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Development and validation of RP-HPLC method for simultaneous estimation of metformin hydrochloride and glipizide in bulk and pharmaceutical dosage form

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ABSTRACT

A simple, accurate, economical and precise reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for simultaneous estimation of Metformin hydrochloride and Glipizide in bulk and pharmaceutical dosage form. The separation of Metformin hydrochloride and Glipizide was achieved by using Cosmosil C 18 (250 mm × 4.6 ID, particle size-5 micron) column as stationary phase with the mobile phase comprising of methanol and water (60:40 ,pH 3 adjusted with ortho-phosphoric acid) in an isocratic mode and flow rate of 0.8 ml/min with UV detection at 226 nm. The retention time of Metformin hydrochloride and Glipizide were found to be 4.159 min and 5.571 min respectively. The proposed method was validated according to ICH guidelines for the parameters like linearity, accuracy, precision, percent recovery, robustness, ruggedness, limit of detection and limit of quantitation. The % RSD is found to be less than 2 % and the tailing factor for both the drugs are found to be less than 2. The number of therotical plates for Metformin hydrochloride and Glipizide were found to be more than 2000.All validation parameters were within the acceptable range. The developed method was successfully applied for the estimation of Metformin hydrochloride and Glipizide in bulk and pharmaceutical dosage forms.

Keywords: Metformin hydrochloride, Glipizide, RP-HPLC, Validation, Simultaneous estimation, ICH guidelines.

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INTRODUCTION

Metformin [Figure 1] (Molecular formula-$C_4H_{11}N_5$) is 1-carbamimidamido-N,N-dimethylmethanimidamide.Metformin is the compound belongs to the class of organic compounds known as biguanides.Biguanides are the organic compounds containing two N-linked guanidine.

Metformin is a antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM).It improves the glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing the insulin-mediated glucose uptake. Metformin is the only oral anti-diabetic agent that is not associated with weight gain. Metformin may induce weight loss and it is the drug of choice for the obese NIDDM patients. [1]

Glipizide [Figure 2] (Molecular formula- $C_{21}H_{27}N_5O_4S$) is a N-[2-(4-{[(cyclohexylcarbamoyl) amino] sulfonyl}] phenyl) ethyl]-5-methylpyrazine-2-carboxamide.lipizide is an oral hypoglycemic agent which is rapidly absorbed and metabolised completely.Glipizide belongs to the class of organic compounds known as benzenesulfonamides. Benzenesulfonamides are the organic compounds containing a sulfonamide group which is S-linked to the benzene ring. Glipizide is a second generation sulfonylurea and it is used with diet to lower blood glucose in patients with type-II diabetes mellitus..Glipizide lowers the blood glucose level acutely by stimulating the release of insulin from the pancreas,this effect is dependent on functioning of beta cells in pancreatic islets. [2].

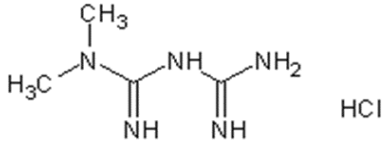

Fig.1: Chemical structure of Metformin Hydrochloride

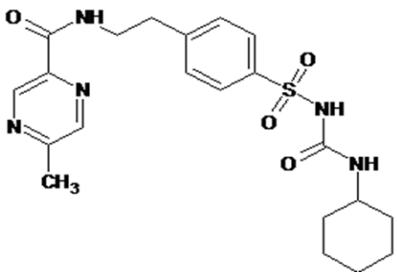

Fig.2: Chemical structure of Glipizide

Literature survey of Metformin Hydrochloride and Glipizide revealed few methods based on Chromatography [3-9] have been have been reported for the determination of both drugs in single and combined dosage forms.The present work describes the development and validation as per ICH guidelines [10] of reverse phase-high performance liquid chromatographic method (RP-HPLC method) , which can quantify these components simultaneously.

MATERIALS AND METHODS

Instrument and equipment

Chemicals and Reagents

Pure samples of Metformin Hydrochloride and Glipizide were provided as a gift sample by Wintech Pharmaceuticals Pvt.Ltd., Sinner. The commercial pharmaceutical preparation Dibizide –M containing 500 mg Metformin Hydrochloride and 5 mg Glipizide respectively (manufactured by Micro Labs Ltd.) were procured from local pharmacy. Methanol HPLC grade, ortho-phosphoric acid (OPA)HPLC grade were procured from Finar chemicals,Ahmedabad,Gujrat and Merk Chemicals, Mumbai respectively.HPLC grade water was obtained from Milli-Q water purification system (Millipore,Milford,USA).

Instrumentation and software

A HPLC (Binary Gradient system, Model: HPLC 3000 series) consisting of P-3000-M Reciprocating pump (40 MPa), UV-VIS detector (Model: series 3000-M), Cosmosil C-18 (250mm × 4.6 ID, particle size-5 micron) and a sample injector system with a 20 µl sample loop and HPLC workstation software on;Ultrasonicator (Wenser Ultra Sonicator,Model:WUC-4L).

Chromatographic System

The chromatographic column used was C-18 (250 mm × 4.6 ID, particle size-5 micron). Mobile phase consists of methanol and water (60:40, pH 3 adjusted with ortho-phosphoric acid).The flow rate of mobile phase was kept at 0.8 ml/min and the column temperature was maintained at ambient and the chromatogram was monitored at 226 nm. The injection volume was monitored at 20µl.

Identification of Metformin Hydrochloride and Glipizide

Melting point determination

Melting point was determined using digital melting point apparatus with one end open capillary method.

Table 1: Melting point determination

Sr.No.	Name	Melting Point Standard Value	Melting Point Observed Value
1.	Metformin Hydrochloride	222°-226°C	220°-222°C
2.	Glipizide	208°-209°C	208°-210°C

Determination of Wavelength Maxima

The standard solution of both the drugs was scanned between 400 nm-200nm.The overlain spectrum of both the drugs was recorded. From the overlain spectrum, 226 nm (λmax of Glipizide) and 238.5 nm (λmax of Metformin Hydrochloride) were selected for estimation of drugs and the isobestic point was found to be at 226nm and it is shown in figure 3.

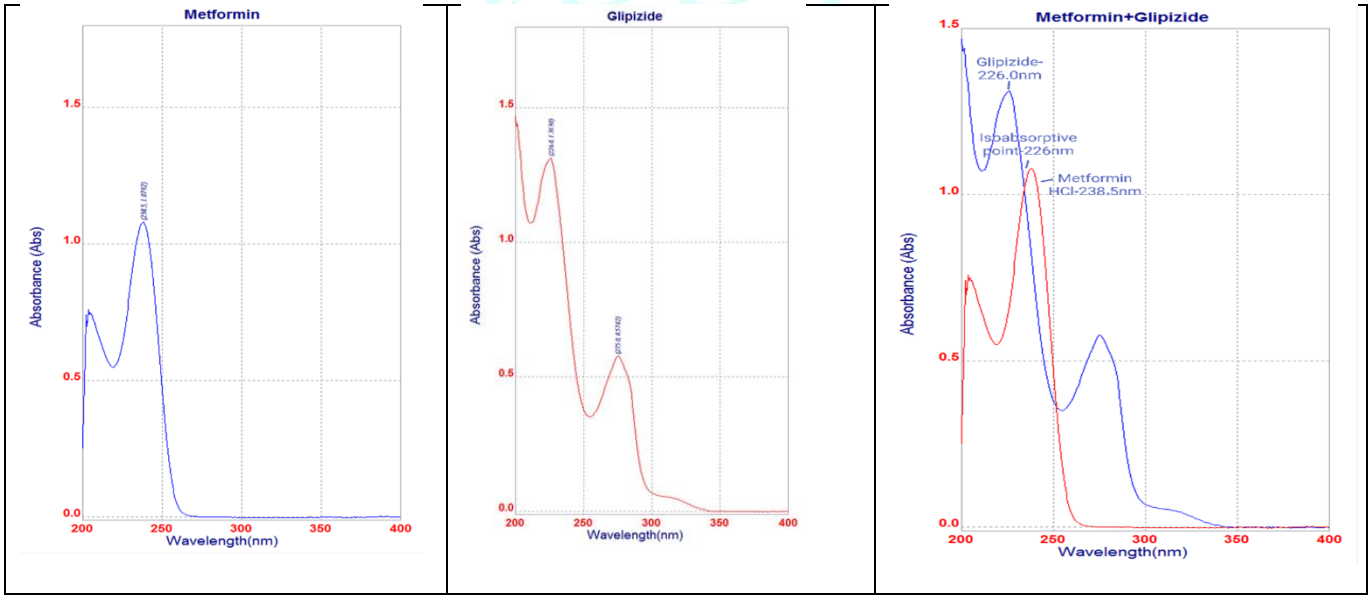

Figure 3: Isobestic point of Metformin HCl and Glipizide

Table No.2: Wavelength maxima of Metformin HCl and Glipizide

Sr.No.	Name of the Sample	Wavelength	Absorbance
1.	Metformin Hydrochloride	238.5	1.0792
2.	Glipizide	226	1.3130
3.	Isobestic point of Metformin HCl and Glipizide	226	1.3102

Preparation of mobile phase

Pipette out 60 ml of methanol and 40 ml of 2 % OPA solution in HPLC grade water (to adjust the pH 3) and Sonicate 5 minutes.

Preparation of standard stock solutions

Accurately weighed 10 mg of pure drugs i.e. Metformin Hydrochloride and Glipizide dissolved individually in 10 ml of mobile phase; this gives 1000 ppm stock solutions of Metformin Hydrochloride and Glipizide (Stock-1) .Standard calibration solutions of Metformin Hydrochloride and Glipizide having concentration in the range of 100 µg/ml-500µg/ml and 1µg/ml-5µg/ml respectively were prepared by diluting stock solution (Stock-1) with mobile phase.

Analytical method validation

Validation is documented evidence, which provide a high degree of assurance for specific method. Validation is analytical process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirement for intended analytical application

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range.

Procedure for preparation of sample solution for determination of Linearity

Linearity was established by calibration curve of five standard drug concentration. From stock solution , pipette out 1ml,2ml,3ml,4ml and 5ml Metformin hydrochloride solution and 0.01 ml,0.02 ml,0.03 ml,0.04 ml, 0.05 ml Glipizide solution and make up the final volume upto the 10ml with the mobile phase.It gives 100 ppm,200 ppm,300 ppm,400 ppm and 500 ppm solutions of Metformin hydrochloride and 1 ml,2 ml,3 ml,4 ml and 5ml solutions of Glipizide respectively .Calibration curve was constructed between concentration and peak area.

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added. The accuracy is calculated from the test results as the triplicates sample preparation.

Procedure for preparation of sample solution for determination of Accuracy

The accuracy was determined by using data obtained from precision study and determined from the calibration curve. Accuracy is done by three level i.e. 100 ppm,300ppm and 500 ppm of metformin hydrochloride and 1ppm,3ppm and 5 ppm of Glipizide .,etc. And the concentration for the accuracy determination is taken 100µg/ml of Metformin hydrochloride and 1 µg/ml of Glipizide respectively, from the stock solution .Find out the Area, Assay and % RSD of sample.

Precision

The precision of an analytical procedure represents the nearness of agreement between a series of measurements got from multiple sampling of the same homogenous sample under the similar analytical conditions and it is divided into 3 categories.

Repeatability: - Precision under same operating conditions, same analyst over a short period of time.

Intermediate precision: - Method is tested on multiple days, instruments, analysts etc.

Reproducibility: -Inter-laboratory studies.

The ICH guidelines suggest that repeatability should be conformed duly utilizing at least 9 determinations with specified range for the procedure (e.g., three concentrations / three replicates each) or a minimum of 6 determinations at 100 % of the test concentration.

Procedure for preparation of sample solution for determination of Precision

The Precision or repeatability of the method was determined by assuming two standard solutions from the stock solution 3ml of Metformin hydrochloride and 0.03 ml of Glipizide pipette out and dilute up to the 10 ml with mobile phase at conc.300µg/ml. and 3µg/ml.The reproducibility of proposed method was determined by performing drug assay at different time intervals on same day (intra-day precision) and on two different days (inter-day precision).Result were recorded to calculate mean, SD, %RSD as per ICH guidelines.

% Recovery

The recovery of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added. The Recovery is calculated from the test results as the percentage of analyte recovered by the assay.

Procedure for preparation of sample solution for determination of % Recovery

The Stock solution equivalent to 300%, 400% and 500% were prepared by using standard stock solution. From the result, % recovery was calculated. Injected standard preparation and sample preparations of recovered solutions into the HPLC and measure the peak responses. Amount of drug recovered was calculated.

Robustness

It is the measure of capacity of the method to remain unaffected by small but deliberate variation in method parameter and provides an indication of its reliability under normal usage.

Determination

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. E.g. flow rate, concentration, run time etc.

Limit of Detection (LOD)

LOQ is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected, but not necessarily quantitated as precise value, under the stated experimental conditions. The detection limit is generally expressed in the concentration of analyte (ppm) in the sample.

The formula for calculating LOD is LOD = 3.3 δ/S

Where δ = standard deviation of intercepts of calibration curves.

S = the slope of linearity plot.

Limit of Quantitation (LOQ)

Limit of quantitation is the least concentration of drug in a sample which is estimated with appropriate precision and accuracy under the affirmed experimental conditions.

The formula for calculating LOQ is LOQ = 10 δ/S

Where δ = standard deviation of response.

S = Mean of slopes of the calibration curves.

System Suitability Parameter

1) Resolution

Resolution value should be greater than 1.75. This parameter is applicable only when there is a combination of two samples. In case of single sample it will show zero „0" value.

2) Theoretical Plates

Number of theoretical plates should be greater than 2000. It indicates the efficiency of column.

3) Tailing / Asymmetry factor

Value of asymmetry factor should be less than 2.

Acceptance criteria

• Asymmetry of both the analytes peak in standard should not be more than 1.0

• Theoretical plates of both the analytes peak in standard should not be less than 2000

• Relative Standard Deviation for five replicates injections of both the standard preparation should not be more than 2.0%

Method development

All the trials are shown in table 1 and selected method chromatogram is shown in fig.4.

Selection of Mobile Phase

Table 3: Observation and Remarks of the Method Development

S.N.	Trials Taken	Observation	Remark
1	Methanol: Water (70:30),pH 3 adjusted with OPA	shows bad peak shape	Not Satisfactory
2	Methanol: Water (60:40),pH 3 adjusted with OPA	Shows sharp peak and stable Retention time	Satisfactory

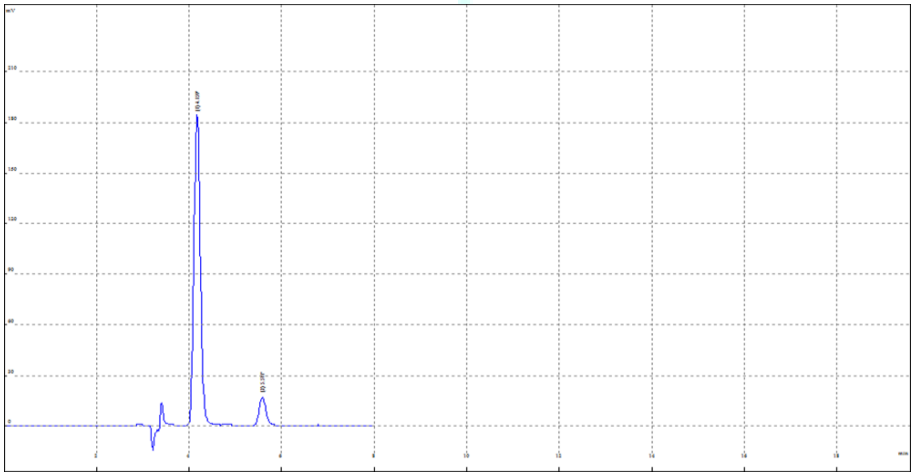

Figure 4: Chromatogram for selected method

Table 4: Final Chromatographic conditions

Column	Cosmosil C18 (250mm × 4.6 ID, Particle size:-5 micron)
Flow rate	0.8 ml/min
Wavelength	226 nm
Injection volume	20 µL
Run time	8.01 min
Mobile phase	Methanol: Water (60:40), pH 3 adjusted with (2 %) OPA
Elution type	Isocratic elution

METHOD VALIDATION

The developed method for Metformin Hydrochloride and Glipizide was validated as per ICH guidelines.

Accuracy

Table 5: Result and statistical data of accuracy for Metformin Hydrochloride

			Standard Deviation		Accuracy	Precision
Sr.No.	Conc.	Area	Mean	SD	%SD	%RSD
1	100 100 100	922877 927432 925460	925256.3333	2284.319665	0.2468851	0.246885061
2	300 300 300	2880002 2871216 2872495	2874571	4746.659984	0.1651259	0.165125856
3	500 500 500	4775306 4764993 4780210	4773503	7767.070168	0.1627122	0.162712167

Table 6: Result and statistical data of accuracy for Glipizide

			Standard Deviation		Accuracy	Precision
Sr.No.	Conc.	Area	Mean	SD	%SD	%RSD
1	1 1 1	137246 136619 138405	137423.3333	906.1094489	0.6593563	0.659356331
2	3 3 3	405362 401759 406520	404547	2482.931533	0.613756	0.613756012
3	5 5 5	638980 633467 634955	635800.6667	2852.131893	0.448589	0.448589006

% Recovery

A] % Recovery of Metformin Hydrochloride-

Table 7: Result and Statistical data of % Recovery of Metformin Hydrochloride

Sr. No.	Level (%)	Conc. Of API spiked (µg/ml)	Conc. Of Sample solution (µg/ml)	Total Concentration (µg/ml)	Area of Standard	Area of Sample	% Recovery
1	50 %	100	200	300	2880002	2868459	99.59
2	100 %	100	300	400	3871538	3872852	100.03
3	150 %	100	400	500	4775306	4751550	99.50

B] % Recovery of Glipizide-

Table 8: Result and Statistical data of % Recovery of Glipizide

Sr. No.	Level (%)	Conc. Of API spiked (µg/ml)	Conc. Of Sample solution (µg/ml)	Total Concentration (µg/ml)	Area of Standard	Area of Sample	% Recovery
1	50 %	1	2	3	405362	401869	99.13
2	100 %	1	3	4	522726	526067	100.63
3	150 %	1	4	5	638980	632059	98.91

Precision

1. Intra- day Precision:-

A. Intra- day Precision for Metformin hydrochloride:-

Table 9: Result of Intra- day Precision for Metformin hydrochloride

	Sr.No.	Conc.(ppm)	R.T.(Min)	Area	Result
Morning	1	300	4.169	2880002	Mean = 2875798.66
	2	300	4.261	2871216	
	3	300	4.208	2872495	S.D.= 4110.354
Evening	4	300	4.195	2872531	
	5	300	4.251	2879103	%R.S.D.= 0.14%
	6	300	4.250	2879445	

B. Intra- day Precision for Glipizide:-

Table 10: Result of Intra- day Precision for Glipizide

	Sr.No.	Conc.(ppm)	R.T.(Min)	Area	Result
Morning	1	3	5.535	405362	Mean = 404558.66
	2	3	5.725	401759	
	3	3	5.658	406520	S.D.= 2037.537
Evening	4	3	5.700	406681	
	5	3	5.698	402581	%R.S.D.= 0.50%
	6	3	5.698	404449	

. Inter- day Precision:-

A. Inter- day Precision for Metformin hydrochloride:-

Table 11: Result of Inter- day Precision for Metformin hydrochloride

	Sr.No.	Conc.(ppm)	R.T.(Min)	Area	Result
Day-1	1	300	4.169	2880002	Mean = 2878954.33
	2	300	4.261	2871216	
	3	300	4.208	2872495	S.D.= 7320.294
Day-2	4	300	4.203	2886219	
	5	300	4.242	2875000	%R.S.D.= 0.25%
	6	300	4.454	2888794	

Inter- day Precision for Glipizide:-

Table 12: Result of Inter- day Precision for Glipizide

	Sr.No.	Conc.(ppm)	R.T.(Min)	Area	Result
Day-1	1	3	5.535	405362	Mean = 403842.66
	2	3	5.725	401759	
	3	3	5.658	406520	S.D.= 2137.702
Day-2	4	3	5.653	401860	
	5	3	5.690	402182	%R.S.D.= 0.529%
	6	3	6.119	405373	

Linearity-

Table 13: Linearity Data for Metformin Hydrochloride

Sr.No.	Concentration (µg/ml)	RT (min)	Area	Plate Count
1.	100	4.210	922877	8735
2.	200	4.177	1894271	8800
3.	300	4.169	2880002	8078
4.	400	4.248	3871538	8836
5.	500	4.250	4775306	8815

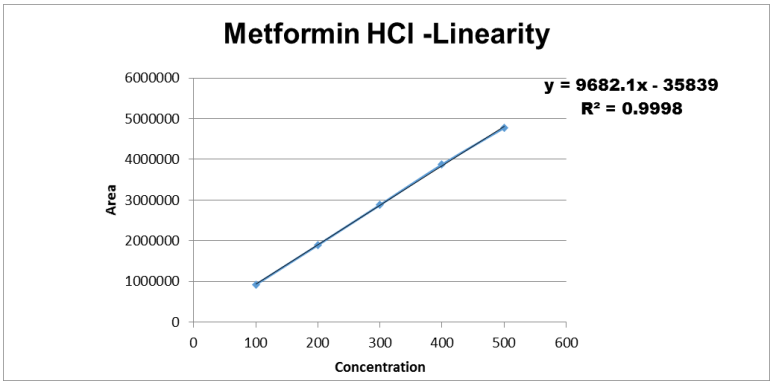

Figure 5: Linearity graph of Metformin HCl.

Table 14: Linearity Data for Glipizide

Sr.No.	Concentration (µg/ml)	RT (min)	Area	Plate Count
1.	1	5.621	137246	8699
2.	2	5.575	283287	8510
3.	3	5.535	405362	8501
4.	4	5.695	522726	8225
5.	5	5.702	638980	8285

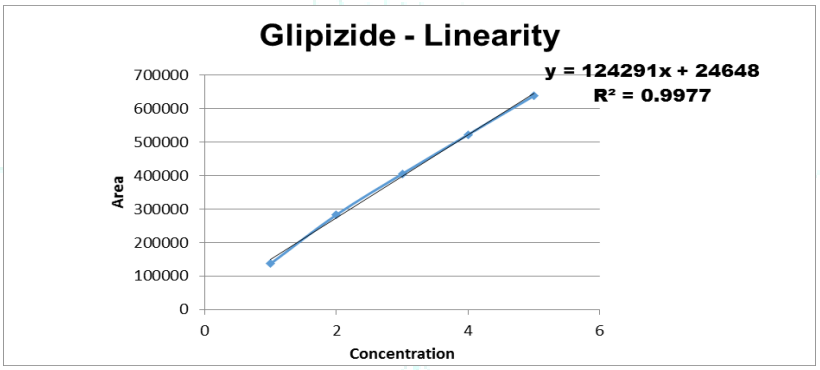

Figure 6: Linearity graph of Glipizide.

ROBUSTNESS:

The robustness of an analytical method 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.

A] Change in Flow rate:-

I) Metformin Hydrochloride -

Table 15: Result and Statistical data of Robustness of Metformin Hydrochloride

Sr.No.	Conc.(µg/ml)	Area of Peak	λmax (nm)	Flow rate (ml/min)	Results
1	200	1890209	226	0.7	**Mean =1890623**
2	200	1887390	226	0.9	**S.D =3459.16**
3	200	1894271	226	1.0	**%S.D. =0.182**

II) Glipizide -

Table 16: Result and Statistical data of Robustness of Glipizide

Sr.No.	Conc.(µg/ml)	Area of Peak	λmax (nm)	Flow rate (ml/min)	Results
1	2	284705	226	0.7	**Mean = 283529**
2	2	282596	226	0.9	**S.D =1075.18**
3	2	283287	226	1.0	**%S.D.=0.379**

B] Change in Wavelength:-

I) Metformin Hydrochloride -

Table 17: Result and Statistical dada of Robustness of Metformin Hydrochloride

Sr.No.	Conc.(µg/ml)	Area of Peak	λmax (nm)	Flow rate (ml/min)	Results
1	200	1894271	226	0.8	**Mean = 1892893**
2	200	1893916	224	0.8	**S.D = 2087.46**
3	200	1890491	228	0.8	**%S.D. = 0.110**

II) Glipizide –

Table 18: Result and Statistical dada of Robustness of Glipizide

Sr.No.	Conc.(µg/ml)	Area of Peak	λmax (nm)	Flow rate (ml/min)	Results
1	2	283287	226	0.8	**Mean =283140**
2	2	281458	224	0.8	**S.D =1614.01**
3	2	284676	228	0.8	**%S.D. = 0.570**

Ruggedness:-

Table 19: Ruggedness Data for Metformin Hydrochloride

Sr.No.	Concentration (µg/ml)	RT (min)	Area	Plate Count
1.	100	4.135	925999	8431
2.	200	4.173	1898856	8654
3.	300	4.252	2888157	8380
4.	400	4.241	3867325	8882
5.	500	4.263	4772516	8288

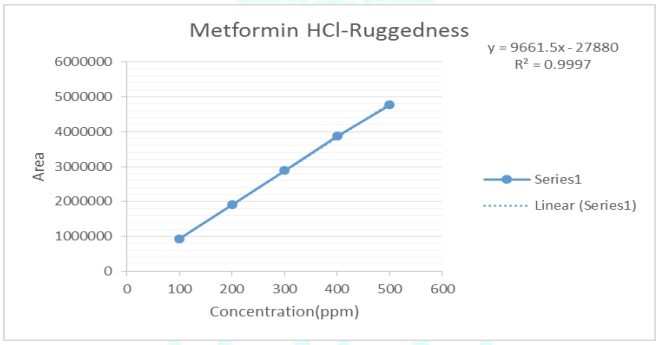

Figure 7: Ruggedness graph of Metformin Hydrochloride

Table 20: Ruggedness Data for Glipizide

Sr.No.	Concentration (µg/ml)	RT (min)	Area	Plate Count
1.	1	5.533	137117	8385
2.	2	5.573	283468	8495
3.	3	5.703	408453	8965
4.	4	5.686	527353	8573
5.	5	5.723	638328	8456

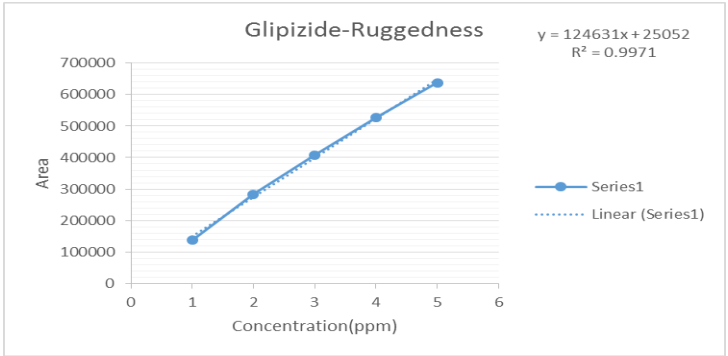

Figure 8: Ruggedness graph of Glipizide

Limit of Detection-

The limit of detection (LOD) may be expressed as: -

$$LOD = 3.3 \times (SD)/S$$

Where, SD= Standard deviation of the Y intercept

S = Slope

A] LOD of Metformin Hydrochloride = 3.3 x 4932.68/ 9682.1 = 1.68 (µg/ml).

B] LOD of Glipizide = 3.3 x 2080.390/ 124291= 0.055 (µg/ml).

The LOD of Metformin Hydrochloride and Glipizide was found to be 1.68 (µg/ml) and 0.055 (µg/ml) respectively.

Limit of Quantitation

The quantitation limit (LOQ) may be expressed as

$$LOQ = 10 (SD)/S$$

Where, SD = standard deviation of Y intercept

S = Slope

A] LOQ of Metformin Hydrochloride = 10 x 4932.68 / 9682.1 = 5.09 (µg/ml).

B] LOQ of Glipizide = 10 x 2080.390/124291= 0.167 (µg/ml).

The LOQ of Metformin Hydrochloride and Glipizide was found to be 5.09 (µg/ml) and 0.167 (µg/ml) respectively.

Analysis of marketed formulation (Assay)-

Table 21: Assay results

Drug	Label Claim (mg/tab)	Taken Concentration (ppm)	Area of Standard	Area of sample	% Assay
Metformin Hydrochloride	500mg	300 ppm	2880002	2872186	99.72%
Glipizide	5 mg	3 ppm	405362	405362	100%

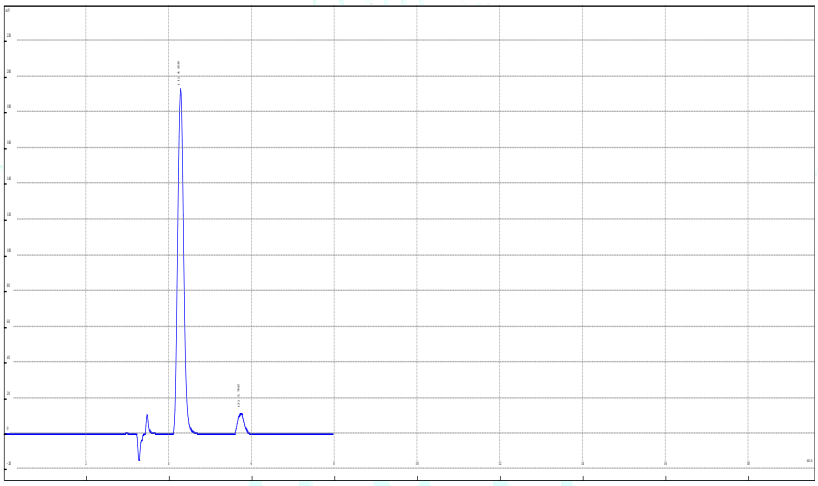

Figure 9: Chromatogram of Assay of Marketed Preparation

SUMMARY AND CONCLUSION

The present work involved the development of simple, accurate, precise and suitable RP-HPLC method.

Literature survey revealed that several methods have been reported for determination of Metformin Hydrochloride and Glipizide individually or in combination with other drugs in pharmaceutical dosage forms.Hence,in the present study, a new, sensitive suitable and cost effective reversed-phase high performance liquid chromatography method was developed and validated for determination of Metformin Hydrochloride and Glipizide in bulk and pharmaceutical dosage form.

In RP-HPLC method, the analytes were resolved by using isocratic program and mobile phase used was Methanol: Water (60:40 v/v; pH adjusted to 3 with OPA), at a flow rate of 0.8ml/min, in HPLC system containing UV-visible detector with HPLC workstation software and Cosmosil C-18 column (250 mm × 4.6 ID, particle size-5 micron) .The detection was carried out at 226 nm. The method gave the good resolution and suitable retention time.

The results of analysis in the method were validated in terms of linearity, accuracy, precision, percent recovery, robustness, ruggedness, limit of detection and limit of quantitation.

The method has several advantages, including simple mobile phase, low cost solvent, rapid analysis, simple sample preparation method and improved selectivity as well as sensitivity. The regression coefficient (R^2) near to 1 which shows good linearity. The percent recovery was in the acceptable range in the tablet dosage form. The % RSD Values are also less than 2 %, showing high degree of precision of the proposed method.

Since the method does not require use of expensive reagent and also less time consuming, it can be performed routinely in industry for routine analysis of marketed products of Metformin hydrochloride and Glipizide.

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