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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN HCL AND GLIMEPIRIDE IN COMBINED TABLET DOSAGE FORM

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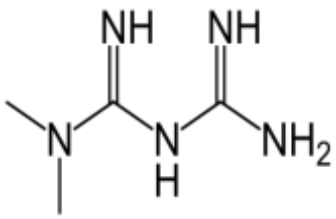
Abstract: An isocratic, reversed phase-liquid-chromatographic method was developed for the quantitative determination of Metformin Hcl And Glimepiride in combined-dosage form. A Waters Symmetry Shielde Rp18, (250*4.6*5µ) column with mobile phase containing pH 9.2 adjusted with ortho phosphoric acid : Methanol in the ratio of (60: 40, v/v) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored at 285 nm. The retention times of Metformin Hcl And Glimepiride were 2.344min and 3.725min, respectively. The correlation co-efficient for Metformin Hcl And Glimepiride was found to be 0.99 and 0.99, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Metformin Hcl And Glimepiride in formulations was found to be in the range of 97-103% and 97-103% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision. The method was successfully applied to the estimation of Metformin Hcl And Glimepiride in combined dosage form.

Key words: RP-HPLC, Metformin Hcl And Glimepiride

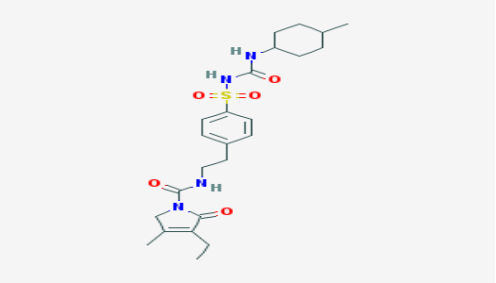
INTRODUCTION

Metformin is anti diabetic drug in biguanide class. Metformin (I, N, N-dimethyldiguanide) and used in the treatment of type 2 diabetes. Molecular formula for Metformin is found to be $C_4H_{11}N_5$ and molecular weight is 129.16 g mol^{-1}. Metformin decreases hepatic gluconeogenesis by interfering with respiratory oxidation in mitochondria. It suppresses gluconeogenesis from several substrates, including lactate, pyruvate, glycerol, and amino acids. In addition, Metformin increases intra-mitochondrial levels of calcium (Ca++), a modulator of mitochondrial respiration. It is a biguanide developed from galegine, a guanidine derivative found in Galega officinalis (French lilac). Chemically, it is a hydrophilic base which exists at physiological pH as the cationic species (>99.9%). Consequently, its passive diffusion through cell membranes should be very limited. Metformin is excreted unchanged in urine. The elimination half-life ($t_{½}$) of Metformin during multiple dosages in patients with good renal function is approximately 5 hours. Lactic acidosis is the feared adverse effect of the biguanide drugs but its incidence is very low in patients treated with Metformin. We suggest that the mean plasma concentrations of Metformin over a dosage interval be maintained below 2.5 mg/L in order to minimize the development of this adverse effect.



Structure of Metformin

Glimepiride (GLM) is a second-generation sulfonylurea, which is chemically known as 3-ethyl-4-methyl-N-{2-[4-({[(4-methylcyclohexyl) carbamoyl] amino} sulfonyl) phenyl] ethyl}-2-oxo-2, 5-dihydro-1H-pyrrole-1-carboxamide . It is an oral hypoglycaemic agent that stimulates insulin release from β cells of pancreases by blocking ATP-sensitive K+channel resulting in membrane depolarization and Ca++ influx.



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Structure of Glimepiride

MATERIAL AND METHODS

Instrumentation: The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software Waters Symmetry Shielde Rp18, (250*4.6*5µ).

Chemicals and Reagents Metformin Hcl And Glimepiride was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Methanol of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Ortho phosphoric acid of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

HPLC Conditions: The mobile phase consisting of water (pH 9.2 adjusted with Ortho phosphoric acid:Methanol (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 60:40v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was 30°C. The detection was monitored at 285nm and the run time was 6min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 15 min. with the mobile phase flowing through the system.

PREPARATIO OF STANDARD SOLUTION

Metformin Hcl: Accurately weighed quantity, 500.0 mg of Metformin was transferred into 100ml of volumetric flask and adds 30ml of mobile phase and sonicate for 15 min. make up the volume with mobile phase. Transferred above solution 5ml into 100ml volumetric flask and diluted to the mark with mobile phase.

Glimepiride: Accurately weighed quantity, 2mg of Glimepiride was transferred into 100ml of volumetric flask and adds 30ml of mobile phase and sonicate for 15mins make up the volume with mobile phase. Transferred above solution 5ml into 100ml volumetric flask and diluted to the mark with mobile phase.

PREPARATION OF SAMPLE SOLUTION:

Accurately weighed 2678.8 mg of sampel. Transfer the sampel powder into 100ml of volumetric flask added 25ml of mobile phase and sonicated for 30mins and make up the volume with mobile phase and filtered through the 0.45µm filter paper Transfer above solution 5ml into 100ml volumetric flask and make up the volume with mobile phase.

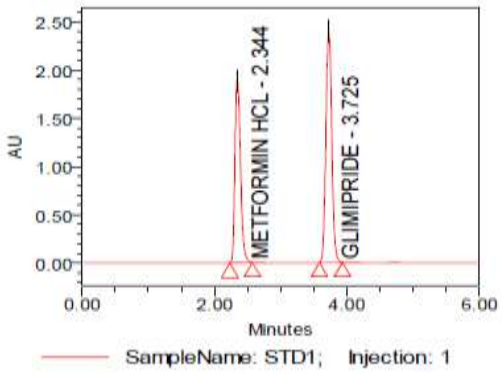


Fig. 1: Standard chromatogram for Metformin Hcl And Glimepiride

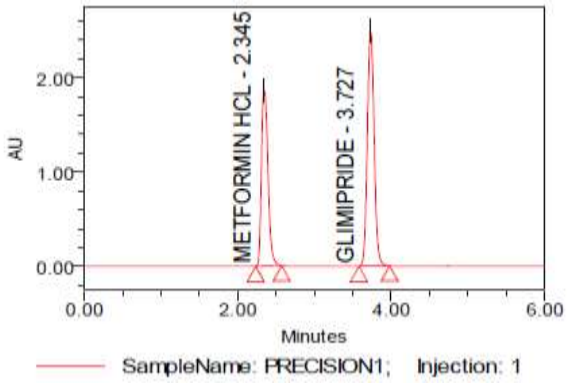


Fig. 2: Formulation chromatogram for Metformin Hcl And Glimepiride

METHOD VALIDATION

System Suitability Studies: The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

Table1: System Suitability Parameters

Parameters	Metformin	Glimepiride
Correlation Coefficient	0.99	0.99
Regression Equation	y = 16616x	y = 19288x
LOD	1.479	0.0051
LOQ	4.931	0.0169
Theoretical plates	3984	8920
Tailing	1.362	1.112

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc

ACCURACY AND PRECISION

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate (Table-3&4). The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. The chromatograms of three different levels shown in Fig 3, 4 &5. From the data obtained, the developed RP-HPLC method was found to be precise (Table-2).

Table 2 : Precision Studies

SNO	Sample Wt(mg)	Area (Met)	Area (Glime)	%Assya (lev)	%Assya(azi)
1	2678.80	11032897	14903226	101	101
2	2678.80	11093430	14934145	101	102
3	2678.80	11009547	14919834	100	101
4	2678.80	11079655	14983578	101	102
5	2678.80	11029324	14955638	101	102
6	2678.80	11022296	14956284	101	102

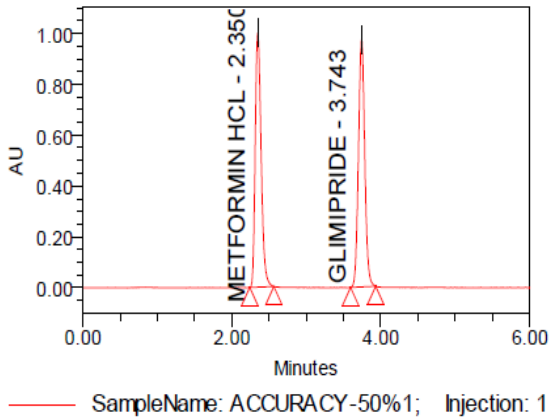


Fig. 3: Accuracy Chromatograms-50% of Metformin Hcl And Glimepiride

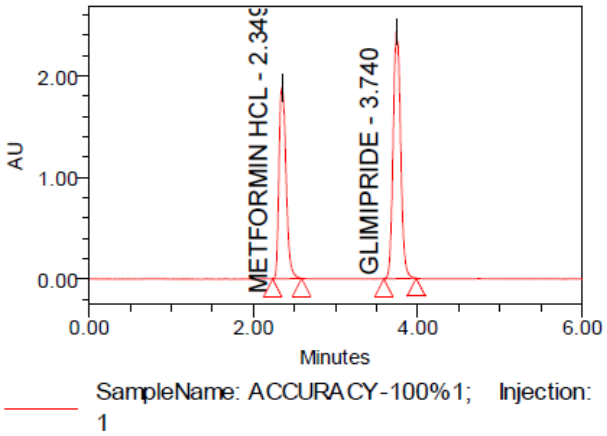


Fig. 4: Accuracy Chromatograms-100% of Metformin Hcl And Glimepiride

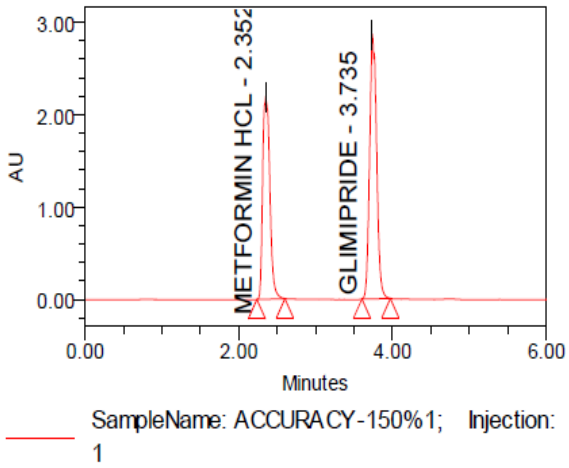


Fig. 5: Accuracy Chromatograms-150% of Metformin Hcl And Glimepiride

Table 3: Accuracy for Metformin

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	1368.45	5515024	247.750	246.21	99	99
50%	1368.45	5510203	247.750	245.99	99	
50%	1368.45	5517495	247.750	246.32	99	
50%	1368.45	5518068	247.750	246.35	99	
50%	1368.45	5515049	247.750	246.21	99	
50%	1368.45	5510366	247.750	246.00	99	
100%	2678.80	11080948	484.981	494.69	102	102
100%	2678.80	11058551	484.981	493.69	102	
100%	2678.80	11065661	484.981	494.01	102	
150%	4047.30	16523664	732.740	737.67	101	101
150%	4047.30	16593428	732.740	740.79	101	
150%	4047.30	16585748	732.740	740.44	101	
150%	4047.30	16584889	732.740	740.41	101	
150%	4047.30	16599730	732.740	741.07	101	
150%	4047.30	16513200	732.740	737.20	101	

Table 4: Accuracy for Glimepiride

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	% recovery	mean
50%	1368.45	7494691	0.997	1.00	100	100
50%	1368.45	7497671	0.997	1.00	100	
50%	1368.45	7494667	0.997	1.00	100	
50%	1368.45	7497215	0.997	1.00	100	
50%	1368.45	7499006	0.997	1.00	100	
50%	1368.45	7494829	0.997	1.00	100	
100%	2678.80	14933392.00	1.952	1.99	102	102
100%	2678.80	14991493.00	1.952	1.99	102	
100%	2678.80	14998326.00	1.952	2.00	102	
150%	4047.30	22479828	2.949	2.99	101	101
150%	4047.30	22454455	2.949	2.99	101	
150%	4047.30	22469210	2.949	2.99	101	
150%	4047.30	22459524	2.949	2.99	101	
150%	4047.30	22495519	2.949	2.99	101	
150%	4047.30	22497628	2.949	2.99	102	

LINEARITY AND RANGE

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 16616x (R^2=0.99) for Metformin and y = 19288x (R^2=0.99) for

Glimepiride The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The overlay chromatograms of Linearity for Metformin Hcl and Glimepiride shows in Fig 6 and the results for calibration curves are given in Fig 7&8.

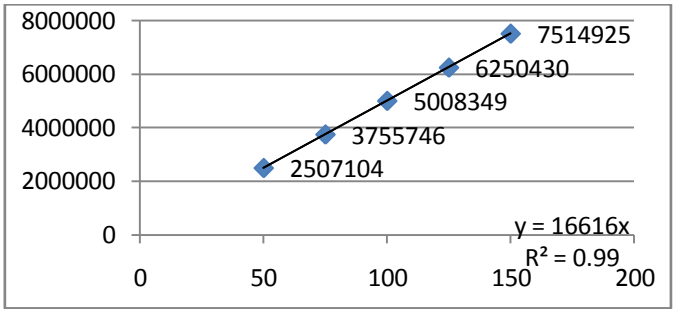


Fig. 7: Linearity Curve for Metformin

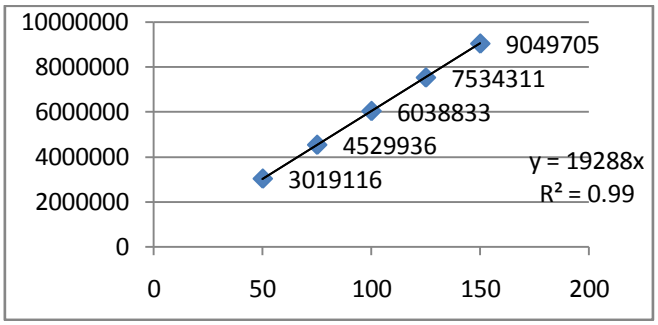


Fig. 8: Linearity Curve for Glimepiride

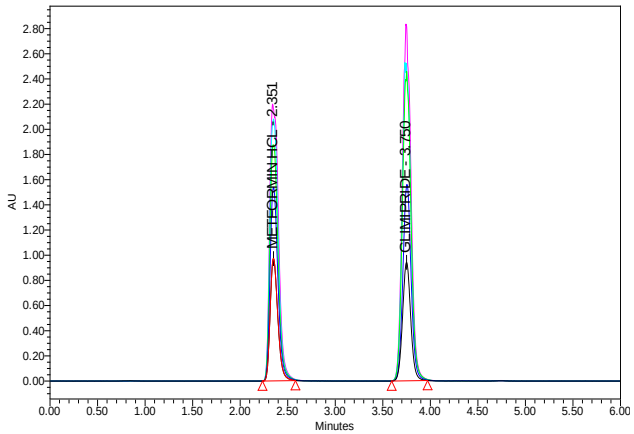
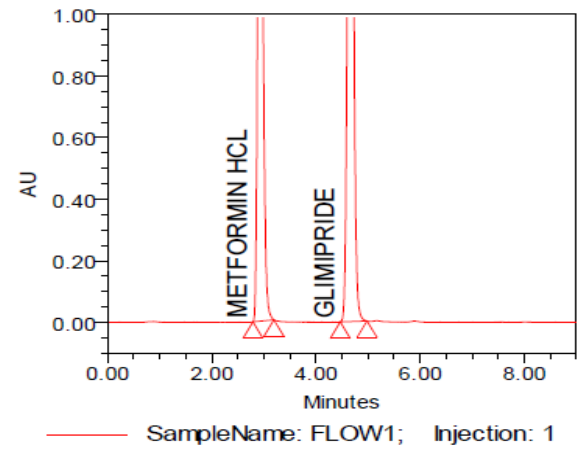
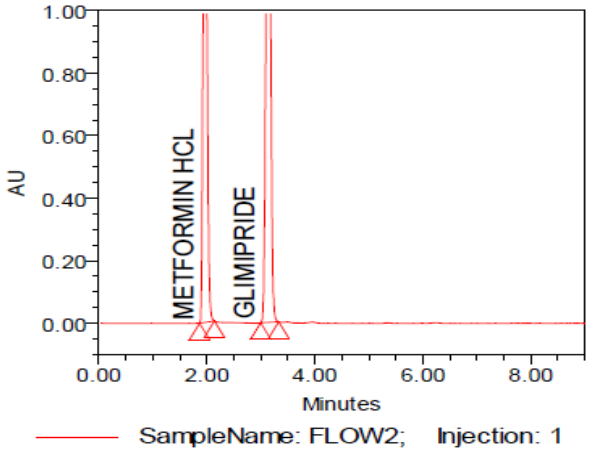


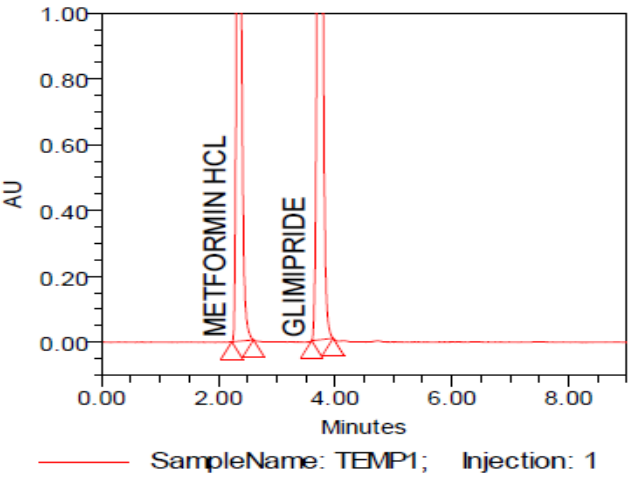
Fig. 9: Overlay chromatograms of Linearity for Metformin Hcl and Glimepiride

ROBUSTNESS

Robustness of the method was determined by making slight changes in the chromatographi conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed, are robust (Table-5&6).







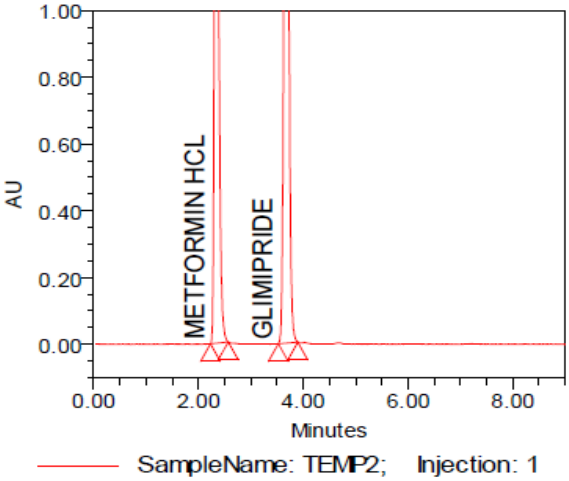


Table5:Robustness for Metformin

	SAMPEL NAME	INJ	NAME	RT	AREA	USP TAILING	USP PLATECOUNT
1	TEMP-1	1	Metformin	2.349	11238736	1.303	3931
2	TEMP-2	1	Metformin	2.339	11142147	1.390	4048
3	FLOW-1	1	Metformin	2.931	13823163	1.193	4133
4	FLOW-2	1	Metformin	1.966	9307321	1.171	3819

Table6:Robustness for **Glimepiride**

	SAMPEL NAME	INJ	NAME	RT	AREA	USP TAILING	USP PLATECOUNT
1	TEMP-1	1	Glimepiride	3.736	15320274	1.152	8510
2	TEMP-2	1	Glimepiride	3.671	15195027	1.174	9367
3	FLOW-1	1	Glimepiride	4.671	19015111	1.006	9589
4	FLOW-2	1	Glimepiride	3.132	12864400	1.105	8177

LOD&LOQ:

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of Metformin Hcl and Glimepiride. Relative standard deviation (σ) method was applied, the LOQ and LOD values were predicted using following formulas (a) and (b). Precision was established at these predicted levels.

(a) LOQ = 10 σ / S
(b) LOD = 3.3 σ / S

Where σ = residual standard deviation of response
S = slope of the calibration curve.

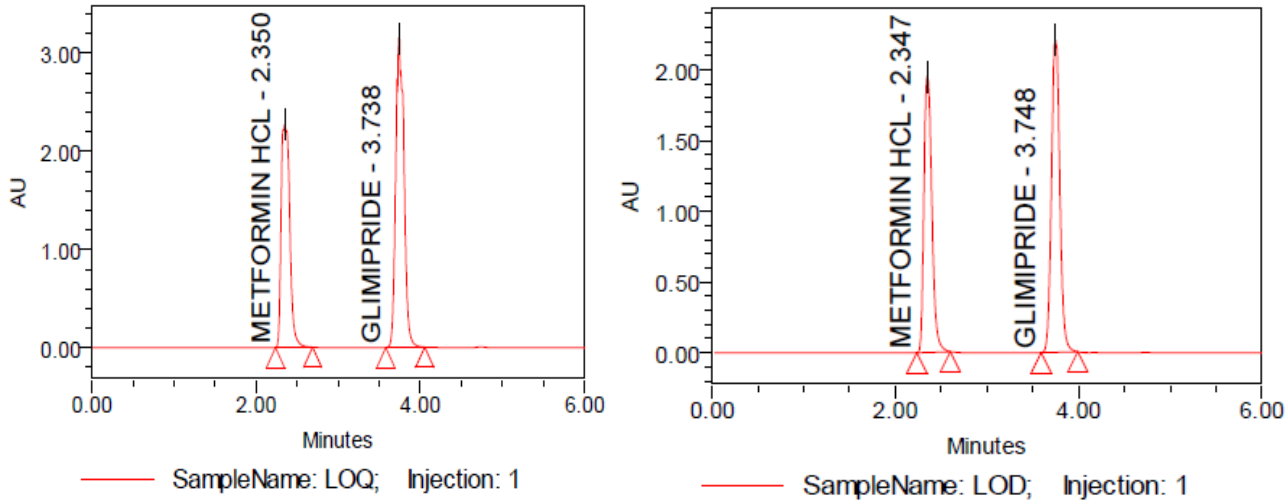


Table 7: LOD and LOQ For Metformin Hcl and Glimepiride

	Sampel name	inj	Name	RT	Area
1	LOD	1	MET	2.347	11781453
2	LOQ	1	MET	2.350	16642607
1	LOD	1	GLIME	3.748	14025433
2	LOQ	1	GLIME	3.738	21998779

RESULTS AND DISCUSSION

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig:1 and fig:2 those figures are Metformin Hcl and Glimepiride standard chromatogram and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method precision passed for both Metformin Hcl and Glimepiride studies. The method accuracy was evaluated by recovery studies. Metformin Hcl and Glimepiride recovery was founded 100% as per ICH 97%- 103% and also percentage RSD was very low so method is accurate shown in table 3&4. Linearity calibration curve was given below fig: 7&8 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation co-efficient.Linear correlation was found to be Y= 16616 for Metformin Hcl and y = 19288 for Glimepiride Method robustness results were given by table 5&6, LOQ and LOD Rsults were given by table 7.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Azithromycin and Levofloxacin in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Azithromycin and Levofloxacin in pure and its pharmaceutical dosage forms.

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