and bound to plasma protein. Metabolism is by oxidative biotransformation, it is hepatic and complete. The medication is metabolized to M1 metabolite by CYP2C9. M1 possesses about 1⁄3 of pharmacological activity. Excretion in the urine is about 65% and the remainder is excreted in the feces.[5-9] The most common technique used for estimation of drug content is HPLC But with HPTLC it allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis there by dramatically reducing analytical time. The same analysis can be viewed using different wavelengths of light thereby providing a more complete profile of Drugs.



In pharmaceutical preparations, multiple analytical procedures have been reported for the analysis of glimepiride when it is used as a single active principle or in combined dosage forms, using HPLC with UV spectrophotometric detection,[10-15] High performance thin layer chromatography and spectrophotometry.[16-23] However, HPLC techniques for routine analysis are often time consuming and expensive. As an alternative to the existing methods, the aim of this study was to develop Simple, Accurate, Precise, Selective, Specific, Reproducible, Highly

sensitive analytical HPTLC methods which would serve as stability indicating assay method for drug product.

EXPERIMENTAL

MATERIALS AND METHODS

Toluene and chloroform were purchased from Merck with percent purity 99.5% and 99.4% respectively. Methanol purchased from Himedia had percent purity 99.8%. Ethanol purchased from Analytical CSS had percent purity 99.8%. Chemicals used were of analytical (AR) grade except specified and purchased from Merck Limited.

INSTRUMENTS

The CAMAG® HPTLC System was equipped with Automatic TLC sampler 4 as sample applicator. Detector used was CAMAG® TLC Scanner 3.Developing chambers usedwere CAMAG TLC chamber and Twin trough chambers CAMAG®. TLC Visualizer 2 was used to visualize standards and samples. Its operational software is VISIONCATs 2.5.

STANDARD PREPARATION

Methanol was selected as diluent due to its best solubility. Accurately weighed GLM (10mg) was transferred in 10ml volumetric flask. The drug was dissolve in methanol with sonication and final volume was adjusted with methanol upto mark to prepare a 1mg/ml stock solution.

SAMPLE PREPARATION

10 tablets were weighed accurately and finely powdered. Powder exactly equivalent to 100mg of GLM was transferred to a 10ml volumetric flask. The powder was dissolved in 10ml of methanol with sonication for 45 minutes followed by centrifugation to prepare 10mg/ml sample solution. Supernant was taken for further analysis.

RESULT AND DISCUSSION

METHOD DEVELOPMENT

The aim of this study was to develop a simple, robust and derivatization-free method for the analysis of Glimepiride in standard and marketed formulation. A systematic method development strategy was utilized to optimize the parameters/conditions for the analysis of Glimepiride including Stationary phase, Mobile phase, Diluent, Saturation Time, Wavelength maxima, Application type and Solvent front.

SAMPLE SOLVENT SELECTION

Development of this HPTLC method was started with selection of a sample solvent that dissolves Glimepiride. Solubility of Glimepiride was performed in different solvents like distilled water, 0.1NHCl, 0.1N NaOH, methanol, dimethyl formamide, acetonitrile, ethanol and chloroform. It was best solubilized in methanol. Therefore, Methanol was selected as sample solvent (diluent).

PREPARATION OF TLC PLATE

Thin layer chromatography was performed on 20×10 cm aluminum backed TLC plates coated with 250 µm layer of silica gel $60F_{254}$ (E. Merck). The plates were prewashed by methanol and activated at 105-110° for 15 min prior to use for chromatography. The samples in methanol were sprayed as 8mm wide bands at a distance of 8 mm from the bottom and 20 mm from the sides of the plate under continuous flow of air by means ofATS-4 as a sample applicator fitted with a 25µl syringe. A constant application rate of 150 nl/s was employed and track distance of 11.8 mm. The plates were then conditioned for 20 min in a pre-saturated twin-trough chamber (CAMAG) with the mobile phase toluene: chloroform: ethanol (4:4: v/v/v). The plate was then placed in the mobile phase and ascending development was performed upto distance of 70 mm from application position at 22°C and 40% relative humidity. After development, plates were air dried and densitometric scanning was performed at a wavelength of 234 nm.

DEVELOPMENT OF MOBILE PHASE

A suitable solvent system for the composition of the mobile phase for development of chromatogram was optimized by testing different solvent mixtures of varying polarity. Various mobile phases were evaluated. Use of chloroform and ethanol as single component and a short saturation time of 15 min gave a necklace effect. So

chloroform: ethanol (5:5 v/v), hexane: ethyl acetate(6:4 v/v), toluene: methanol (7:3, 8:2 v/v), toluene: chloroform: ethanol (4:4:1 v/v/v), toluene: ethyl acetate: methanol (6:5:0.5 v/v/v) were tried. The best results were obtained using toluene: chloroform: ethanol (4:4:1 v/v/v). This mobile phase showed a good resolutionand a compact bandof Glimepiride. Densitometric scanning of all the tracks at λmax 234 nm showed compound with Rf value 0.51±0.05 identified as glimepiride. The method was further used in the analysisof Glimepiride from tablet formulation without interference of the formulation excipients.

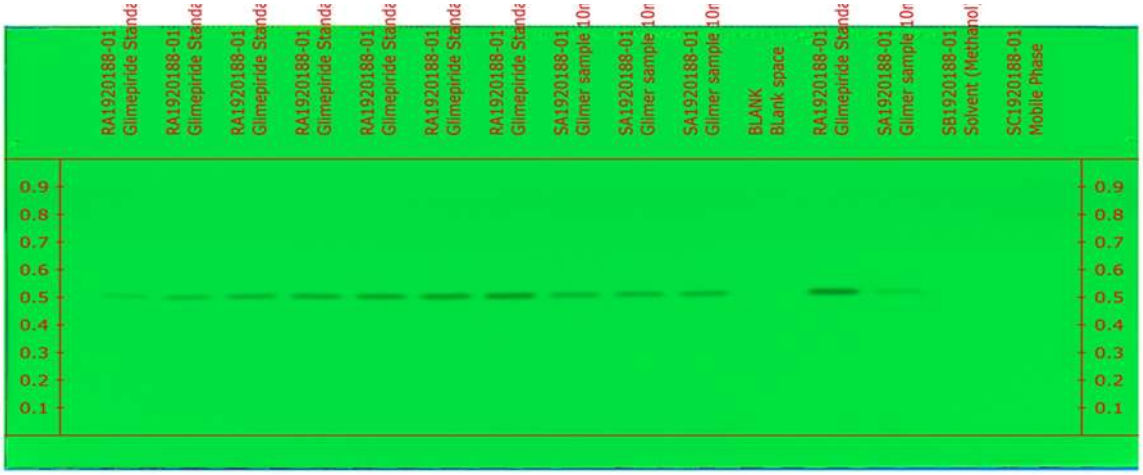


Figure:-1 Developed Plate Visualized Under 254nm

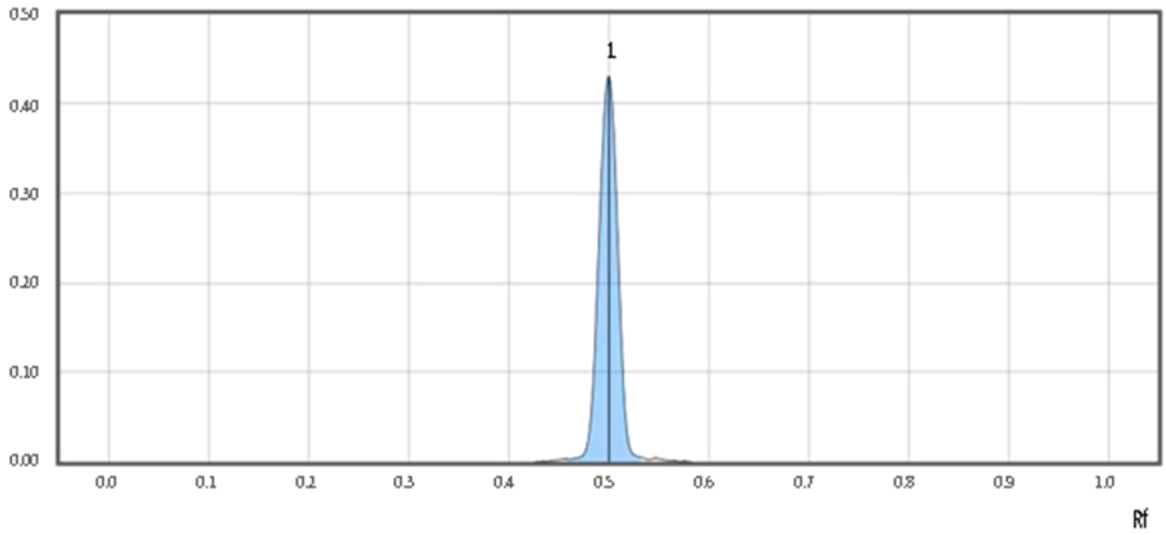


Figure :-2 Chromatogram of Standard Glimepiride

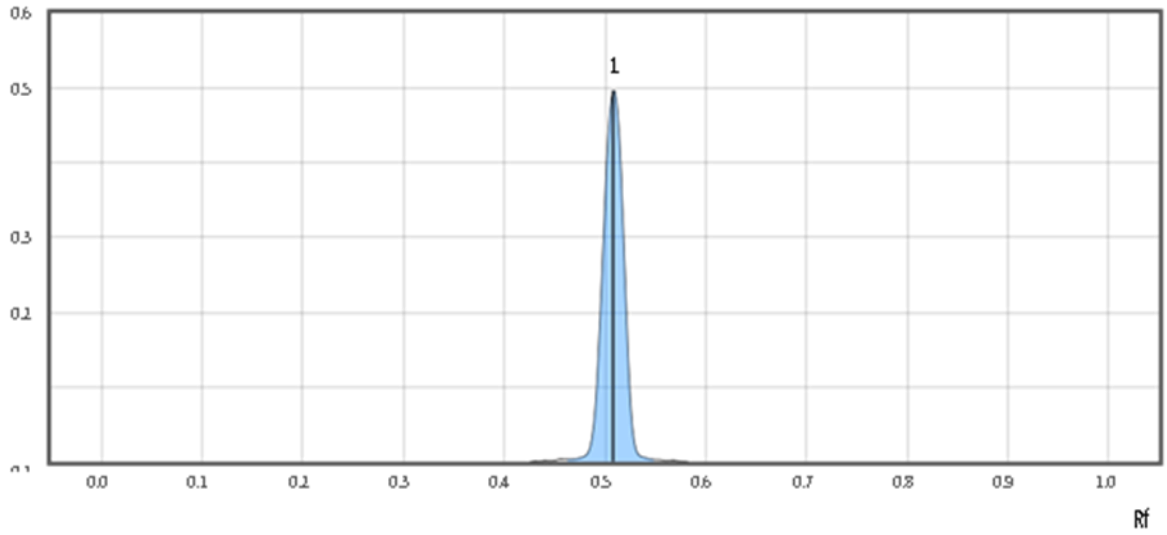


Figure :-3 Chromatogram of Sample Glimepiride

VALIDATION

The proposed method was validated for linearity, recovery, precision, specificity, Limit of detection, limit of quantification and robustness as per the ICH method validation guidelines.

1. LINEARITY

Standard stock of Glimepiride was prepared (1mg/ml). From this stock 0.5, 1, 1.5, 2, 2.5, 3 and 3.5µl were sprayed in form of band. Each volume triplicate was taken. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves. Regression equation was found to be y=0.0034x+0.0062 and correlation coefficient R2=0.9948.

Sr. No	Conc (µg)	Area
1	1	0.00955
2	1.5	0.01197
3	2	0.01364
4	2.5	0.01523
5	3	0.01658
6	3.5	0.01777

Table no:-1 Data of Calibration Curve

Parameters	Results
Wavelength	234nm
Linearity range(µg)	1-3.5µg
Regression equation	y=0.0034x+0.0062
Slope	0.0034
correlation coefficient (R2)	0.9948
Coefficient of variation	2.09%

Table no:-2 Data of Linearity

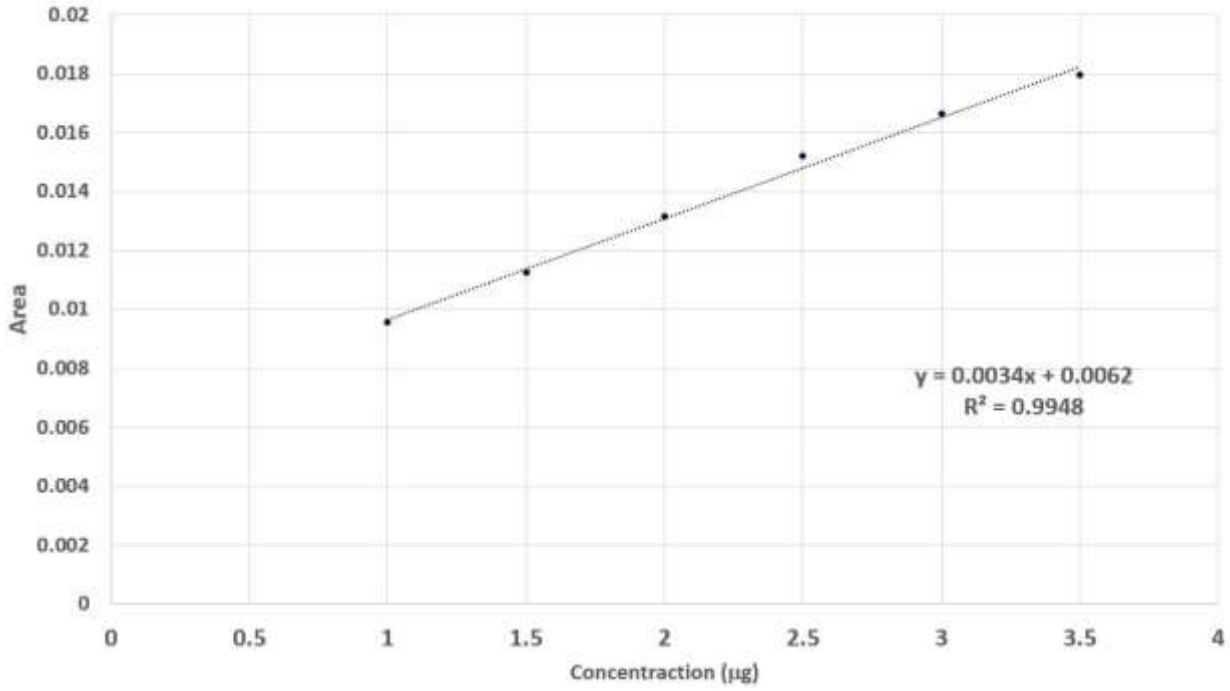


Figure: - 4 Calibration plot obtained by Chromatography of Glimepiride

2. SPECIFICITY

Specificity of the method was determined by analyzing standard drug and sample. The specificity of the method was ascertained by analyzing Glimepiride. The ability of the method to separate the drug from tablet excipients indicates the specificity of the method. There was no interference or co elution from excipients at Rf value (0.51) of drug, The band of Glimepiride was confirmed by comparing the Rf of the sample with that of standard, indicating absence of interference of mobile phase, diluent and excipients.

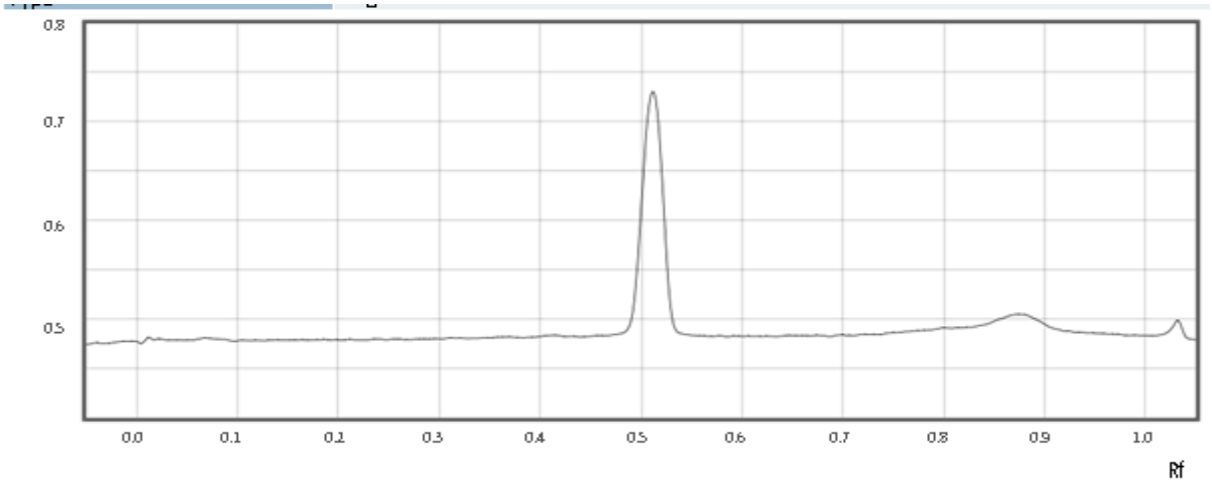


Figure: - 5 Chromatogram of Standard Glimepiride

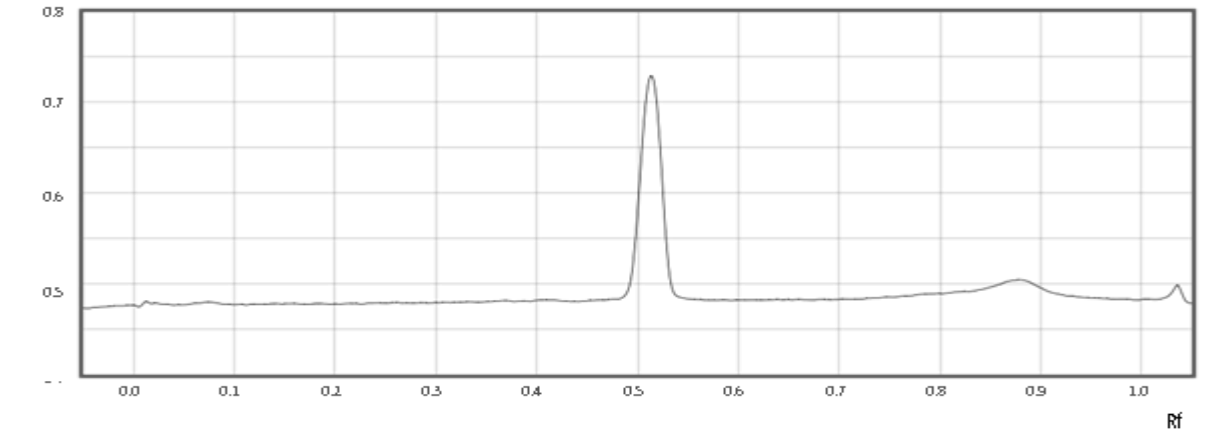


Figure: - 6 Chromatogram of Sample Glimepiride

PRECISION

Precision was determined by demonstrating interday variation. 6 replicates of standard solution of 2µl were applied on two plates on two different days (Inter-day precision) and the coefficient of variation was found to be 1.85% and 1.75% respectively.

3. DATA OF REPEATABILITY

Conc (2µg)		CV=1.85%	
Sr.No	Rf	Peak area	Deviation
1	0.555	0.01552	2.85%
2	0.556	0.01525	1.09%
3	0.555	0.01494	-1.02%
4	0.548	0.01481	-1.88%
5	0.542	0.01484	-1.66%
6	0.56	0.01518	0.61%
		Avg=0.015	

Table no: - 3 Data of Repeatability (n=6)

INTERDAY PRECISION DATA

Conc (2µg)		CV=1.85%	
Sr.No	Rf	Peak area	Deviation
1	0.555	0.01552	2.85%
2	0.556	0.01525	1.09%
3	0.555	0.01494	-1.02%
4	0.548	0.01481	-1.88%
5	0.542	0.01484	-1.66%
6	0.56	0.01518	0.61%
		Avg=0.015	

Table no:-4 Day 1

Conc (2µg)		CV=1.74%	
Sr.No	Rf	Peak area	Deviation
1	0.555	0.01532	2.52%
2	0.556	0.01512	1.21%
3	0.555	0.01481	-0.90%
4	0.547	0.01463	-2.09%
5	0.54	0.01474	-1.35%
6	0.56	0.01504	0.62%
		Avg=0.015	

Table no:-5 Day 2

4. RECOVERY

Recovery was carried out to determine accuracy of the method. Recovery was determined at 3 concentration level at 80% (4µl), 100%(5µl) and 120%(6µl). Recovery percentage was found to be 99.6254187%. Both sample and standard were diluted 1:20 in methanol.

Percent Amount spiked	Area x 106	Average Area	Area Obtained	Excepted Area	Percentage	Mean Percentage
Sample(100%)	0.000411337					
5µl	0.000448895	0.00043				
Standard (80%)	0.002820197					
4µl	0.002853377	0.00283678	0.003279	0.00326644	100.396809	
Standard (100%)	0.003350541					
5µl	0.003280882	0.003316	0.003788	0.003745365	101.148722	99.6254187
Standard(120%)	0.003899043					
6µl	0.003977168	0.003938	0.004251	0.004367759	97.3307248	

Table no:-6 Data of Recovery

5. ROBUSTNESS

It demonstrates reliability of the analysis with respect to deliberate variations in method parameter. No change in Rf was found even after change in the composition of mobile phase. Also Relative Standard deviation lies within range.

Amount of drug spiked(µl)	Change in mobile phase composition		%CV
	toluene: chloroform: ethanol (4:4:1 v/v/v).	toluene:chloroform:ethanol(4.5:4:1v/v/v)	
1	0.5	0.51	1.3933
2	0.503	0.5	0.4229
3	0.503	0.495	1.1336
			Avg=1.0507

Table no: -7 Data of Robustness

6. LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

LOD and LOQ of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations as per ICH guideline.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response.

S = slope of the calibration curve.

LOD was found to be 15.1809 ng/ band and LOQ was found to be 46.00273ng / band.

7. ANALYSIS OF MARKETED FORMULATION

Twenty tablets were weighed, their mean weight was determined and was finely powdered and powder equivalent to1tablet of glimepiride was weighed. Sample preparation (10mg/ml) was made and drug content per tablet was determined by performing assay. The percent content was found to be 110.16%

CONCLUSION

The subject matter of this report is to develop some new method for analysis of pharmaceuticals by using HPTLC technique. This technique is preferred over other modern analytical techniques because of its versatility in terms of application, selectivity, sensitivity and speed. In today's regulated pharmaceutical industry, it is essential to use very specific, sensitive, simple and accurate techniques, such as HPTLC to ascertain the quality of these pharmaceuticals.

The HPTLC method was developed for estimation of Glimepiride in standard and sample. The sample preparation was simple since no derivatization was required. The method was validated and proved to be specific, precise and accurate for analysis of Glimepiride. ICH guidelines were followed throughout the study for method validation and this method can be further used for research support and to check validation of different marketed preparation.

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