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Simultaneous Estimation of Glipizide and Metformin Hydrochloride in Marketed Formulation

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ABSTRACT

The developed HPLC method was found to be accurate, precise, and specific and can be routinely used for simultaneous estimation of Glipizide and Metformin hydrochloride in marketed formulations. The percentage assay of Glipizide and Metformin hydrochloride in the marketed formulation was found to be in the range of 98.82% to 101.06% and 99.79 to 100.07% respectively. The results show that there is no interference from excipients and no impurities were observed in samples for the proposed method.

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INTRODUCTION:

ANALYTICAL METHOD DEVELOPMENT [1-5]:

Analytical method development and validation play important roles in the discovery, development and manufacture of pharmaceuticals. Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product. This presentation will discuss the development and validation of analytical method (Spectrophotometric & High-performance liquid chromatography (HPLC)) for drug products containing more than one active ingredient. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products**.**

Basic criteria for new method development of drug analysis:

- The drug or drug combination may not be official in any pharmacopeias,
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations,
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients,
- Analytical methods for the quantitation of the drug in biological fluids may not be available,
- Analytical methods for a drug in combination with other drugs may not be available,
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

SYSTEM SUITABILITY [6-11]:

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Before performing any validation experiment, you should establish that the HPLC and procedure are capable of providing data of acceptable quality. These tests are used

to verify that the resolution and repeatability of the system are adequate for the analysis to be performed. It is based on the concept that equipment, electronics, analytical operations and sample constitute an integral system that can be evaluated as a whole.

The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagents, columns, analysts) is suitable for the intended application.

The parameters that are affected by the changes in chromatographic conditions are,

- Resolution (R_s),
- Capacity factor (k'),
- Selectivity (α),
- Column efficiency (N) and
- Peak asymmetry factor (As).

Methodology:

Sample: Glipizide and Metformin hydrochloride Tablet

Brand name: Glynase –MF (5 mg of Glipizide and 500 mg of Metformin hydrochloride).

Manufacturer: USV Pvt Ltd, Mumbai, India.

Preparation of Sample Solution:

Sample stock solution:

Tablet of Glynase –MF containing Glipizide and Metformin hydrochloride. 662 mg of tablet powder equivalent to 5mg of Glipizide and 500mg of Metformin hydrochloride was transferred into a clean and dry 100 mL volumetric flask and dissolved in few mL of methanol by sonication for 3 min. The resulting solution was filtered through Whatmann filter and volume of filtrate was made up to 100 mL with methanol (stock I).

Working sample solution:

2.5, 5, 10, 12.5, and 15 mL of sample stock solution were transferred to separate 100mL volumetric flasks and volume made up to 100 mL with methanol to obtain the concentration of 0.125, 0.25, 0.5. 0.625 and 0.725µg/mL of Glipizide and 12.5, 25, 50, 62.5 and 75 µg/mL of Metformin hydrochloride.

RP-HPLC method for simultaneous estimation of Glipizide and Metformin hydrochloride was developed and validated.

Table.No.1 Instrument Specification:

Instrument	SHIMADZU UFLC-2000 Prominence LC-20AD Binary Gradient System SPDM 20 A Detector
Injector	Rheodyne
Column	Enable C-18 Gcolumn250×4.6mm&5µm
Detector	PDA Detector
Wavelength	258 nm
Flow rate	1.0 mL/min
Injection volume	20 µL
Mobile phase	Phosphate buffer: Acetonitrile (60:40) pH 5.8

Reagents and chemicals:-

1. Potassium dihydrogen Phosphate (Thermo Fisher Scientific India Pvt. Ltd, Mumbai)

2. Sodium hydroxide (Thermo Fisher Scientific India Pvt. Ltd, Mumbai))

3. Acetonitrile (Thermo Fisher Scientific India Pvt. Ltd, Mumbai))

4. Millipore water

5. Glipizide (Supra Chemicals, Thane, Mumbai, India)

6. Metformin Hydrochloride (Dr. Reddy's, Hyd, India)

7. Glynase-MF Tablets (Glipizide 5mg and Metformin Hydrochloride 500mg) were manufactured by USV Pvt. Ltd. Mumbai, India purchased from local market.

Development of HPLC Method for Simultaneous Determination of Glipizide and Metformin Hydrochloride:

A method was developed for the simultaneous estimation of Glipizide and Metformin Hydrochloride on HPLC by selecting the appropriate λ max, optimum mobile phase and flow rate which gives good peaks and sharp Resolution.

Selection of Mobile Phase

Several solvent systems were tried to get good optimum resolution of Glipizide and Metformin hydrochloride in the present method. The observation obtained with various mobile phases in different rations are given below.

Table.No.2 Different Mobile Phase used and their observations

Mobile Phase used	**Ratio**	**Observation**
Methanol: Water	50:50	Split peak
Methanol: Water	70:30	Split peak
Methanol: Water:0.01M Phosphate buffer	70:25:5	Tailing
Methanol: 0.05M Phosphate buffer (pH 7±0.05)	85:15	Split peak
Acetonitrile: Water	50:50	Split peak
Acetonitrile: Phosphate buffer (pH 3.0)	35:65	Single peak
0.2MPhosphate buffer (pH 5.8): Acetonitrile	**60:40**	**Satisfactory resolution**

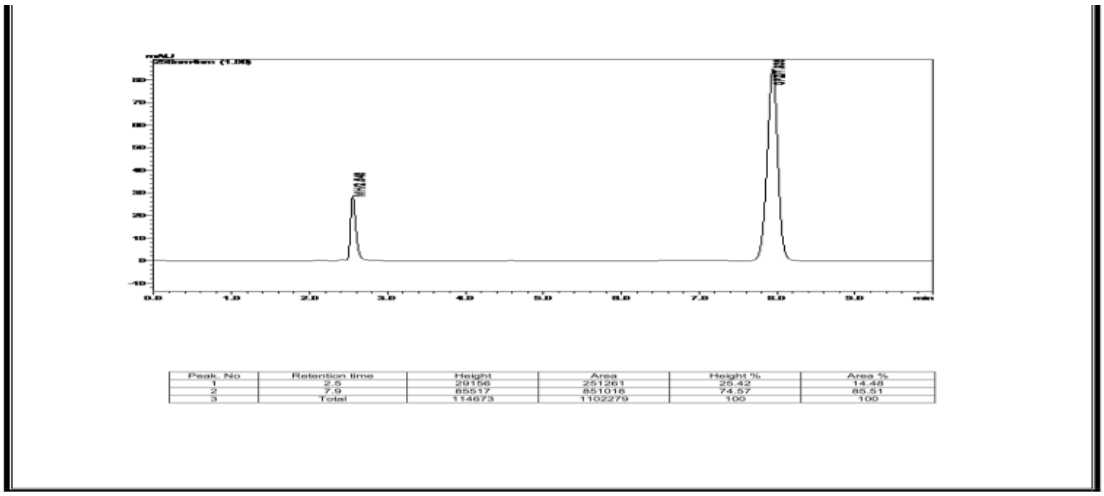


Fig No.1 Chromatogram showing Glipizide and Metformin hydrochloride peaks with good resolution with the mobile phase Phosphate buffer (pH 5.8) and Acetonitrile (60:40 v/v)

(c) Determination of Retention Time for Glipizide and Metformin hydrochloride

20µL of standard mixture solution of Glipizide and Metformin hydrochloride (100µg/mL) was injected at a flow rate of 1mL/min and UV detection at 258 nm. The chromatogram obtained is presented below.

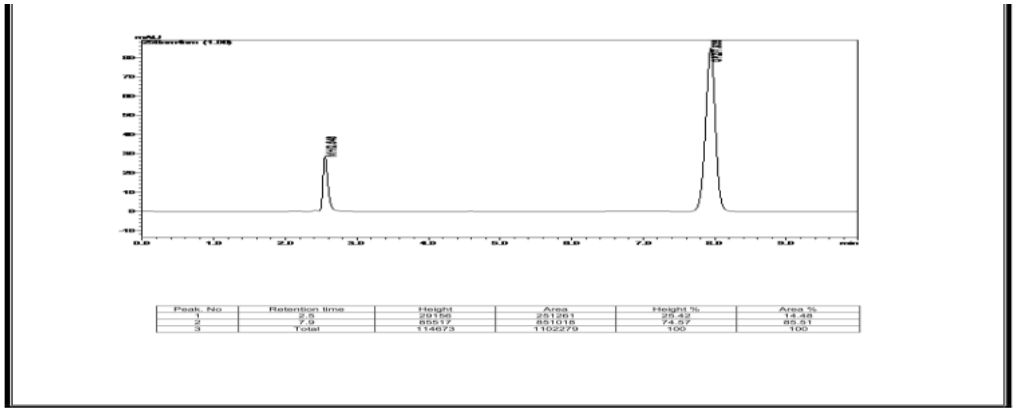


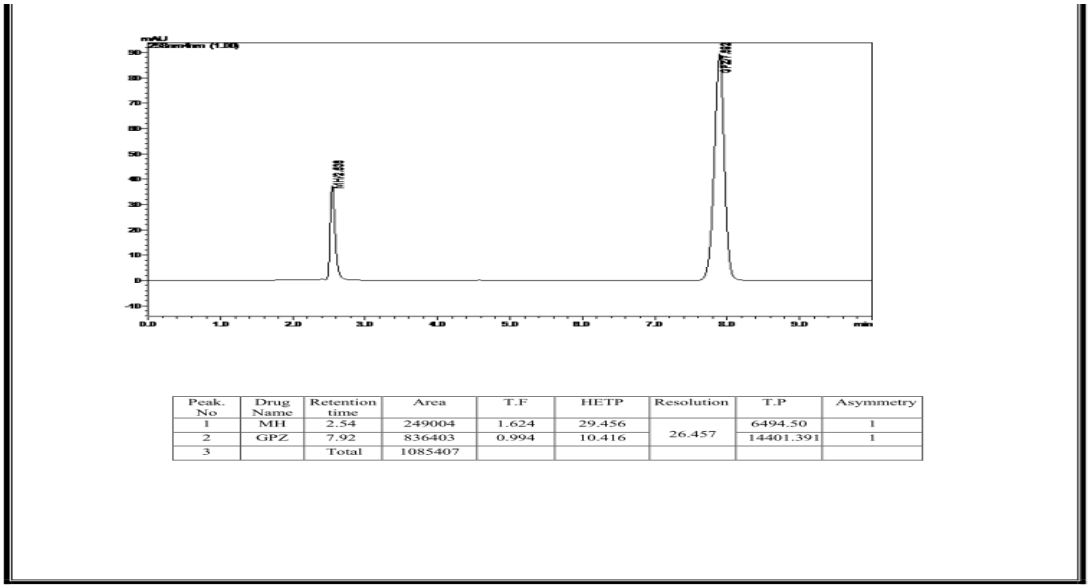
Fig No.2 Chromatogram for retention time of Glipizide and Metformin hydrochloride in combination

Report:

The retention time for Glipizide was found to 7.92 min and the retention time for Metformin hydrochloride were found to be 2.54 min indicating no change in retention time for Glipizide and Metformin hydrochloride in combination.

System suitability:

This parameter ensures that the analytical system is working properly and can give accurate and precise results. A 20 µL solution of Glipizide and Metformin hydrochloride (100µg/mL) each was injected and the system suitability parameters like theoretical plates per column and tailing factor resolution were calculated from the following chromatogram.



Peak. No	Drug Name	Retention time	Area	T.F	HETP	Resolution	T.P	Asymmetry
1	MH	2.54	249004	1.624	29.456	26.457	6494.50	1
2	GPZ	7.92	836403	0.994	10.416		14401.391	1
3		Total	1085407					

Fig No.3 Chromatogram for System Suitability Parameters for Glipizide and Metformin Hydrochloride

Table No.3 System Suitability Data of Glipizide and Metformin hydrochloride

System Suitability Factor	Glipizide	Metformin hydrochloride	Acceptance Criteria
Tailing factor	0.994	1.624	2
HETP (mm)	10.416	29.45	-
Resolution	26.45		-
Theoretical plates	14401.391	6494.50	> 6000
Asymmetry	1	1	1

Report:

The system suitability parameters were calculated for Glipizide and Metformin hydrochloride and the results obtained were found to be within the acceptance criteria.

Validation of analytical method for the assay of glipizide and metformin hydrochloride.

The HPLC method developed was validated by performing the various method validation parameters like specificity, LOD, LOQ, linearity, range, precision, robustness, accuracy, and system suitability parameters as per ICH guidelines. This is to ensure that the performance characteristics of the HPLC method developed meets the requirements for the intended analytical applications.

Following parameters were performed for method validation:

1. Accuracy

2. Precision

a) System precision

b) Method precision

c) Intermediate precision (Ruggedness)

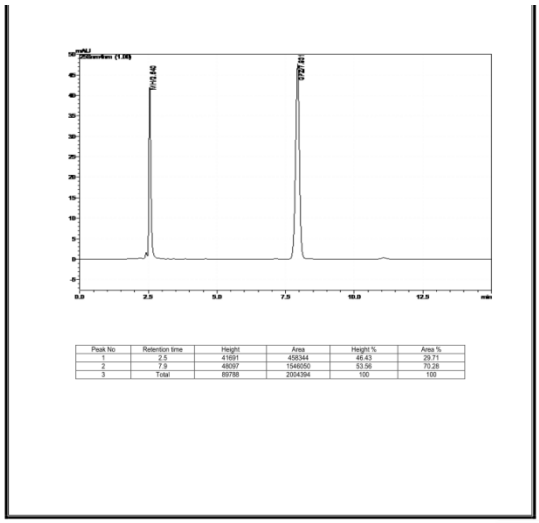
3. Specificity

4. Limit of Detection (LOD)

5. Limit of Quantification (LOQ)

6. Linearity and Range

7. Robustness



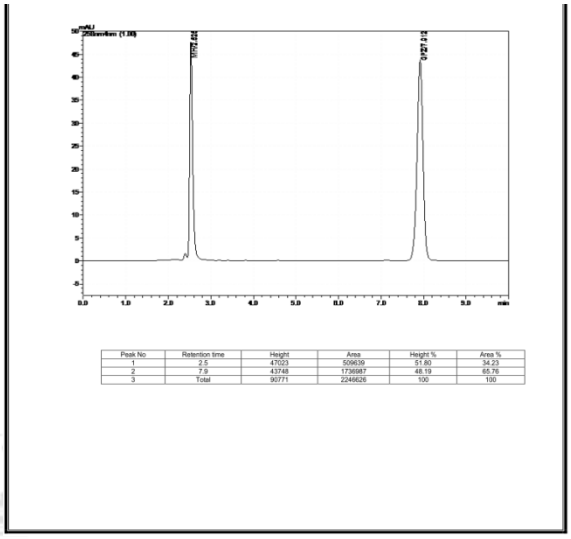


Fig No.4: Chromatogram for Recovery Studies at 80% level

Fig No.5: Chromatogram for Recovery Studies at 100% level

Accuracy:

This parameter is performed to determine the closeness of test results with that of the true value which is expressed as % recovery. These studies were performed at three different levels (80%, 100% and 120%) and the % recovery of Glipizide and Metformin hydrochloride was calculated. 20 µL of three different solutions of Glipizide and Metformin hydrochloride were injected repeatedly into the chromatograph, the peak area and chromatogram were recorded and are presented below.

Table No.4 Recovery study data for Glipizide

Level	Standard conc (µg/mL)	Sample conc (µg/mL)	Total conc (µg/mL)	Peak area	Avg. (µg/mL)	STD	% RSD	% Recovery
I	80	100	180	1706050	180.7	0.76	0.76	100.9%
II	100	100	200	1896987	201.7	0.60	0.59	101.7%
III	120	100	220	2063568	219.8	0.47	0.44	100.3%

***Average of three**

Table No.5 Recovery study data for Metformin hydrochloride

Level	Standard conc (µg/mL)	Sample conc (µg/mL)	Total conc (µg/mL)	Peak area	Avg (µg/mL)	STD	% RSD	% Recovery
I	80	100	180	458344	179.6	0.51	0.51	100.0%
II	100	100	200	509639	199.3	0.71	0.74	99.3%
III	120	100	220	568546	219.8	0.39	0.31	102.5%

***Average of three readings**

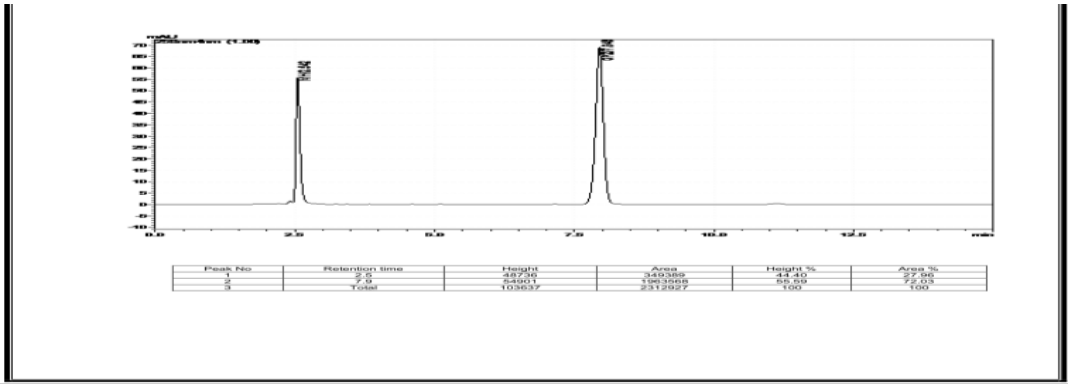


Fig No. 6 Chromatogram for Recovery studies at 120% level

Report:

The mean percentage recovery for Glipizide and Metformin hydrochloride at three different levels was found to be between 100.3 % to 101.7 % and 99.3% to 102.5% respectively, which are well within the limit of 90% to 110% and hence the method was found to be accurate.

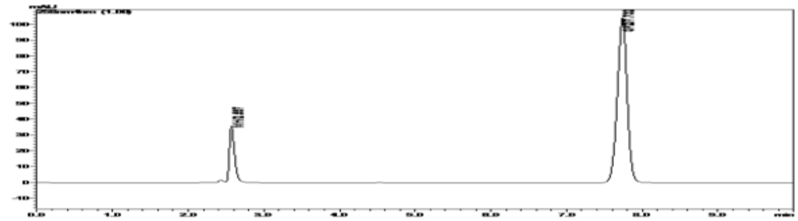
Precision

Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) it is performed to see the closenes of agreement between the series of measurements. It is determined by assaying sufficient number of samples and their relative standard deviation is determined.

(a) System precision: This method validation parameter was performed to ensure the closeness of results between true value and experimental value. Six injections of 20 µL of concentration 100µg/mL of Glipizide and Metformin hydrochloride were injected into the system. The peak area was recorded and is presented below.

Table No. 6 System Precision Data of Glipizide and Metformin hydrochloride

Replicates	GPZ Peak area	MET Peak area
1	967487	254271
2	968141	256247
3	981091	255284
4	978156	253423
5	969658	257192
6	977360	253585
Average	973648.83	255000.33
STD	5894.13	1514.0
%RSD	0.60	0.59



Peak No	Retention time	Height	Area	Height %	Area %
1	2.5	37133	255284	29.11	17.02
2	7.9	90414	845091	70.88	82.97
3	Total	127547	1100375	100	100

Fig.No.7 Chromatogram for System Precision

Report:

The %RSD values of peak areas for six replicate injections of Glipizide and Metformin hydrochloride were found to be 0.60 and 0.59 respectively which are well within the acceptance criteria limit of NMT 2%.

(b) Method Precision (Repeatability):

The method precision was performed to standardize methodology i.e. to check whether the developed method is precise i.e. whether the method is giving consistent results. Six injections of 20 µL working standard solution of Glipizide and Metformin hydrochloride were injected. The peak area and Chromatograms were recorded and presented below. The % RSD for peak area and assay was calculated.

Table No.7 Method Precision Data of Glipizide and Metformin hydrochloride

Replicates	GPZ		MET	
	Peak area	**Concentration**	**Peak area**	**Concentration**
1	967487	99.63	254271	99.81
2	954141	98.16	258547	101.49
3	975091	100.47	254984	100.09
4	968156	99.70	256423	100.65
5	849658	97.66	257192	100.95
6	963360	99.17	249585	97.97
Average	**962982.16**	**99.13**	**255167**	**100.16**
STD	**9481.85**	**1.04**	**3133.61**	**1.22**
%RSD	**0.98**	**1.04**	**1.22**	**1.22**

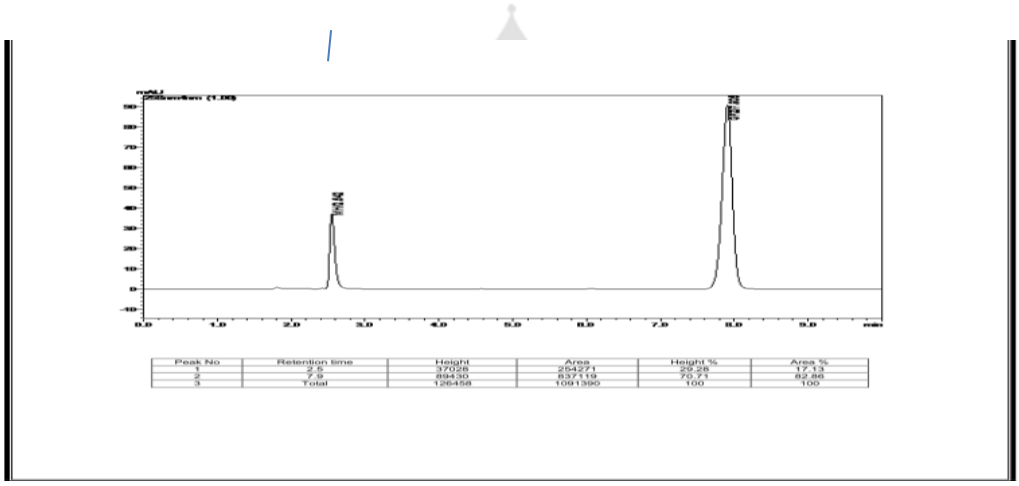


Fig.No.8 Chromatogram for Method Precision

Report:

The %RSD values of concentration for six replicate injections of Glipizide and Metformin hydrochloride were found to be 1.04 and 1.22 respectively which are well within the acceptance criteria limit of NMT 2%.

(c) Intermediate Precision (Ruggedness):

Intermediate precision was performed to ensure whether the precision is maintained with in lab variations like different days. Six injections of 20µL working standard solution of Glipizide and Metformin hydrochloride were injected. The % RSD for peak area and assay was calculated.

Table. 8 Intraday Precision Data of Glipizide and Metformin Hydrochloride

Replicates	GPZ		MET	
	Peak area	Concentration	Peak area	Concentration
1	967487	99.63	254271	99.81
2	964132	99.26	257143	100.93
3	965091	99.36	253610	99.55
4	975156	100.47	255243	100.20
5	965658	99.47	247201	97.65
6	973360	100.27	256240	100.58
Average	968482	100.54	253951.33	99.78
STD	4639.93	0.51	1652.69	0.64
%RSD	0.47	0.51	0.64	0.64

Report:

The %RSD values of concentration for six replicate injections of Glipizide and Metformin hydrochloride were found to be 0.512 and 0.646 respectively which are well within the acceptance criteria limit of NMT 2%.

Table. No.9 Interday Precision Data of Glipizide and Metformin hydrochloride

Replicates	Date interval	GPZ		MET	
		Peak area	Concentration	Peak area	Concentration
1	27/3/12	967487	99.63	254271	99.81
2	27/3/12	974141	100.36	255805	100.41
3	28/3/12	965091	99.36	249837	98.07
4	28/3/12	976456	100.63	254395	99.85
5	29/3/12	975658	100.53	253609	99.55
6	29/3/12	963360	99.17	259827	101.99
Average		**970365.5**	**99.94**	**254624**	**99.94**
STD		**6250**	**0.623**	**2788.71**	**1.09**
%RSD		**0.634**	**0.623**	**1.08**	**1.08**

Report:

The %RSD values of concentration for six replicate injections of Glipizide and Metformin hydrochloride were found to be 0.623 and 1.08 respectively which are well within the acceptance criteria limit of NMT 2%.

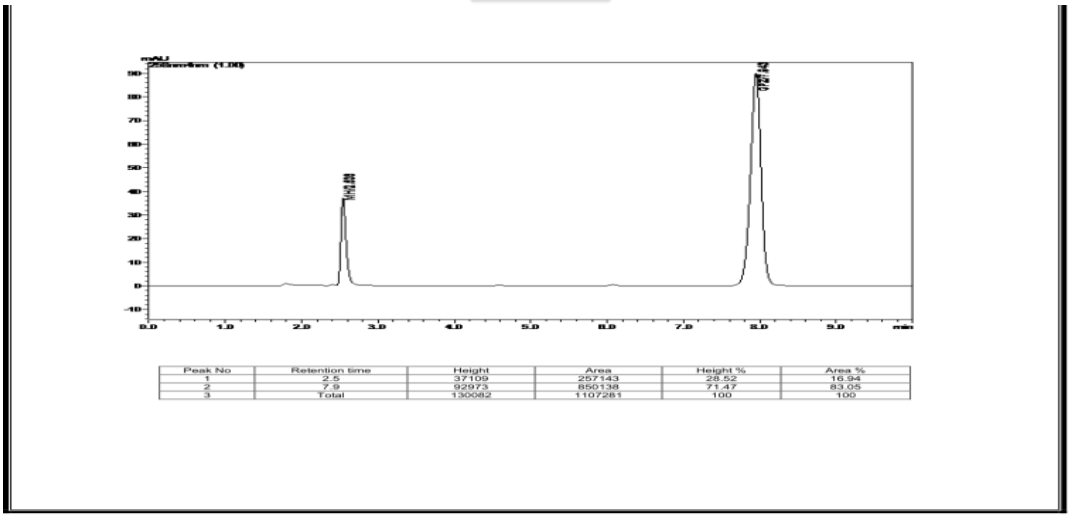
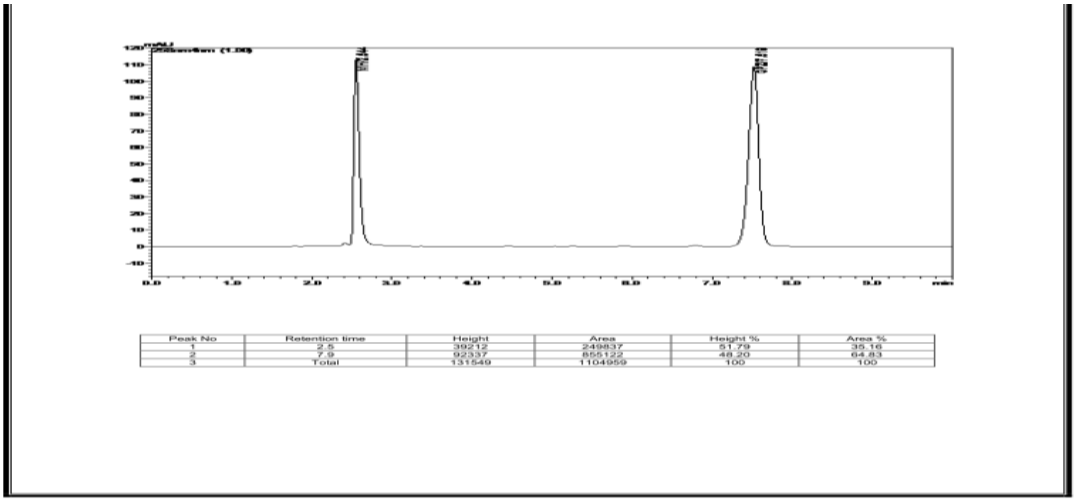


Fig No.9 Chromatogram for Intraday precision



Peak No	Retention time	Height	Area	Height %	Area %
1	2.5	39212	249837	51.79	35.16
2	7.9	92337	855122	48.20	64.83
3	Total	131549	1104959	100	100

Fig No.10 Chromatogram for Inter day precision

Specificity:

Specificity was performed to assess and ensure that the impurities, degraded products do not interfere with peaks of analyses.

Determination: Volume of 20 µL of Blank sample, Standard sample were injected in to the chromatograph and the chromatograms were recorded and the peak purity of the analyse peaks were evaluated and presented below.

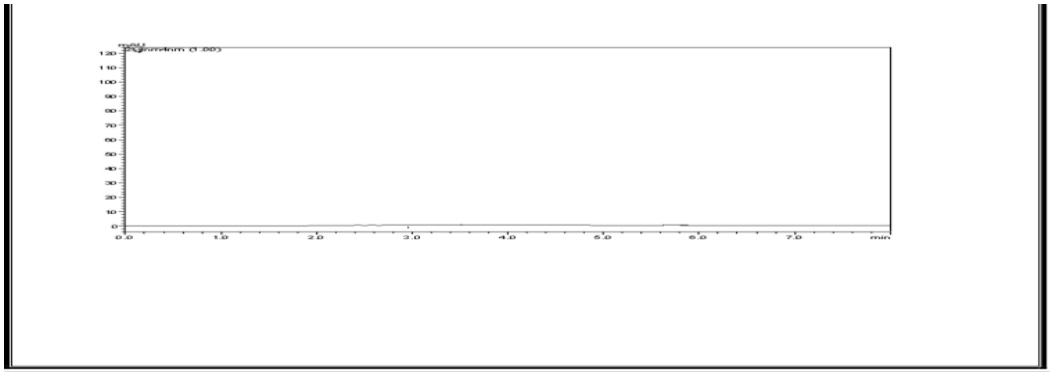
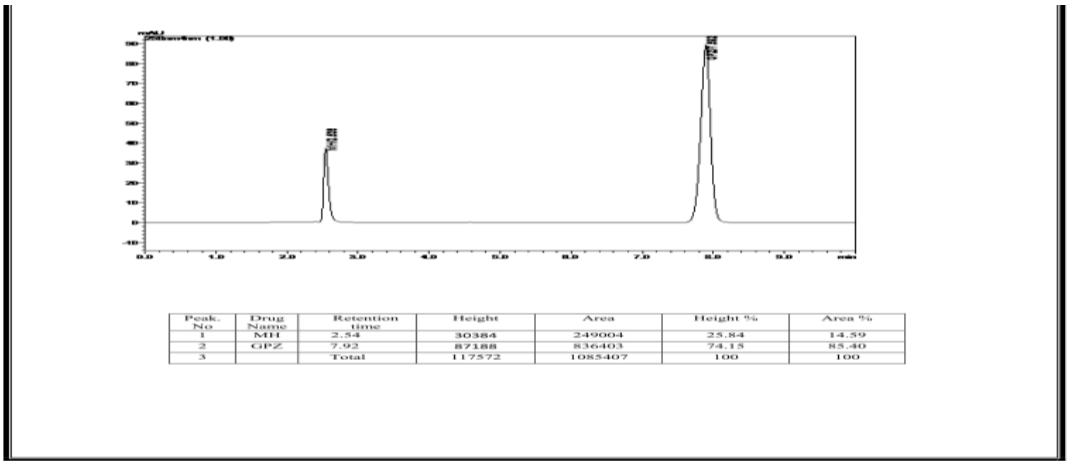


Fig.No.11 Chromatogram for Specificity (Blank)



Peak No.	Drug Name	Retention time	Height	Area	Height %	Area %
1	MH	2.54	30384	249064	25.84	14.59
2	GPZ	7.92	87188	836403	74.15	85.40
3		Total	117572	1085467	100	100

Fig. No.12 Chromatogram for Specificity (Standard sample)

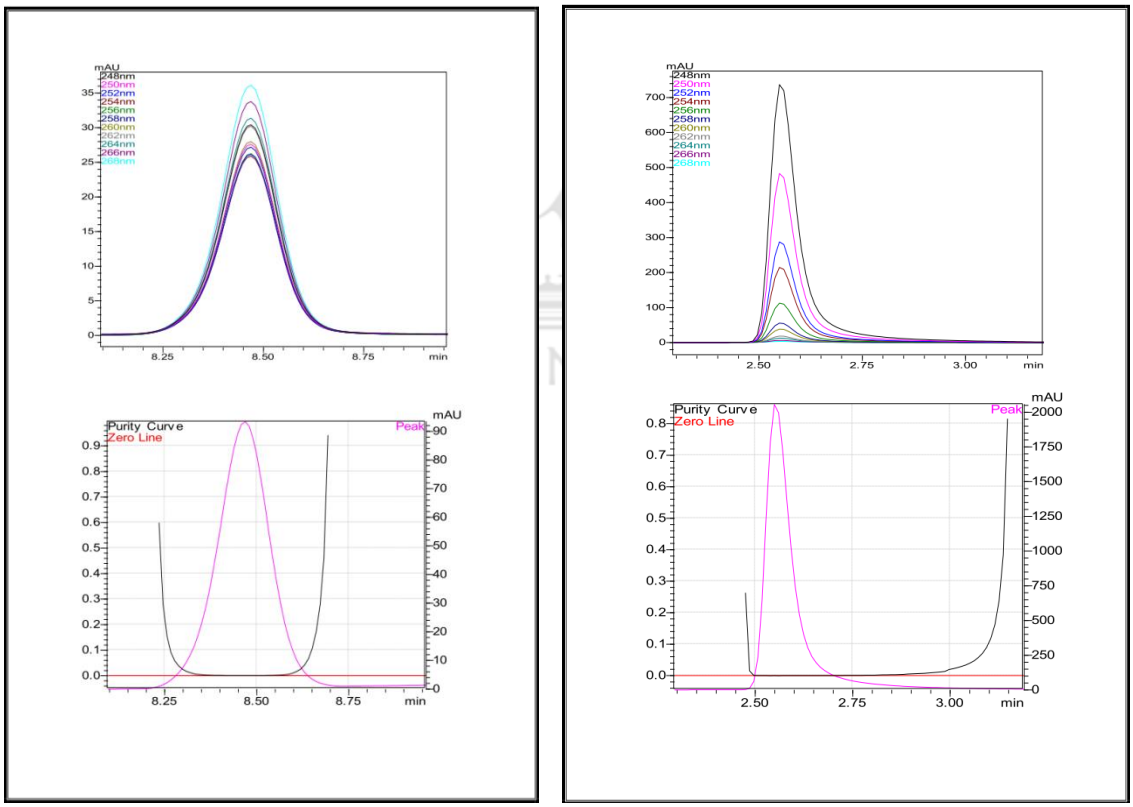


Fig No.13 Chromatogram for Specificity (GPZ&MH Peak Profile &Peak Purity)

GLIPIZIDE	METFORMIN HYDROCHLORIDE
Impurity: Not detected	Impurity: Not detected
Peak Purity: 1.00000	Peak Purity: 0.999900
Single point threshold: 0.999557	Single point thresholds: 0.999969
Minimum Peak purity index: 443	Minimum Peak purity index: 63

Observation: The analytes did not have any interference with the excipients and impurities.

Report:

As there is no interference with the excipients at a retention time of 7.9 (Glipizide) and 2.5min (Metformin hydrochloride), hence the proposed method was specific for the detection of Glipizide and Metformin hydrochloride.

Limit of Detection (LOD):

Limit of detection is the lowest concentration of the analyte that can be detected by injecting a decreasing amount, not necessarily quantity by the method, under the stated experimental conditions.

The minimum concentration at which the analyse can be detected is determined from the standard deviation of the response and the slope by applying the formula.

$$\text{Limit of detection} = \frac{\sigma}{S} \times 3.3$$

For Glipizide:

Limit of detection = 31.6678/9070 x 3.3

$= 0.0115 \times 10^{-3}$

=11.5ng/mL

For Metformin hydrochloride:

Limit of detection = 31.6678/2549 x 3.3

$= 0.04099 \times 10^{-3}$

= 40.99ng/mL

Report:

The lowest concentration of Glipizide and Metformin hydrochloride that can be detected, was determined from standard deviation was found to be 11.5 ng/mL and 40.99ng/mL.

Limit of Quantification (LOQ):

Limit of quantification is the lowest concentration of the analyse in a sample that can be estimated quantitatively. By injecting decreasing amount of drug, with acceptable precision and accuracy under the stated experimental conditions of the method. Limit of quantification can be obtained from the standard deviation of the response and the slope by applying the following formula.

$$\text{Limit of quantification} = \frac{\sigma}{S} \times 10$$

For Glipizide:

Limit of quantification = 31.6678/9070 x 10

$= 0.03491 \times 10^{-3}$

= 34.91ng/mL

For Metformin hydrochloride:

Limit of quantification = 31.6678/2549 x 10

$= 0.12423 \times 10^{-3}$

= 124.23ng/mL

Report:

The lowest concentration at which peak can be quantified is called LOQ, was found to be 34.91ng/mL for Glipizide and for Hydrochlorothiazide was found to be 124.23ng/mL.

Linearity and Range

The linearity was performed to ensure that the test results are directly proportional to the concentration of analyte sample.

20µL of each of working standard solution of Glipizide and Metformin hydrochloride were injected in to the chromatograph. The results obtained are tabulated below.

Table.No.10: Linearity and Range data for Glipizide

Volume of stock solution (mL)	Volume adjusted to (mL)	Concentration (µg/mL)	GPZ Peak Area
6	100	60	600833
8	100	80	797214
10	100	100	967397
12	100	120	1166271
14	100	140	1323525

***Average of three readings**

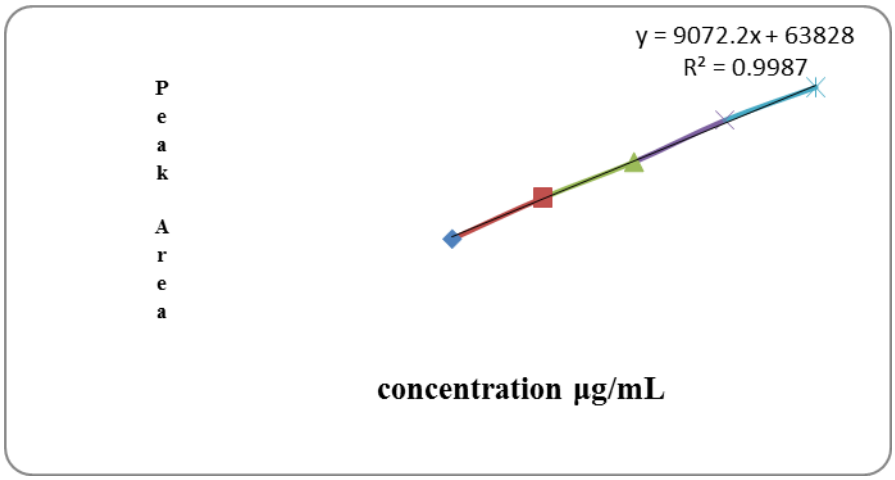


Fig No.14 Linearity Range Graph of Glipizide

Table No.11 Linearity and Range data for Metformin hydrochloride

Volume of stock solution (mL)	Volume Adjusted to (mL)	Concentration (µg/mL)	MET Peak Area
6	100	60	155683
8	100	80	203217
10	100	100	250832
12	100	120	306654
14	100	140	358862

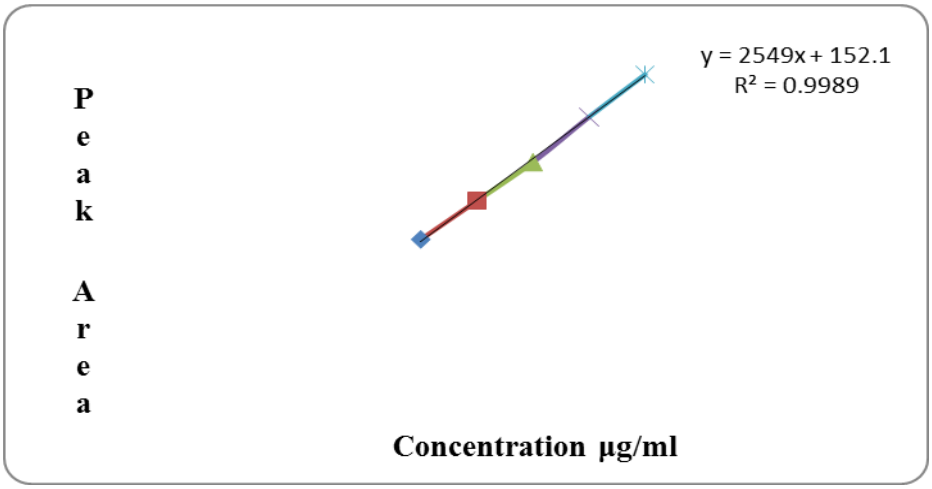


Fig No.15 Linearity Range Graph of Metformin hydrochloride

Table No.12 Linearity report of Glipizide and Metformin hydrochloride

Parameters	GPZ	MET	Acceptance Criteria
Linearity range	60 – 140 µg/mL	60 – 140 µg/mL	-
Regression Equation	9072.x + 63828	2549x + 152.1	-
Correlation co-efficient	0.998	0.998	0.99
Percentage curve fitting	99.8 %	99.8%	99%
Intercept	63828	152.1	-
Slope	9070	2549	-

Report:

The linearity in response for Glipizide and Metformin hydrochloride was observed in the concentration range of 60–140µg/mL respectively, with percentage curve fittings found to be well within the limits of acceptance criteria (99%).

Robustness:

Robustness was performed to check the ability of the system to give unaffected results for small deliberate changes in system parameters and method parameters.

(a) Change in Flow Rate:

20 µL working standard solutions were prepared in mobile phase and were injected in chromatograph at flow rate 0.8 and 1.2mL/min; the % assay was calculated and presented below.

Table No.13 Robustness Data for Change in Flow Rate

Drug	Change in flow rate(mL)	Peak area	%Assay
GPZ	0.8mL	977336	100.74
	1.2mL	967336	99.25
MET	0.8mL	260050	102.08
	1.2mL	249004	97.74

***Average of three readings**

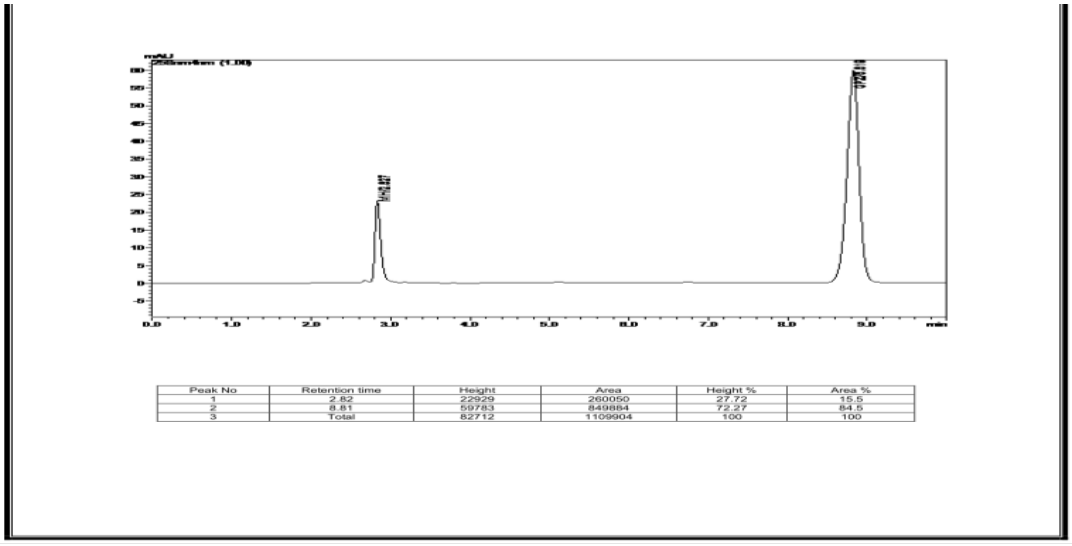


Fig No.16 Chromatogram for Change in Flow rate (0.8mL/min)

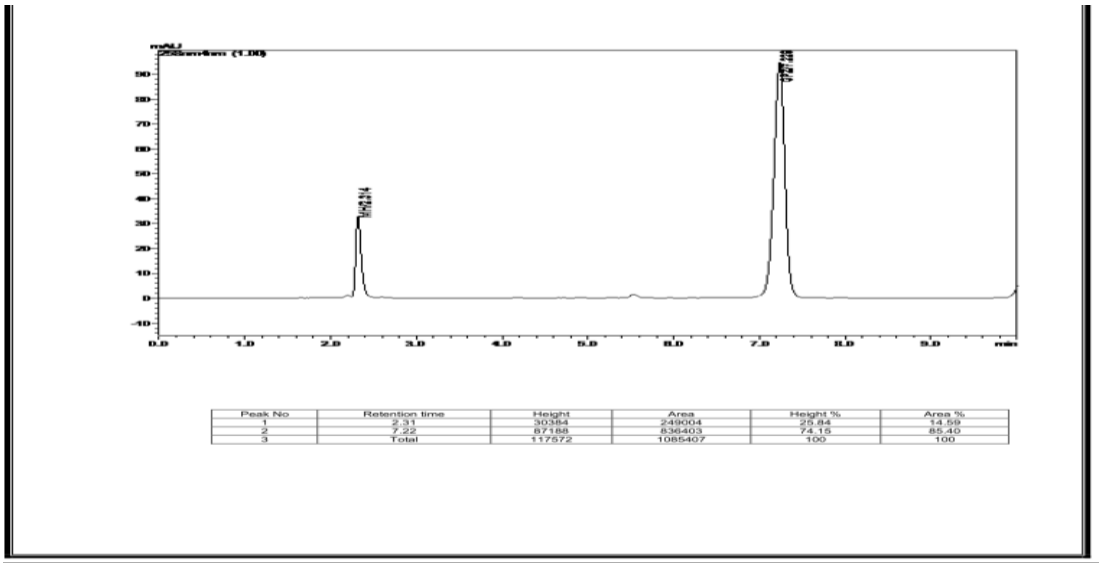


Fig No.17 Chromatogram for Change in Flow rate (1.2mL/min)

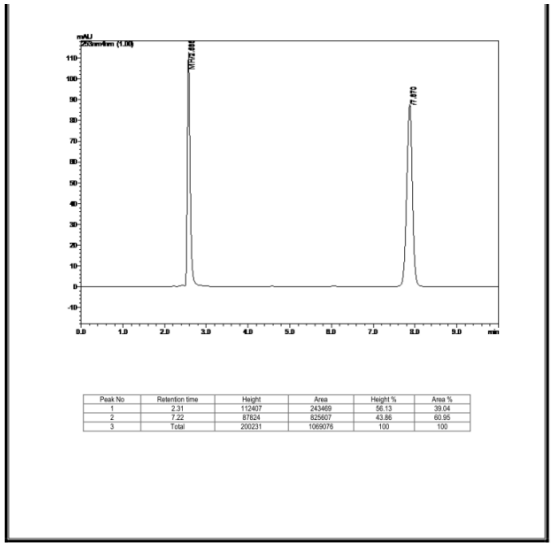
Report:

The % assay were found to be 100.74%, 99.25 % for Glipizide and 102.08%, 97.74% for Metformin hydrochloride when the flow rate was changed to 0.8mL and 1.2mL respectively, indicating that the method was found to be robust with deliberate change in flow rate.

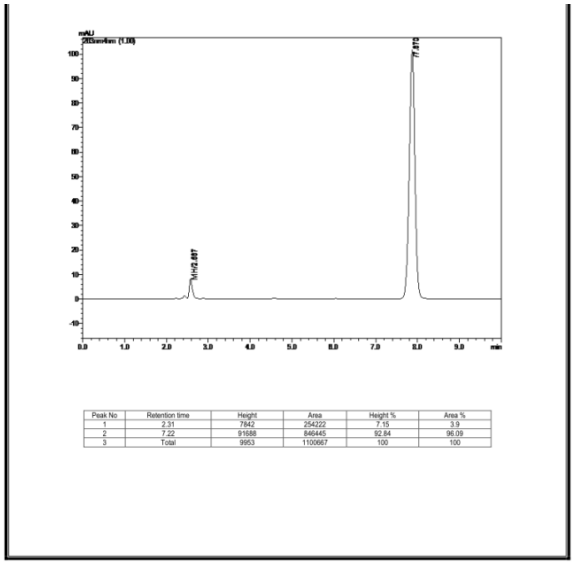
b) Change in Detection wavelength:

For the method developed, detection wavelength of 258nm was used. For Robustness study, detection wavelength was changed to 253 nm and 263 nm.

Table No 17: Robustness Data for Change in Detection wavelength



Peak No	Retention time	Height	Area	Height %	Area %
1	2.31	112407	243469	56.13	39.04
2	7.22	87824	825607	43.86	60.95
3	Total	200231	1069076	100	100



Peak No	Retention time	Height	Area	Height %	Area %
1	2.31	7842	254222	7.15	3.9
2	7.22	91688	846445	92.84	96.09
3	Total	9953	1100667	100	100

Fig No.18 Chromatogram for Change in Detection wavelength (253 nm)

Fig No. 19 Chromatogram for Change in Detection wave length (263 nm)

Report:

The % assay were found to be 98.06%, 100.36% for Glipizide and 95.57% and 99.78% for Metformin hydrochloride when the detection wavelength was changed to 253 nm and 263 nm respectively, indicating that the method was found to be robust with deliberate change in detection wavelength.

Application of developed hplc method for the simultaneous estimation of glipizide and metformin hydrochloride in tablet dosage form:

The RP-HPLC method developed and validated for estimation of Glipizide and Metformin hydrochloride was applied to determine Glipizide and Metformin hydrochloride was in marketed formulations (Tablets).

Brand name: Glynase–MF (5mg of Glipizide and 500mg of Metformin hydrochloride).

Manufacturer: USV Pvt. Ltd., Mumbai, India

Table. No. 14 Instrument Specifications

Instrument	SHIMADZU UFLC-2000 Prominence LC-20AD Binary Gradient System SPDM 20 A Detector
Injector	Rheodyne
Column	Enable C-18 Gcolumn250×4.6mm&5µm
Detector	PDA Detector
Wavelength	258 nm
Flow rate	1.0 mL/min
Injection volume	20 µL
Mobile phase	Phosphate buffer: Acetonitrile (60:40) pH 5.8

Procedure:

20 µL of each working stock sample solution of concentration of 60, 100 and 140 µg/mL were injected into chromatograph at a flow rate of 1.0 mL/min and UV detection at 258 nm. The peak area and chromatogram obtained were recorded. The concentration of drug in sample solution was determined and the % assay for the amount of Glipizide and Metformin hydrochloride present in marketed dosage form was calculated. The results obtained are presented in table below.

Table No. 15 Assay report of Glipizide and Metformin hydrochloride

Volume of stock solution	**GPZ**			**MET**		
	Peak Area*	**Concentration (µg/mL)**	**% Assay**	**Peak Area***	**Concentration (µg/mL)**	**% Assay**
6	611883	60.42	100.70	152907	60.046	100.07
10	980465	101.06	101.06	254235	99.79	99.79
14	1318704	138.34	98.82	353389	138.69	99.64

***Average of three readings**

Report:

The % assay of Glipizide and Metformin hydrochloride in marketed formulation was found to be in the range of 98.82% to 101.06% and 99.79 to 100.07% respectively. The results show that there is no interference from excipients and no impurities were observed in samples for the proposed method.

Hence the developed HPLC method was found to be accurate, precise, and specific and can be routinely used for simultaneous estimation of Glipizide and Metformin hydrochloride in marketed formulations.

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