Journal of Pharmacreations

PharmaCreations

ISSN: 2348-6295

Pharmacreations | Vol.6 | Issue 4 | Oct - Dec - 2019

Journal Home page: www.pharmacreations.com

Research article *Open Access*

Simultaneous estimation of new analytical method development and validation of glipizide and metformin by reverse phase-high performance liquid chromatography

Sandhya Rani*, G.Saikiran, N.Sriram

Holy Mary Institute of Science and Technology, (College of Pharmacy), Bogaram, Keesara, Hyderabad.

***Corresponding Author**: **Sandhya Rani**

ABSTRACT

A new, simple and accurate, precise RP-HPLC method was developed for simultaneous determination of Glipizide and Metformin in bulk and in combined pharmaceutical dosage form. The separation of Glipizide and Metformin was achieved within 6 minutes on an Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column using Methanol: Acetate Buffer pH-3.8 (24:76v/v) as the mobile phase. Detection was carried out using wavelength at 262nm. The method showed adequate sensitivity concerning linearity, accuracy and precision over the range 100-500µg/ml and 30-70µg/ml for Glipizide and Metformin, respectively. Careful validation proved advantages of high sensitivity, accuracy, precision, selectivity, robust and suitability for quality control laboratories. The developed method was robust as the %RSD was within the range and without effecting system suitability parameters. The proposed method is suitable for simultaneous determination of Glipizide and Metformin in bulk and pharmaceutical dosage form.

Keywords: Glipizide and Metformin, RP-HPLC, Validation, Precision, Robustness.

INTRODUCTION

Glipizide is an oral hypoglycemic agent in the second-generation sulfonylurea drug class that is used to control blood sugar levels in patients with type 2 diabetes mellitus. It was first introduced in 1984 3 and is available in various countries including Canada and the U.S. According to the 2018 Clinical Practice Guidelines by Diabetes Canada, sulfonylurea drugs are considered a second-line glucose-lowering therapy following Metformin. Because sulfonylureas require functional pancreatic beta cells for their therapeutic effectiveness, sulfonylureas are more commonly used for early-stage type 2 diabetes when there is no progressed pancreatic failure. The IUPAC Name N-[2-(4-{[(cyclohexyl carbamoyl) amino] sulfonyl} phenyl) ethyl]-5-methylpyrazine-2-carboxamide and the chemical formula is $C_{21}H_{27}N_5O_4S$.

Metformin is an antihyperglycemic agent of the biguanide class, used for the management of type II diabetes). Currently, Metformin is the first drug of choice for the management of type II diabetes and is prescribed to at least 120 million people worldwide. Metformin is considered an antihyperglycemic drug

because it lowers blood glucose concentrations in type II diabetes without causing hypoglycemia. Metformin is commonly described as an insulin sensitizer leading to a decrease in insulin resistance and a clinically significant reduction of plasma fasting insulin levels. The IUPAC Name 1-carbamimidamido-N, N-dimethyl methanimidamide and the chemical formula is $C_4H_{11}N_5$. The Chemical Structures of Glipizide and Metformin are as follows

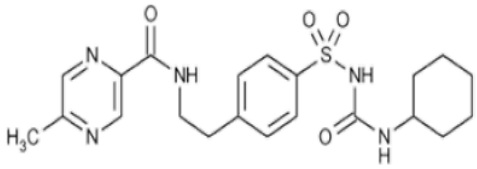

Fig-1: Chemical Structure of Glipizide

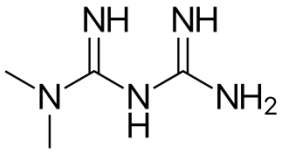

Fig-2: Chemical Structure of Metformin

MATERIALS AND METHODS

Instruments Used

Table-1: Instruments used

S.No.	Instruments And Glass wares	Model
1	HPLC	WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra Sonicator	Labman

Chemicals Used

Table-2: Chemicals used

S.No	Chemical	Brand names
1	Glipizide	Sura labs
2	Metformin	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT

Preparation of standard solution

Accurately weigh and transfer 10 mg of Glipizide and Metformin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 3ml of Glipizide and 0.5ml of Metformin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak

elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water, Acetonitrile and water with varying proportions. Finally, the mobile phase was optimized to Methanol: Acetate Buffer pH-3.8 in proportion 24:76 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

- Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
- Temperature : 37ºC
- Column : Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column
- Mobile phase : Methanol: Acetate Buffer pH-3.8 (24:76v/v)
- Flow rate : 1ml/min
- Wavelength : 262nm
- Injection volume : 10 µl
- Run time : 6 min

METHOD VALIDATION

Preparation of mobile phase

Accurately measured 240 ml (24%) of Methanol and 760 ml of Acetate Buffer (76%) a were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

METHOD VALIDATION PARAMETERS

System Suitability

Accurately weigh and transfer 10 mg of Glipizide and 10mg of Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3ml of Glipizide and 0.5ml of Metformin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Glipizide and 10mg of Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3ml of Glipizide and 0.5ml of Metformin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Glipizide and Metformin sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3ml of Sample solution from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY

Accurately weigh and transfer 10 mg of Glipizide and 10mg of Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

- **Preparation of Level – I (100ppm of Glipizide & 30ppm of Metformin)**
- Pipette out 1ml of Glipizide and 0.3ml of Metformin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.
- **Preparation of Level – II (200ppm of Glipizide & 40ppm of Metformin)**
- Pipette out 2ml of Glipizide and 0.4ml of Metformin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.
- **Preparation of Level – III (300ppm of Glipizide & 50ppm of Metformin)**
- Pipette out 3ml of Glipizide and 0.5ml of Metformin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.
- **Preparation of Level – IV (400ppm of Glipizide & 60ppm of Metformin)**
- Pipette out 4ml of Glipizide and 0.6ml of Metformin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.
- **Preparation of Level – V (500ppm of Glipizide & 70ppm of Metformin)**
- Pipette out 5ml of Glipizide and 0.7ml of Metformin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

Repeatability

Preparation of Glipizide and Metformin Product Solution for Precision

Accurately weigh and transfer 10 mg of Glipizide and 10mg of Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3ml of Glipizide and 0.5ml of Metformin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure

- **Day 1:** The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
- **Day 2:** The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

ACCURACY

For preparation of 50% Standard stock solution

Accurately weigh and transfer 10 mg of Glipizide and 10mg of Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.5ml of Glipizide and 0.25ml of Metformin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution

Accurately weigh and transfer 10 mg of Glipizide and 10mg of Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3ml of Glipizide and 0.5ml of Metformin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution

Accurately weigh and transfer 10 mg of Glipizide and 10mg of Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 4.5ml of Glipizide and 0.75ml of Metformin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Glipizide and Metformin and calculate the individual recovery and mean recovery values.

ROBUSTNESS

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution

Accurately weigh and transfer 10 mg of Glipizide and 10mg of Metformin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3ml of Glipizide and 0.5ml of Metformin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of flow conditions

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e. Methanol: Acetate Buffer was taken in the ratio and 29:71, 19:81 instead (24:76), remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

Optimized Chromatographic Condition

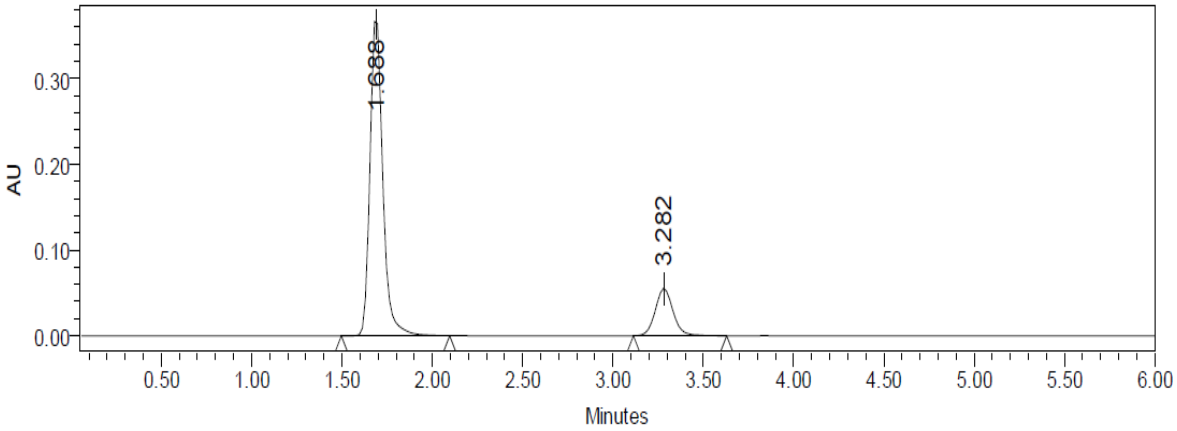

Fig-3: Optimized Chromatogram

Table-3: Observation of Optimized Chromatogram

S.No.	Peak Name	Retention Time	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Glipizide	1.688	1658785	385669	1.69	7586	10.85
2	Metformin	3.282	425631	65245	1.58	6235	

METHOD VALIDATION

Specificity (Assay)

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The percentage purity of the given Marketed Formulation was found to be 99.86%.

SYSTEM SUITABILITY PARAMETERS

Table-4: System Suitability Parameters

S. No.	Parameter	Glipizide	Metformin
	Retention Time (min)	1.688	3.282
	Theoretical Plates	7586	6235
	Tailing factor	1.69	1.58
	Area	1658768	426589
	Resolution	10.89	

The system suitability parameters were found to be within the specified limits for the proposed method.

ACCURACY

Table-5: Accuracy Observation of Glipizide

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	879537	150	150.048	100.032	
100%	1743252	300	300.521	100.172	100.112%
150%	2609693	450	450.598	100.132	

Table-6: Accuracy Observation of Metformin

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	224271	25	25.114	100.456%	
100%	445748.3	50	49.952	99.904%	100.16%
150%	670006.3	75	75.101	100.134%	

The accuracy studies were shown as % recovery for Glipizide and Metformin at 50%, 100% and 150% the limits of % recovery should be in range of 98-102%.

The results obtained for Glipizide and Metformin were found to be within the limits. Hence the method was found to be accurate.

The accuracy studies showed % recovery of the Glipizide 100.112%- and Metformin 100.16%.

The limits of % recovery of drugs were 98-102 % and from the above results it indicates that the commonly used excipients present in the pharmaceutical formulation do not interfere in the proposed method.

PRECISION

Table-7: Observation of System Precision

S. No.	Sample Area 1 (Glipizide)	Sample Area 2 (Metformin)
1	1658254	426598
2	1658952	426589
3	1654857	426985
4	1659854	426587
5	1653298	426515
Mean	**1657043**	**426654.8**
Std.dev	**2820.29**	**187.5692**
%RSD	**0.1702**	**0.043963**

Acceptance Criteria

In the precision study %RSD was fond to be less than 2%. For Glipizide 0.17% and Metformin 0.04% which indicates that the system has good reproducibility.

For precision studies 5 replicated injections of Glipizide and Metformin formulation was performed. %RSD was determined for peak areas of Glipizide and Metformin.

The acceptance limits should be not more than 2% and the results were found to be within the acceptance limits.

ROBUSTNESS

(Day-1)

Table-8: Observation of Robustness Day1

S. No.	Sample Area 1 (Glipizide)	Sample Area 2 (Metformin)
1	1665985	436598
2	1662598	436855
3	1668484	436598
4	1664598	436587
5	1663579	436741
6	1664587	432659
Mean	**1664972**	**436006.3**
Std. Dev.	**2060.327**	**1643.285**
% RSD	**0.123745**	**0.376895**

Acceptance Criteria

%RSD of five different sample solutions should not more than 2.

(Day-2)

Table-9: Observation of Robustness Day2

S. No.	Sample Area 1 (Glipizide)	Sample Area 2 (Metformin)
1	1648598	415985
2	1642587	415267
3	1649852	415986
4	1648754	415265
5	1645289	415874
6	1647581	415632
Mean	**1647110**	**415668.2**
Std. Dev.	**2699.291**	**337.2106**
% RSD	**0.16388**	**0.081125**

Acceptance Criteria

%RSD of five different sample solutions should not more than 2.

LINEARITY

Table-10: Linearity Observation of Glipizide

S. No.	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1	I	100	585985
2	II	200	1182468
3	III	300	1768785
4	IV	400	2326852
5	V	500	2856874
Correlation coefficient			0.999

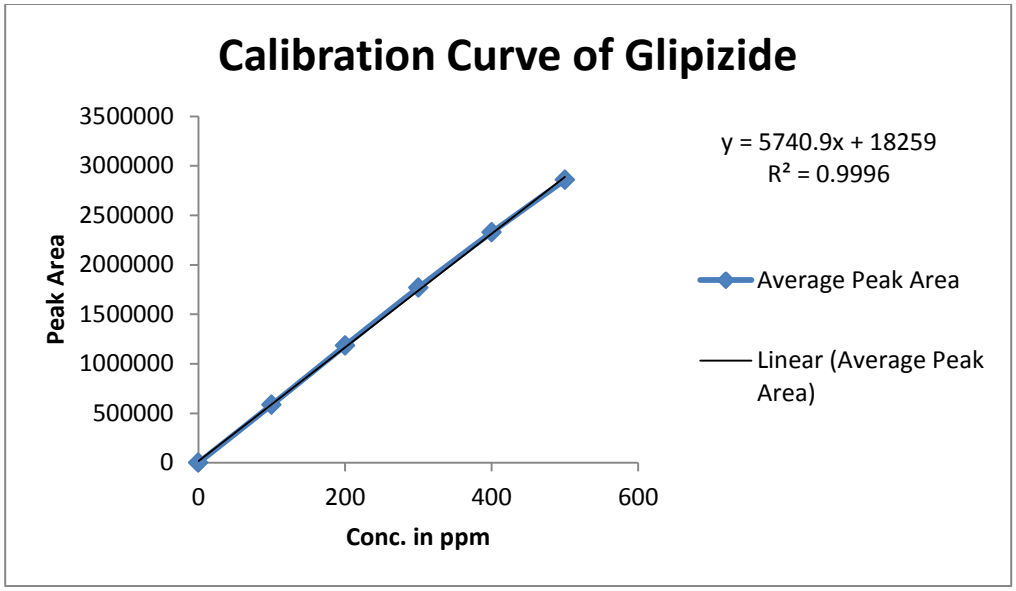

Fig-4: Calibration Curve for Glipizide

Table-11: Linearity Observation of Metformin

S. No.	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1	I	30	268764
2	II	40	356958
3	III	50	445631
4	IV	60	535186
5	V	70	624698
Correlation coefficient			0.999

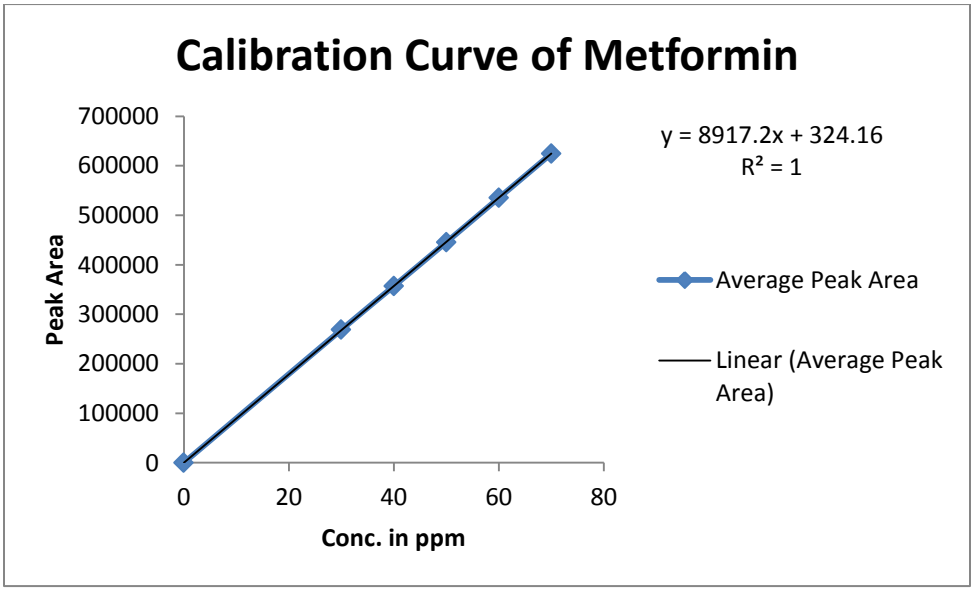

Fig-5: Calibration Curve for Metformin

The linearity range was found to be 100-500 and 30-70µg/ml for both Glipizide and Metformin respectively. Calibration curve was plotted and correlated Co-efficient for both the drugs found to be 0.999.

Hence the results obtained were within the limits.

LIMIT OF DETECTION (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × S.D / Slope

The Limit of Detection (LOD) values of Glipizide and Metformin was found to be 2.1µg/ml and 1.28 µg/ml respectively

LIMIT OF QUANTITATION (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10× S.D / Slope

The Limit of Quantification (LOQ) values of Glipizide and Metformin was found to be 6.3µg/ml and 3.84µg/ml respectively

ROBUSTNESS

Flow Rate: (ml/min)

System Suitability Results for Glipizide

Table-12: Flow Rate Observation of Glipizide

	System suitability Results		
Flow Rate	**USP Plate**	**USP**	**Retention Time**
Less Flow	7365	1.62	1.868
Actual Flow	7586	1.69	1.688
More Flow	7254	1.61	1.544

Results for actual flow rate have been considered from assay standard.

System Suitability Results for Metformin

Table-13: Flow rate Observation of Metformin

	System suitability Results		
Flow Rate	**USP Plate**	**USP**	**Retention Time**
Less Flow	6284	1.51	3.621
Actual Flow	6235	1.58	3.282
More Flow	6168	1.56	2.998

On evaluation of the above results, it can be concluded that the variation in flow rate not affect the method significantly.

Organic Composition

Table-14: System Suitability Results Glipizide

Organic phase		**System suitability Results**		
		USP Plate	**USP**	**Retention Time**
Less organic	5	7269	1.61	1.868
Actual organic	5	7586	1.69	1.688
More organic	6	7496	1.64	1.675

Table-15: System Suitability Result Metformin

Organic phase		**System suitability Results**		
		USP Plate	**USP**	**Retention Time**
Less organic	5	6182	1.54	3.621
Actual organic	5	6235	1.58	3.282
More organic	6	6322	1.56	2.302

Acceptance Criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Glipizide and Metformin was done by RP-HPLC. The Phosphate buffer was p^H 3.8 and the mobile phase was optimized with consists of Methanol: acetate buffer (pH-3.8) mixed in the ratio of 24:76%v/v. An Agilent Zorbax (C18) (150mm x 4.6mm, 5µm) column or equivalent chemically bonded to porous silica particles were used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Glipizide and Metformin were found to be from 100-500µg/ml, 30-70µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Glipizide and Metformin. LOD and LOQ were found to be within limits.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

REFRENCES

[1]. Dr. Kealey and P.J Haines, Analytical Chemistry, Bios Publisher, 1, 2002, 1-7.
[2]. A. Braith Wait and F.J.Smith, Chromatographic Methods, Kluwer Academic Publisher, 5, 1996, 1-2.
[3]. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, Academic press, 1, 1997, 24-37.
[4]. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, Wiley Interscience A JohnWiley & Sons, Inc., Publication, 1, 2007, 15-23.
[5]. Meyer V.R. Practical High-Performance Liquid Chromatography, England, John Wiley & Sons Ltd, 4, 2004, 7-8.
[6]. Sahajwalla CG a new drug development, Marcel Dekker Inc., New York, 141, 2004, 421–426.
[7]. Introduction to Column. (Online), URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf
[8]. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, IFPMA, Switzerland, 60, 1995, 1126.
[9]. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 1996, 1- 8.
[10]. Data elements required for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.
[11]. Snyder LR practical HPLC method development, John Wiley and sons, New York, 2, 1997, 180-182.
[12]. Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. 1994, 1-5.
[13]. Sharma B K, Instrumental method of chemical analysis Meerut. 1999, 175-203.
[14]. Breaux J and Jones K: Understanding and implementing efficient analytical method development and validation. Journal of Pharmaceutical Technology, 5, 2003, 110-114.
[15]. Willard, H. y. Merritt L.L, Dean J.A and Settle F.A "Instrumental methods of analysis" CBS publisher and distributors, New Delhi, 7, 1991, 436-439.
[16]. ICH Q2A, "validation of analytical methods, definitions and terminology", ICH Harmonized tripartite guideline, 1999.
[17]. https://www.drugbank.ca/drugs/DB01067
[18]. https://pubchem.ncbi.nlm.nih.gov/compound/glipizide
[19]. https://en.wikipedia.org/wiki/Glipizide
[20]. https://www.drugbank.ca/drugs/DB00331
[21]. https://pubchem.ncbi.nlm.nih.gov/compound/4091

[22]. https://en.wikipedia.org/wiki/Metformin
[23]. Sri Lakshmi D*, Jane T Jacob, Srinivas D, Satyanarayana D, Simultaneous Estimation Of Metformin And Glipizide By RP-HPLC And Its Validation, World Journal of Pharmacy and Pharmaceutical Sciences, 4(9), 740-750.
[24]. Bagadane Snehal Bapusaheb*, Jadhav Prerana B, Development and validation of RP-HPLC method for simultaneous estimation of Metformin hydrochloride and Glipizide in bulk and pharmaceutical dosage form, Journal of Drug Delivery and Therapeutics, 9(3), 2019, 146-155.
[25]. K. Ganesh1, G. Nikitha1, D. Sireesha2*, B. Vasudha3, Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Metformin and Glipizide in Tablet Dosage Form, International Journal of Applied Pharmaceutical Sciences and Research, 1(2), 2016, 56-59.
[26]. Suresh Kumar GV, D.Triveni, S.B. Puranik, N. Sateesh Kumar4 K.A.SR, Development and validation of RP HPLC method for simultaneous estimation of Glipizide and Metformin in bulk and tablet dosage form, International Research Journal of Pharmacy 3(9), 2012, 260.
[27]. Ahmed Gedawy a, Hani Al-Salami a, b, Crispin R. Dass a, b,*, Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, Metformin and Gliclazide, Journal of food and drug analysis, 27, 2019, 315-322.