STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF METFORMIN AND GLIPIZIDE

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Abstract:

A novel, precise, and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Metformin and Glipizide in bulk and pharmaceutical dosage forms. The chromatographic analysis was carried out using a C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of phosphate buffer (pH 3.5) and acetonitrile in the ratio of 60:40 v/v. The flow rate was set at 1.0 mL/min, and detection was performed at 230 nm using a UV detector. The method provided well-resolved peaks for both Metformin and Glipizide with retention times of approximately [insert retention times, if known]. Method validation was carried out in accordance with ICH Q2(R1) guidelines, evaluating parameters such as linearity, accuracy, precision, specificity, robustness, and sensitivity. Both drugs showed good linearity over the concentration ranges of [insert range] µg/mL, with correlation coefficients (R^2) greater than 0.999. Recovery studies confirmed the accuracy of the method, while %RSD values in precision studies were within acceptable limits. Forced degradation studies were conducted under various stress conditions including acidic, basic, oxidative, thermal, and photolytic degradation. The method effectively separated the drugs from their degradation products, confirming its stability-indicating capability. This validated RP-HPLC method is simple, accurate, and suitable for routine quality control and stability analysis of Metformin and Glipizide in combined dosage forms.

Introduction:

The increasing prevalence of type 2 diabetes mellitus (T2DM) has led to the widespread use of combination therapies aimed at achieving optimal glycemic control. Among the various antidiabetic agents, Metformin and Glipizide are frequently prescribed together to manage blood glucose levels effectively. Metformin, a biguanide, primarily reduces hepatic glucose production and improves insulin sensitivity, whereas Glipizide, a sulfonylurea, promotes insulin secretion from pancreatic beta cells. The fixed-dose combination of these two drugs is commonly formulated in oral dosage forms for convenient administration and enhanced patient compliance. Given their concurrent usage, it becomes essential to establish a reliable and validated analytical method for the simultaneous estimation of Metformin and Glipizide, particularly one capable of distinguishing the active pharmaceutical ingredients (APIs) from their degradation products.[2]

Stability-indicating methods play a crucial role in pharmaceutical analysis, as they help monitor the stability of a drug and ensure its safety and efficacy throughout its shelf life. A stability-indicating method is an analytical technique that can accurately quantify the active ingredients without interference from degradation products, excipients, or other impurities. Regulatory authorities such as the International Council for Harmonisation (ICH) emphasize the need for stability-indicating assays as part of the validation process. These methods are critical not only for routine quality control but also for supporting stability studies and ensuring regulatory compliance.[3]

Reversed-phase high-performance liquid chromatography (RP-HPLC) is a widely accepted technique for the analysis of pharmaceutical compounds due to its high resolution, accuracy,

reproducibility, and ability to separate complex mixtures. It is particularly suitable for stability studies because it enables the separation of drugs from their potential degradation products under various stress conditions. Although individual methods have been reported for the estimation of Metformin and Glipizide, there exists limited literature on a validated stability-indicating RP-HPLC method that can simultaneously analyze both drugs in bulk and tablet dosage forms.[4]

The primary aim of the present study is to develop and validate a simple, rapid, precise, and stability-indicating RP-HPLC method for the simultaneous estimation of Metformin and Glipizide. The method is intended to be applicable for routine analysis in quality control laboratories and capable of distinguishing the drugs from their respective degradation products under various stress conditions including acid, base, oxidative, thermal, and photolytic degradation. The method is validated in accordance with ICH Q2(R1) guidelines by evaluating parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantification (LOQ), and system suitability.[5]

By developing a robust and stability-indicating analytical method, this study contributes to ensuring the consistent quality, safety, and therapeutic efficacy of Metformin and Glipizide formulations. Furthermore, the method provides a scientific foundation for drug stability studies and supports the regulatory requirements for pharmaceutical development and manufacturing. This will ultimately benefit pharmaceutical companies in their product development cycle, while also ensuring patient safety through reliable quality control mechanisms.[5]

Materials and Methods:

Metformin hydrochloride and Glipizide pure drug standards were procured as gift samples from a certified pharmaceutical company. The combined tablet dosage form containing Metformin (500 mg) and Glipizide (5 mg) was purchased from a local pharmacy. All solvents and reagents used in the study were of HPLC or analytical grade. HPLC-grade methanol, acetonitrile, and water were obtained from Merck (India), and analytical grade orthophosphoric acid and sodium dihydrogen phosphate were used for buffer preparation. All solutions were filtered through a 0.45 µm membrane filter and degassed prior to use.[8]

The analysis was performed using a Shimadzu LC-20AT HPLC system equipped with a quaternary pump, UV-Visible detector (SPD-20A), and manual injector with a 20 µL loop. Data acquisition and interpretation were carried out using LabSolutions software. Chromatographic separation was achieved using a reversed-phase C18 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of a mixture of phosphate buffer (pH adjusted to 3.0 with orthophosphoric acid) and acetonitrile in the ratio 65:35 v/v. The mobile phase was pumped at a flow rate of 1.0 mL/min, and the detection wavelength was set at 230 nm. The total run time for the analysis was 10 minutes, and the column temperature was maintained at ambient room temperature (25 ± 2°C).[9]

Standard stock solutions were prepared by dissolving 100 mg of Metformin and 10 mg of Glipizide separately in 100 mL of the mobile phase to obtain concentrations of 1000 µg/mL and 100 µg/mL, respectively. Working standard solutions were prepared by appropriate dilution with the mobile phase to achieve concentration ranges of 10–100 µg/mL for Metformin and 1–10 µg/mL for Glipizide. Sample preparation involved accurately weighing and

powdering 20 tablets. A quantity of tablet powder equivalent to 500 mg of Metformin and 5 mg of Glipizide was transferred to a 100 mL volumetric flask, dissolved in the mobile phase with sonication for 20 minutes, filtered, and diluted to achieve the required concentration for analysis.[10]

Forced degradation studies were conducted under various stress conditions including acid hydrolysis (0.1N HCl for 1 hour), alkali hydrolysis (0.1N NaOH for 1 hour), oxidative degradation (3% H_2O_2), thermal degradation (exposure to 80°C for 2 hours), and photolytic degradation (exposure to UV light for 24 hours). These conditions were used to assess the stability-indicating capability of the developed method by identifying any additional peaks corresponding to degradation products and ensuring resolution from the main drug peaks.[11]

The developed RP-HPLC method was validated according to ICH Q2(R1) guidelines. System suitability was evaluated by injecting six replicates of the standard solution and calculating retention time, tailing factor, and theoretical plates. Linearity was assessed using standard solutions at multiple concentration levels, and calibration curves were plotted. Precision was evaluated through intra-day and inter-day analysis, while accuracy was determined using recovery studies at 80%, 100%, and 120% levels. LOD and LOQ were determined based on the standard deviation of the response and slope of the calibration curve. Robustness was assessed by making deliberate changes in flow rate, mobile phase composition, and detection wavelength.[13]

Results and Discussion:

Solubility Studies

These studies are carried out at 25^0C

Metformin:

Metformin is a white to off-white powder. It is soluble in water, strong acids, and strong bases. It is also soluble in methanol, chloroform, ethyl acetate, and acetonitrile.

Glipizide:

Glipizide is a white or almost white, crystalline powder. It is very slightly soluble in water, freely soluble in alcohol and also soluble in acetonitrile.

Determination of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of Metformin

10 mg of Metformin was weighed and transferred in to 100 ml volumetric flask. It was dissolved and made up to mark with acetate buffer. The resulting solution was futher diluted to obtain 10 µg/ml of solution.

Preparation of standard stock solution of Glipizide

10 mg of Glipizide was weighed and transferred in to 100 ml volumetric flask. It was dissolved and made up to mark with acetate buffer. The resulting solution was futher diluted to obtain 10 µg/ml of solution.

Results

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in acetate buffer were scanned using UV-Visible spectrophotometer within the wavelength region of 200-400 nm against methanol as blank. The resulting spectra are shown

in the Figure 8.1, 8.2 and 8.3 and the absorption curve shows characteristic absorption maxima at 232 nm for Metformin, 274 nm for Glipizide and 257 nm for the combination.

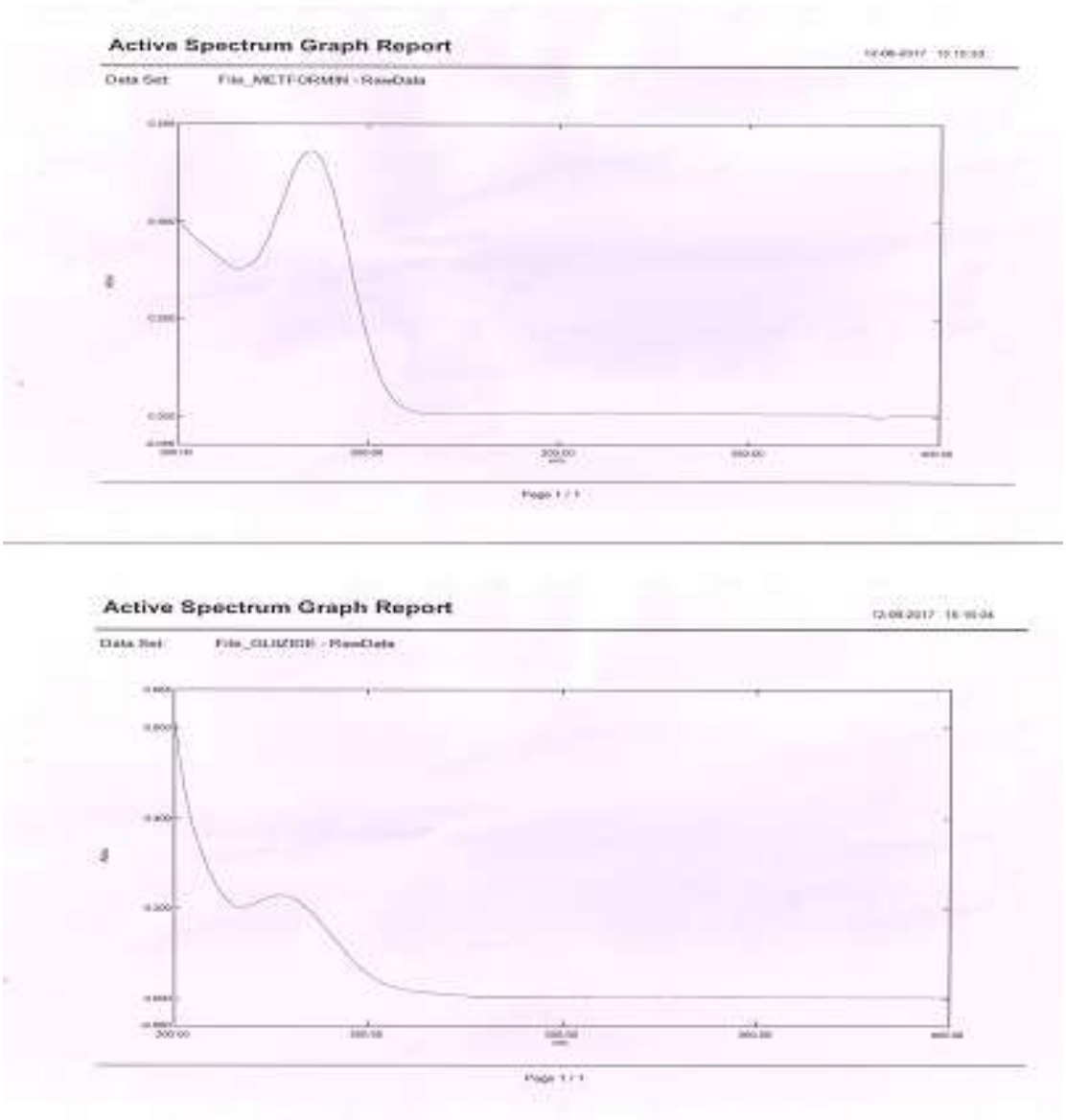

Fig. 1: UV spectrum of Metformin and Glipizide

Observation: λ_{max} was found to be 232 nm and 274 nm for Metformin and Glipizide respectively as shown in the Figure 7.2.1.

Method Development of Metformin and Glipizide

Trial - 1

Chromatographic conditions

Mobile phase : Acetonitrile:water

Ratio : 50:50 v/v

Column : Mircosorb-MV C18 (250×4.6×5µ)

Wavelength : 228 nm

Flow rate : 1.0 ml/min

Injection Volume : 20 µl

Run time : 8.0 min.

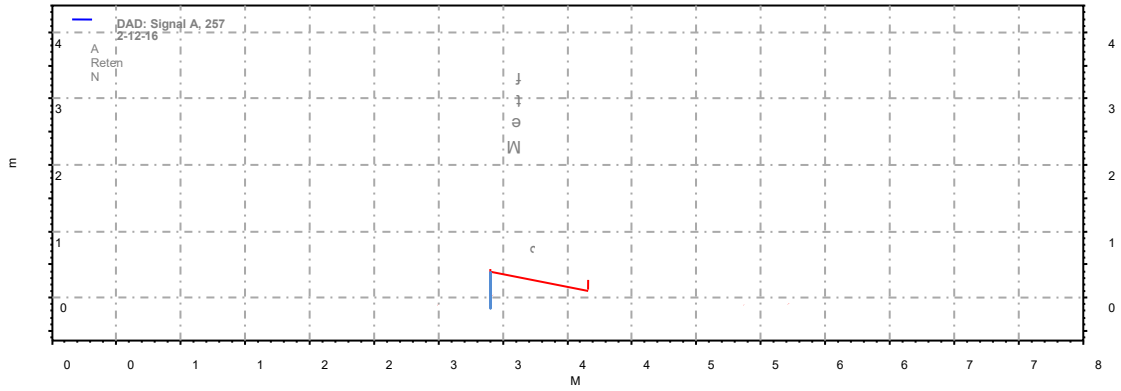

Fig.: Chromatogram of trial-1

Observation: Glipizide was not eluted. The peak of Metformin was not good. Hence it was not taken for optimization.

Trial - 2

Chromatographic conditions

Mobile phase : Acetonitrile:buffer

Ratio : 50:50 v/v

Buffer : 0.1% othro phosphoric acid

Column : Mircosorb-MV C18 (250×4.6×5µ)

Wavelength : 228 nm

Flow rate : 1.0 ml/min

Injection Volume : 20 µl

Run time : 8.0 min.

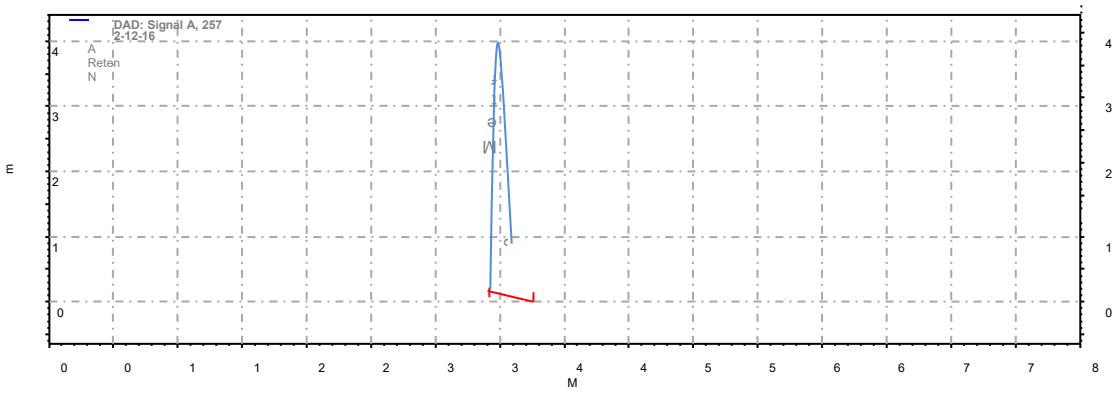

Fig. : Chromatogram of trial-2

Observation: Good peak shape was obtained for Metformin. But glipzide was not eluted in proposed run time. Hence it was not taken for optimization.

Trial - 3

Chromatographic conditions

Mobile phase : Acetonitrile:buffer

Ratio : 50:50 v/v

Buffer : 0.1M Acetate buffer

Column : Mircosorb-MV C18 (250×4.6×5µ)

Wavelength : 228 nm

Flow rate : 1.0 ml/min

Injection Volume : 20 µl

Run time : 8.0 min.

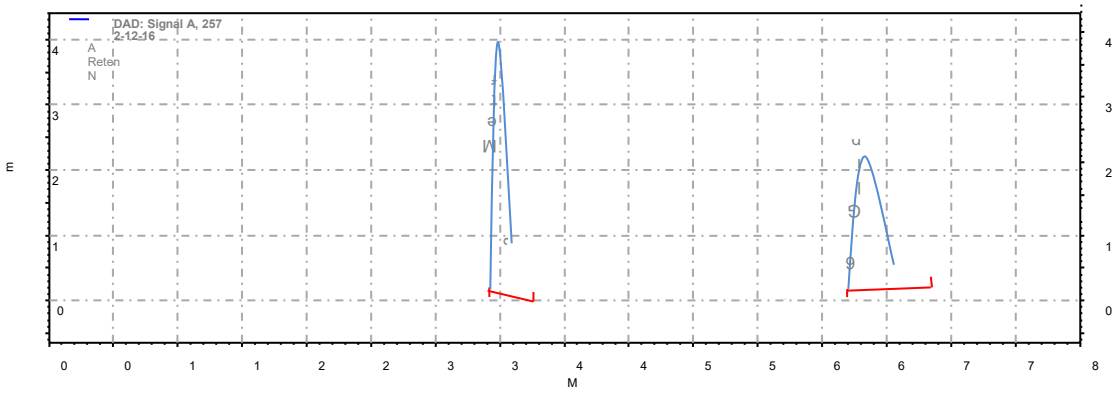

Fig.: Chromatogram of trial-3

Observation: Both analytes were eluted. But the tailing for Glipizide was more. Hence it was not taken for optimization.

Trial - 4

Chromatographic conditions

Mobile phase : Buffer:acetonitrile

Ratio : 60:40 v/v

Buffer : 0.1M Acetate buffer

pH : 4.0

Column : Mircosorb-MV C18 (250×4.6×5µ)

Wavelength : 228 nm

Flow rate : 1.0 ml/min

Injection Volume : 20 µl

Run time : 8.0 min.

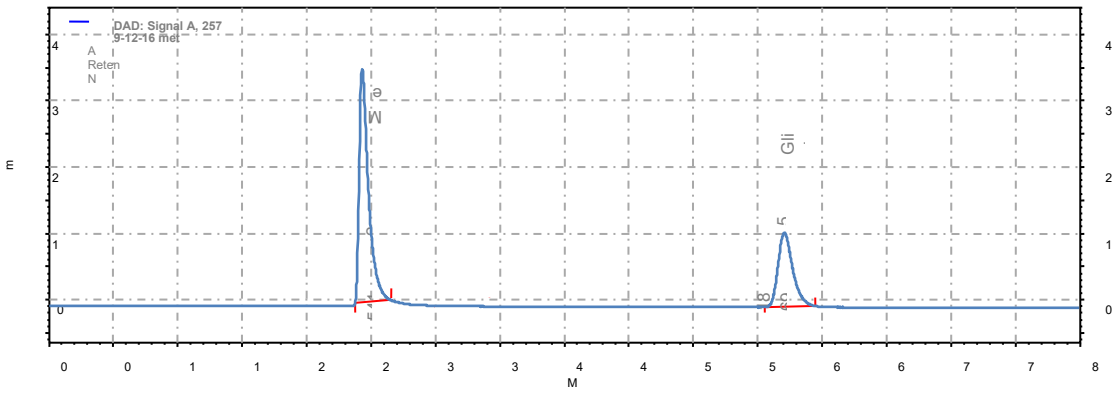

Fig.: Chromatogram of trial-4

Observation: Both analytes were eluted. The peak resolution and peak shape was good. Hence it was taken for optimization.

Table: Optimized chromatographic conditions

Parameter	**Conditions**
Mobile phase	Acetate Buffer (pH 4.0) :Acetonitrile 60:40 v/v
Column	Mircosorb-MV C18 (250×4.6×5µ)
Flow rate	1.0 ml/min
Wavelength	257 nm
Injection volume	20 µl
Run time	8 minutes
Retention time	2.433 min for Metformin and 5.710 min for Glipizide

Observation: The efficiency for both Metformin and Glipizide is within the limits (limit->2000). The asymmetry factor is less than 2.0. The run time was 8 minutes. All the system suitability parameters were satisfied. Hence this method was optimized.

Assay

Preparation of samples for Assay

Standard sample

Standard stock solutions of Metformin and Glipizide (µg/ml) were prepared by dissolving 500 mg of Metformin and 5 mg of Glipizide dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50 ml with mobile phase. Further dilutions of 140 µg/ml of Metformin and 50 µg/ml of Glipizide were made by adding 1 ml of stock solution to 50 ml of mobile phase.

Tablet sample

20 tablets (each tablet contains 500 mg of Metformin and 5 mg of Glipizide) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Metformin and Glipizide (µg/ml) were prepared by dissolving weight equivalent to 500 mg of Metformin and 5 mg of Glipizide and dissolved in sufficient mobile phase. After that filtered the solution using 0.45 µ syringe filter and Sonicated for 5 min and dilute to 100 ml with mobile phase. Further dilutions are prepared in 5 replicates of 140 µg/ml of Metformin and 50 µg/ml of Glipizide was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Metformin and Glipizide present in the formulation by using the formula given below, and results shown in above table:

$$\% Assay = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Average Peak area due to assay preparation

WS: Weight of Metformin / Glipizide n mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

AW: Average weight

P: Standard purity

LC: Label claim

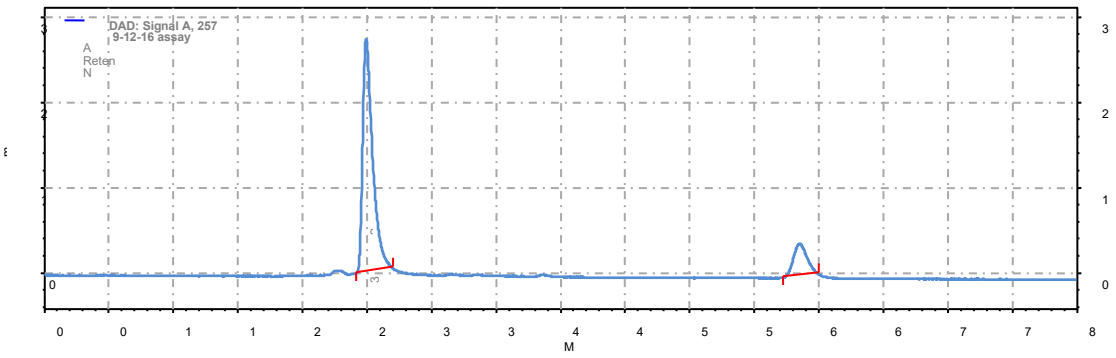

Fig. : Chromatogram of Assay

Table : Assay Results

S. No.	Metformin %Assay	Glipizide %Assay
1	99.65	99.46
2	98.41	100.01
3	99.39	100.97
4	98.16	98.33
5	98.77	98.39
6	99.59	98.51
AVG	98.99	99.28
STDEV	0.68	1.07
%RSD	0.64	1.08

Observation: The amount of Metformin and Glipizide present in the taken dosage form was found to be 98.99 % and 99.28 % respectively.

Conclusion :

Five consecutive injections of the mixture of standard solution showed % RSD (% Relative Standard Deviation) less than 2 concerning retention time and peak areas for both the drugs which indicate the method developed and optimized is system precise.

Six consecutive injections of the sample showed % RSD less than 2 concerning % assay and peak areas for both the drugs which indicate the method developed and optimized is intraday precise, by the test of repeatability and hence can be understood that the method gives consistent results.

Six consecutive injections of the sample solution on the other consecutive day, showed % RSD less than 2 on different days and between days for % assay for both the drugs, which indicate the method developed and optimized is inter day precise.

A linear relationship between peak areas versus concentrations was observed for Metformin and Glipizide in the range of 60 to 140 and 10-50 µg/ml respectively. The correlation coefficient for linear curve obtained between concentration vs. area for standard preparations of Metformin and Glipizide is 0.998 and 0.999, which meet the method validation acceptance criteria, an indication of method being linear in the range of 60 to 140 and 10-50 µg/ml for Metformin and Glipizide respectively

Accuracy studies revealed that found desirable recoveries were achieved (98-102%) as per acceptance criteria of method validation. % RSD for Metformin HCl and Glipizide was less than two. Hence, the method developed and optimized is accurate.

Method developed is found to be robust as it is found that the results of peak performance parameters are:

- Resolution factor (Rs) >2.0
- Tailing factor < 2.0 and
- Number of theoretical plates (Efficiency) more than 2000, which are in acceptance criteria to method validation despite deliberate variations done concerning flow rate, % organic phase and column temperature.

The LOD for this method was found to be 0.287 µg/ml & area 45865.92 for Metformin and 0.06 µg/ml & area 16937.03 for Glipizide. The LOD for this method was found to be 0.287 µg/ml & area 45865.92 for Metformin and 0.065 µg/ml & area 16937.03 for Glipizide. The degradation behavior of Metformin and Glipizide in different stress conditions like acidic,

alkaline, oxidative, thermal and neutral studies were performed and observed that Metformin and Glipizide was sensitive for acid degradation compared to other stress conditions.

From all the above validation conclusions, it is very clear that the Reverse Phase HPLC isocratic method developed and validated as per ICH guidelines is sensitive, accurate, precise, linear and convenient for intended applications in any pharmaceutical industries.

Table: Summary table for Metformin and Glipizide

Parameter	**Metformin**	**Glipizide**
Calibration range	60 - 140 µg/ml	10-50µg/ml
Optimized wavelength	257 nm	257 nm
Retention time	2.433 min.	5.710 min.
Regression equation	y = 2754.x + 45142	y = 3405.x + 16717
Correlation Coefficient (r^2)	0.998	0.999
Precision (%RSD)	0.37	0.31
% Assay	98.99	99.29
LOD	0.287 µg/ml	0.065 µg/ml
LOQ	0.870 µg/ml	0.196 µg/ml

9. REFERENCES

1. Skoog, Holler and Crouch, Liquid Chromatography. In Instrumental Analysis, Cengage Learning India Pvt. Ltd, New Delhi, 2011; 893.

2. R.G. Chatwal and K.S. Anand, High Performance Liquid Chromatography. Instrumental Methods of Chemical Analysis, 5th ed.; Himalaya Publishers, Mumbai, 2010; 2.570-2.629.
3. B.K. Sharma, High Performance Liquid Chromatography. Instrumental Methods of Chemical Analysis, 24th ed.; Goel Publishers, Meerut, 2005; 295-300.
4. Douglas, F. Skoog, H. James and R.C. Stanley, Liquid Chromatography. Instrumental Analysis, 9th ed.; Cengage Learning India Pvt. Ltd, New Delhi, 2007; 893 - 934.
5. Types of HPLC -http://www.chem.agilent.com/Library/primers/Public/59896639EN.pdf
6. Types of elution – http://chemwiki.ucdavis.edu/@api/deki/pages/402/pdf
7. Types of elution - http://hplc.chem.shu.edu/NEW/HPLC_Book/Rev.-Phase/rp_grad.html
8. Diagram of HPLC - http://hiq.lindegas.com/international/web/lg/spg/like35lgspg.nsf/docbyalias/image_hplc
9. Solvent Delivery System - http://www.monzirpal.net/Instrumental%20Analysis/Lectures/Lectures%2021-/L39.pdf
10. Injection valves - http://www.dolomitemicrofluidics.com/webshop/flowaccessoriesinjection-valves-c-17_18/sample-injection-valve-p-783
11. Injection valves - http://weather.nmsu.edu/Teaching_Material/SOIL698/Student_Material/Hplchp1090/Hplcinj.html
12. Flow path of a Manual Injector - http://polymer.ustc.edu.cn/xwxx_20/xw/201109/P020110906263097048536.pdf

13. A Braithwaite and FJ Smith, Liquid Phase Chromatography on Columns. Chromatographic Methods, 5th ed.; Kluwer Academic Publishers, Netherlands, 1999; 129.
14. Columns - International Pharmacopeia, 4th edition – http://apps.who.int/phint/en/p/docf/
15. Detectors – http://lipidlibrary.aocs.org/topics/detect92/file.pdf
16. Detectors – http://www.shodex.net/index.php?seitenid=1&applic=1485
17. Method development - http://www.pharmainfo.net/reviews/introductionanalyticalmethoddevelopmentpharmaceutical-formulations
18. The Drugs and Cosmetics Act and Rules, 1940.
19. Validation of Analytical Procedures: Text and methodology, Q2(R1), ICH Harmonised Tripartite Guidelines, USA, 2005; 1-13.
20. International Conference on Harmonization: Q2A, Text on Validation of Analytical Procedures. Federal Register, 1995(60); 11260–11262.
21. International Conference on Harmonization: Q2B, Validation of Analytical Procedures: Methodology, Availability. Federal Register, 1997(62); 27463–27467.
22. Methods of Analysis – http://www.pharmatutor.org/pharma-analysis
23. G Mubeen and N Khalikha. Spectrophotometric method for analysis of Metformin Hydrochloride. Indian Journal of Pharmaceutical Sciences. 2009; 100-102.
24. GVS Kumar, D Triveni, SB Puranik, NS Kummar and KA Sridhar. Development and validation of RP-HPLC method for simultaneous estimation of Glipizide and Metformin in

bulk drugs and tablet dosage form. International Research Journal of Pharmacy. 2012; 3(9): 260-263.

25. S Rahila and K Asif. RP-HPLC Method for the analysis of Glipizide in pharmaceutical dosage forms. International Journal of Ayurvedic Research and Pharmacy. 2010; 1(2): 455-458.
26. M Kar and PK Choudhury. HPLC Method for estimation of Metformin in formulated microspheres and tablet dosage form. Indian Journal of Pharmaceutical Sciences. 2009; 71(3): 318-320.
27. SM Charde, G Yadav, AS Welankiwar, K Jitendra and RP Marathe. Development of stability indicating assay method for the simultaneous estimation of Metformin Hydrochloride and Glipizide by RP-HPLC method. International Journal of Advances in Pharmaceutical Research. 2014; 4(3): 256-262.
28. N Sultana, N Shafi, FA Siddiqui and A Hussain. Development and validation of new assay method for the simultaneous analysis of Diltiazem, Metformin, Pioglitazone and Rosiglitazone by RP-HPLC and its applications in pharmaceuticals and human serum. Journal of Chromatographic Sciences. 2011; 49(10): 774-779.
29. RA Tengli, BM Gurupadayya, N Soni1 and B Vishwanathan. Method development and validation of Metformin, Pioglitazone and Glibenclamide in tablet dosage form by using RP-HPLC. Biochemistry & Analytical Biochemistry. 2013; 2(2): 1-5.
30. S Mandal and A Bose. Stability indicating assay method by HPLC for simultaneous estimation of Metformin and Glipizide in bulk and pharmaceutical dosage forms. World Journal of Pharmacy and Pharmaceutical Sciences. 2015; 4(6): 1410-1431.

31. S Goyal, A Gupta and R Rani. Development and validation of RP-HPLC method for estimation of Glipizide in bulk and pharmaceutical dosage form. International Journal of Pharm Tech Research. 2013; 5(1): 183-188.
32. Effect of flow rate – http://www.ionsource.com/tutorial/chromatography/rphplc.html
33. Effect of flow rate – http://www.ionsource.com/Card/linvelocity/linvol.html
34. Effect of temperature – http://www.pharmtech.com/pharmtech/Analytical/UsingHighTemperatureHPLC--for-ImprovedAnalysis/ArticleStandard/Article/detail/97082
35. Effect of pH- http://www.laserchrom.co.uk/LaserchromHPLC-pHBufferGuide.html
36. Effect of pH - Technical tips-selecting buffer ph in reverse phase HPLC
37. Effect of pH- http://www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/Fluka/General_InformatIon/analytixnotes_lpc_lowres.pdf
38. Effect of ion-pair reagent- http://www.standardbase.com/tech/HPLC%20validation%20PE.pdf
39. Peak shapes - http://www.chem.agilent.com/Library/eseminars/Public/secrets%20of%20good%20peak%20shape%20in%20hplc.pdf
40. Metformin – http://www.drugbank.ca/drugs/DB00331
41. Glipizide – http://www.drugbank.ca/drugs/DB01067