Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

https://doi.org/10.36673/AJPAMC.2025.v13.i01.A02

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION USING HPLC FOR ROUTINE QUALITY CONTROL OF GLICLAZIDE IN PHARMACEUTICAL PREPARATIONS

Neeru Sharma[1], Varsha Rani[1], Damodaran Venugopal[1], Meena Yadav[*1]

[1*]Department of Chemistry, School of Physical Sciences, Starex University, Gurugram, Haryana, India.

ABSTRACT

Level of blood sugar is elevated due to deficiency or complete unavailability of insulin hormone in human body. This is the characteristic feature of diabetes mellitus. Sulfonyl urea drugs belong to the second-generation anti-diabetic drugs. These drugs work by enhancing the insulin secretion in pancreas. In pharmaceutical drug form of any drug there is always need to develop a selective method to measure the accurate amount of active ingredient due to presence of various inactive ingredients. Optimization of chromatography parameter inclusive of its composition in its mobile phase in order to increase the separation with resolution of drug are the significant points within the present study. For this purpose, a hypersil column (C18 OSD at temp 25°C), rate of flow 1.00mL/minute, a volume of 20µL, 15 min running time and λ = 228nm, phosphate buffer and acetonitrile (10:90 v/v) with pH = 3 were opted. Further specificity and precision of technique as well as along with appropriateness, linearity, accuracy its robustness was also studied to validate the method. LOQ for Gliclazide was found to be as 0.0909mg/mL and LOD was observed as 0.0299mg/mL for the linear range from 20-70mg/mL. Results show that the present designed methodology can be used for investigation of Gliclazide drug forms with accuracy, cost economy and more sensitivity and preciseness.

KEYWORDS

Validation, HPLC, Gliclazide, Insulin and Antidiabetic.

Author for Correspondence:
Meena Yadav,
Department of Chemistry,
School of Physical Sciences, Starex University,
Gurugram - 122413, Haryana, India.
Email: meenuyadav333@gmail.com

INTRODUCTION

Diabetes mellitus is a metabolic disorder in human body resulting in the increased blood sugar level. Body blood sugar level is controlled by a hormone recognized as insulin which is emitted by beta cells of pancreas[1,2]. In such metabolic disorder pancreas beta cells become incapable for the secretion of insulin hence leading to the disease diabetes

mellitus[3]. Gliclazide is an anti-diabetic drug which is also known as (1-(3-azabicyclo [3.3.0] oct-3-yl))-1-(hexahydrocyclopenta [c] pyrrol-2(1H)-yl). This is a sulphonyl urea drug and can be used for the treatment of hypoglycemic condition of body as reported by[4].

Gliclazide is second generation antidiabetic drug working by enhancing the secretion of insulin hormone from pancreas beta cells along with increasing the peripheral body sensitivity towards insulin hormone and improving the whole metabolic dynamics of insulin within the body as reported by[5,6]. Almost 96% of this drug is absorbed and metabolized and approximately 4% is excreted in the form of urine[7,8]. Specific properties are provided to the sulfonyl urea multi by the presence of an "azabicyclo-octyl" group as reported in earlier report[9,10]. In addition, molecule reduces the production of glucose along with increased glucose clearance. However not any changes have been reported yet in the insulin receptors. This can be suggested that action of insulin can be affected post reception by means of active glycogen synthase in muscles as well as fructose -2, 6- bisphosphatase moiety[10,11]. Gliclazide also increases the process of fibrinolysis by the reduction in adhesion of platelets aggregation along with their hyper activity[12,13]. These are the key benefits of Gliclazide, avoiding the diabetic micro angiopathy. Further the drug is metabolized by liver and products are excreted in urine and feces as reported by Bolla *et al*[14]. Tablets forms of Gliclazide are being prescribed by physicians as an anti-diabetic tablet to regulate blood sugar level of patient[15]. There are various brand names available in India for the Gliclazide composition as 30 milligram 60 milligram and 80 milligrams[16].

Research literature review suggests about the number of High-Performance Liquid Chromatography (HPLC) methods in order to estimation of Gliclazide content and its combination with other drugs[17-19]. However, a quick repeatable and more precise methods are still required to be developed for determination of Gliclazide and its combination in pharmaceutical drug forms in continuation to optimization of methods and technology[20-22]. The present study was aimed to develop a more precise accurate, time saving and cost economic approach as well as the validation of method in order to determine the Gliclazide in its pharmaceutical dose forms.

MATERIAL AND METHODS

Chemicals and Reagents

Standard reference samples for Gliclazide were purchased from the commercial market of India (Reddy's laboratory). Other sample were purchased from retail drug market as pharmaceutical doses (Cipla Ind). All other chemical and reagents were purchase from market for HPLC procedure. (HPLC grade mark India). All the chemical and reagents used were of analytical grade.

Development of HPLC Method

Instrumentational Condition

HPLC instrument Shimadzu LC 2080 India model, was used with PDA detector Colum (18mm × 4.6mm x 2.6mm, 5m) temperature condition was kept as 10˚C and 25˚C for auto sampler and column respectively. Mobile phase was prepared with PO_4 buffer mixed with acetonitrile HPLC grade 10:90 v/v, pH = 3 with a flow rate of 1.0mL/min at wavelength (λ = 228nm), for optimization process. Different mobile phases and trails running time are represented as Table No.1.

Buffer Preparation

For buffer preparation, 0.3g disodium hydrogen phosphate and 1.6 g potassium dihydrogen phosphate were dissolved in 550mL of double distillated water. Solution prepared was filtered by using 0.5µm membrane filter.

Mobile Phase

Filtration of buffer solution as well as acetonitrile solution was done along with the process of degasification as 10:90 v/v in order to prepare the mobile phase. pH level was optimized up to 3 using the orthophosphoric acid to the prepared mobile phase solution.

Stock and Standard Solutions

A 10mg of Gliclazide was dissolved into adequate amount of moveable phase using a 100mL flask. Furthermore, the solution was sonicated nearly 12 minutes in order to dissolve the Gliclazide content

properly. The final 100mL volume of solution was obtained by adding mobile phase. The resulting 100mL solution thus obtained was referred as stock solution with a concentration of 100g/mL. Now various concentrations were prepared by using stock solution such as 10mg/mL to 70mg/mL by taking the 0.1mL to 0.7mL of stock solution in a 10 mL volumetric vessel.

Sample Solution

20 numbers of non-damaged tablets of Gliclazide were crushed and mixed well with the help of mortar. Powder obtained was weighed equal to 10mg Gliclazide. Further, it was mixed with enough diluents up to 100mL in a volumetric flask. This solution was used as the stock solution. In order to ensure proper mixing Sonication was also performed for 15 minutes. To obtain proper Gliclazide concentrations more diluted solutions were prepared by using this stock solution.

Validation of Method

Method validation was performed as per the International Council for Harmonisation (ICH) guidelines and the developed method was tested for its appropriateness of system, specificity, and the precision of HPLC system used. The ruggedness as well as the linearity of developed method was also tested[23,4].

Suitability of System

System variability was also studied and two different HPLC system were used for this purpose. For each 5 samples were prepared and investigated for their respective chromatography pattern along with the theoretical plates. The retention time, trail factor (T) and % RSD were also investigated and the technique was confirmed for its resolution and repeatability[24]. Following criteria was adopted for the selection of acceptance criteria for the study of system suitability. Each of the standard solution 5 replications of injections were investigated and the maximum % RSD was restricted as not more than 2% for primary peak retention. Further % RSD was restricted as not more than 3.0% for primary peak area responses. The minimum number of theoretical plates and maximum trailing factor T were kept as 2000 and less than 2.0, respectively.

Linearity

The measured stock solution was kept in a volumetric vessel of 10mL capacity and it was diluted with mobile phase. The concentration was kept between 20 to 70mg/mL and it was injected in triplicate time period of 15 minutes with a flow rate 1.0mal/min at λ = 228nm). The calibration was received with correlation coefficient (R^2) 0.9990 min, Y intercept % of 2.0.

Precision

The repeatability criterion was tested and confirmed by precision. For précised system, standard solution of drug was inserted by means of 5 injections along with maintaining protocols. Percentage RSD for peak area was restricted up to less than equal to 3.0%.

For specificity, the reference and standard solution of the drug molecule were used for injections and the interference peak was analyzed for sample. Retention time for the standard as well as test was found almost equal.

LOD and LOQ was calculated using the below mentioned formulae[22,25] in equations 1-2:

$LOD = 3.3\times\sigma/S$ (1)

$LOQ = 10\times\sigma/S$ (2)

Where σ = response standard deviation and S = slope for calibration curve of analyte.

Accuracy (Recovery)

Gliclazide concentration as 50% 100% and 150% were used in triplicate for process the assay. Further comparison of accuracy was performed to the standard, based on their concentrations. The rate of recovery was completed and the percentage of recovery was between 90% to 110%.

Robustness

The international flow rate fluctuations were used to demonstrate the toughness of method developed here in. The standard solution was prepared in accordance of test procedure and further it was injected in HPLC system with a flow rate of 0.80mL/min, 1.0mL/min and 1.2mL/min. Further the appropriateness criteria of the system were investigated with a protocol of maximum T is equal to 2.0.

Ruggedness

System to system variability was also studied and in this respect system variability was investigated for number of HPLC system with same experimental condition. [% RSD = 3.0% Maximum recovery = 90% - 110%].

RESULTS AND DISCUSSION

Method Development and Conditional Optimization

Selection of stationary phase is one of the important processes during the development of HPLC analytical techniques. Selection of stationary phase is done by the help of molecular solubility and weight. As from the literature reference it was revealed that reverse phase chromatography is the most efficient technology with this regard, hence a C-18 column was selected for this study. While the study of some buffer solutions as well as acetonitrile concentration was also performed and optimization was done to obtain the symmetric peaks in a shorter run time. The optimization conditions for chromatographic study are depicted in Table No.2.

Acetonitrile

A buffer (90:10 % v/v), was used to produce symmetric peaks. Retention time was found as 9.53 minutes and different mobility phases were studied as portrayed in Table No.3. Standard and test chromatographs are denoted as in Figure No.1(a) and 1(b), respectively.

System Suitability

Five replications of injections were processed in HPLC and results were recorded. It can be observed that drug Gliclazide shown are good repeatability for all injections. (% RSD = 0.03%) (Threshold NMT = 1% retained; separation time = 9.5 minute). Trailing factor of 0.90 denotes good peak symmetry. This coupled the criteria of acceptance as NMT 2%. High column efficiency was indicated by more than 6000 theoretical plates (approval criteria as NLT 2000). It was noted that for the major peaks the peak area response was observed as 3351663, % RSD = 0.02%, Acceptance < 2% as shown in Table No.4.

Linearity Study

The calibration curve was linear for 20-70mg/mL, Gliclazide concentrations. Figure No.2 represents the graph along with limit of acceptance as 0.990. The coefficient of regression (R^2) was found to be at 0.999. Table No.5 represents the concentration range and area response.

Specificity

Not any interference was found in between retention time for test and standard solutions. Thus, the same retention time for test as well as reference meets the acceptance criteria for the specificity. Retention time was 9.52 minute for test as well for the standard solution.

LOD and LOQ

LOQ for Gliclazide was found to be as 0.0909 mg/mL and LOD was observed as 0.0299 mg/mL. These LOQ and LOD were considered as the upper limit of sensitivity for the method developed. Hence the method showed a good sensitivity for LOD as well as for LOQ.

Robustness

The robustness of the method was examined by increasing the flow rate from 0.8 - 1.2mL/min and it was discovered to be within reasonable bounds. The retention durations of all other peaks, which were isolated from gliclazide, were comparable to those observed for mobile phase flow rates of 1.0mL/min. When the chromatographic conditions were slightly changed, no significant changes were observed, demonstrating that the method is robust against small, deliberate changes in flow rate. The results are shown in Table No.6. The robustness of the method was demonstrated by the discovery of an RSD of less than 1%.

Robustness

System to system variability was also taken in concern for the study of test method flexibility. It was demonstrated by the comparison of result of strength for two separate HPLC systems. It was observed that there was not any significant difference in between results of two different HPLC systems. The % RSD for the drug was obtained as 0.03% from 6 sample. (An acceptance criterion was < 2.0). The assay percentage for the drug was found

to be 90% to 110% Table No.7, (Data of two separate systems).

Ruggedness

The assay test procedure's resilience to system-to-system variability is demonstrated by comparing the results from the two distinct HPLC systems. Ruggedness was also expressed as a percentage of relative standard deviation, and statistical analysis showed no discernible differences between the results of the two alternative methods. Although the percent relative standard deviation of Gliclazide from the six sample preparations was found to be 0.03% (Acceptance criteria: NMT 2.0%), the percent assay of Gliclazide was estimated to be between 90.0% and 110.0%. Data from two distinct systems are shown in Table No.7.

Accuracy (Recovery)

The validity and recovery of method was shown and confirmed in the term of the degree to which the measured analyte value was found to be agreed with that of theoretical dilutions at several levels. Greater than 99% of the drug was recaptured from the experiments. (Acceptance criteria = 90% to 110% Table No.8 resulted the drug recovery).

Precision

Furthermore, the repeatability with 0.00% and 0.0 1% RSD was noted for the peak area and retention time with 6 standard drug solutions. (Acceptance < 2%). Thus, the precision and repeatability of the system can be received here in. (Table No.9, system precision and repeatability).

In order to test the method accuracy 6 samples were processed individually. The peak area and drug assay were found to be in the acceptable range. (Gliclazide assay = 100.14%, acceptance 90%-110%). This indicates a high degree of repeatability as well as precision as shown in Table No.10.

Table No.1: Shows mobile phases trials at various time intervals

S.No	Acetonitrile %	Phosphate Buffer %
1	50	50
2	60	40
3	70	30
4	80	20
5	90	10

Table No.2: Portrays optimization – Chromatographic conditions

S.No	Parameters	Details
1	Flow rate	1.0mL/min
2	Column	Thermo- Hypersil ODS –C18, 4.6mm× 250mm, 5µm column
3	Detector wavelength	228nm
4	Column temp	25ºC
5	Injection volume	20µl
6	Run time	15 min
7.	Retention time	9.528 min

Table No.3: Represents compositions of mobile phases and their observations

S.No	Acetonitrile %	Phosphate Buffer %	Observation
1	50	50	The peak was splited and an extra peak observed
2	60	40	Asymmetric peak but a broad peak observed
3	70	30	Symmetric peak with a broad peak observed
4	80	20	Symmetric peak with a slightly sharp peak
5	90	10	Well resolved and excellent symmetrical sharp peak

Table No.4: Shows system suitability

Injection	RT	Peak Area	Theoretical Plates	Trailing Factor
1	9.534	3551456	6485	0.901
2	9.530	3355554	6478	0.910
3	9.528	3352681	6472	0.910
4	9.528	3351507	6505	0.908
5	9.528	3348116	6544	0.907
Mean	9.530	3351663	6495	0.907
SD	0.003	594.685	…	…
% RSD	0.03	0.02	…	…

Table No.5: Depicts concentrations and area count for test molecule

S.No	Concentration (mg/mL)	Area	Statistical Analysis
1	0	0	Linearity Equation (Y) = 65422x + 4232 Correlation Coefficient (R^2) = 0.999
2	20	76424	
3	30	131953	
4	40	198502	
5	50	268752	
6	60	335703	
7	70	392154	

Table No.6: Shows impact on flow rate variations

S.No	-	Std Area	Tailing factor	-	Std Area	Tailing factor	-	Std Area	Tailing factor
1		2682318	0.942		3351541	0.906	-	4023478	1.003
2	Flow	2684237	0.943	Flow	3352144	0.906	Flow	4026486	1.003
3	0.80mL/min	2685263	0.944	1.0mL/min	3354683	0.906	1.20mL/min	4024585	1.004
4	-	2682455	0.942	-	3352563	0.906	-	4026483	1.003
5	-	2683542	0.943	-	3353147	0.907	-	4023567	1.001
6	Avg	2683563	0.943	Avg	3352815	0.906	Avg	4024919	1.002
7	SD	1237.17	0.001	SD	1197.55	0	SD	1493.11	0.002
8	% RSD	0.046	0.09	% RSD	0.036	0.05	% RSD	0.037	0.141

Table No.7: Represents peak area and % assay by - system 1 and system 2

S.No		Injection	System 1		System 2	
			Peak area	% Assay	Peak area	%Assay
1	50ppm	1	758426	100.00	3355786	100.10
		2	758314	99.99	3353478	100.04
		3	758236	99.98	3358248	100.18
		4	758461	100.00	3354124	100.05
		5	758245	99.98	3361124	100.26
		6	758862	100.06	3359482	100.21
2	Statistical Analysis	Mean	753424	100.00	3357040	100.14
		SD	10233.23	-	3061.539	0.091
		% RSD	1.597	0.03	0.091	0.09

Table No.8: Depicts the recovery data for Gliclazide

S.No	Concentration (%) spiked level	Area	Amount Added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery (Mean)
1	50% Sample1	5052346	300	303	100.33	99.96%
2	50% Sample2	5031197	300	301	99.91	
3	50% Sample3	5018776	300	298	99.67	
4	100% Sample1	6747651	600	604	100.50	100.73%
5	100% Sample2	6781223	600	605	101.00	
6	100% Sample3	6756044	600	603	100.63	
7	150% Sample1	8392602	900	904	100.00	100.06%
8	150% Sample2	8395956	900	902	100.04	
9	150% Sample3	8406027	900	900	100.16	

Table No.9: Shows repeatability study

S.No	Concentration	Injection	RT	Peak area
1	60ppm	1	9.527	3352425
		2	9.531	3352187
		3	9.527	3352441
		4	9.527	3352127
		5	9.527	3352247
2	Statistical Analysis	Mean	9.527	3352285
		SD	0.001	141.375
		% RSD	0.01	0.00

Table No.10: Represents data of repeatability (Method precision)

S.No	Concentration	Injection	Peak area of Gliclazide	% Assay
1	50ppm	1	3355786	100.10
		2	3353478	100.04
		3	3358248	100.18
		4	3354124	100.05
		5	3361124	100.26
		6	3359482	100.21
2	Statistical Analysis	Mean	3357040	100.14
		SD	3061.539	0.091
		% RSD	0.09	0.09

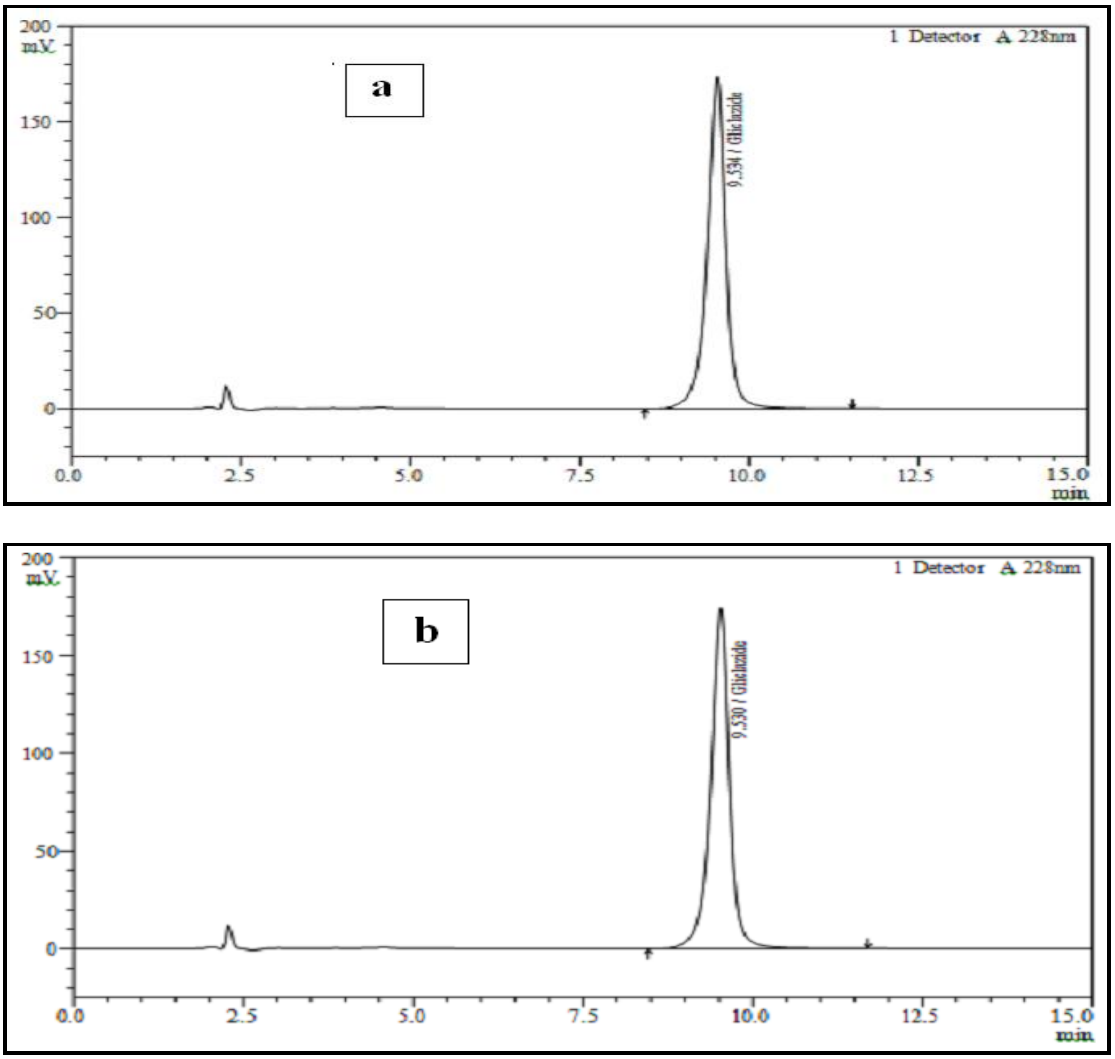

Figure No.1: HPLC Chromatogram for Gliclazide as (a) Standard and (b) Test

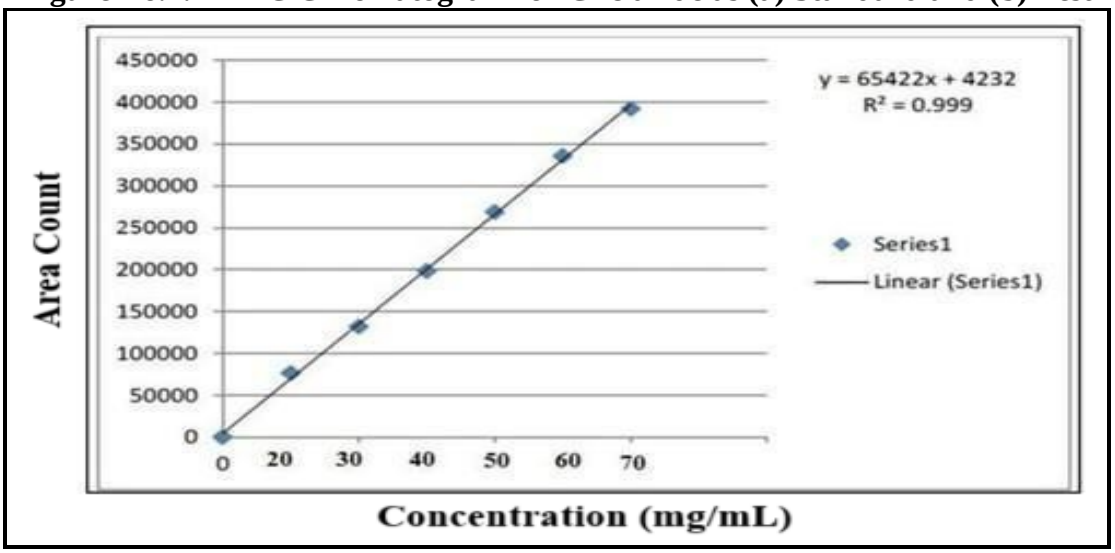

Figure No.2: Calibration curve different Gliclazide concentrations

CONCLUSION

To conclude, a method was developed to estimate the drug Gliclazide by using reverse phase HPLC technique. The current conventional methods can be substituted by newly developed method in a precise manner. LOQ for Gliclazide was found to be as 0.0909mg/mL and LOD was observed as 0.0299mg/mL for the linear range from 20-70mg/mL. The linear regression coefficient (R^2) was observed to be at 0.999. The newly developed method can be applied to the raw drug material as well as in its pharmaceutical dosages. Method was found offering a high degree of sensitivity as well as accuracy. Moreover, the new method is more cost economic, time saving and easier having strength to substitute the conventional methods.

STATEMENTS AND DECLARATIONS

ACKNOWLEDGEMENT

The authors are obligated to Kurukshetra University, Kurukshetra, Haryana, India and Starex University, Gurugram, Haryana, India for providing necessary facilities.

COMPETING INTERESTS

The authors declare no conflict of interest.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Neeru Sharma

Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Resources; Validation; Roles/Writing - original draft.

Varsha Rani

Formal analysis, Data curation, Resources

Damodaran Venugopal

Formal analysis, Data curation, Resources

Meena Yadav

Conceptualization; Supervision; Methodology; Formal analysis, Investigation, Validation; Visualization; Writing - review and editing.

BIBLIOGRAPHY

1. Tests D. 2. Classification and diagnosis of diabetes, *Diabetes Care*, 38(1), 2015, S8-S16.
2. Bruen D, Delaney C, Florea L, Diamond D. Glucose sensing for diabetes monitoring: Recent developments, *Sensors (Switzerland)*, 17(8), 2017, 1-21.
3. Sabhyatha T S, Narayana Babu. Analytical method validation of gliclazide related substances by high performance liquid chromatography method, *Int J Curr Pharm Res*, 14(4), 2022, 36-41.
4. Senkardes S, Guniz Kucukguzel. Development and validation of a reversed-phase HPLC method for the determination of lisinopril and gliclazide in pharmaceuticals, *Marmara Pharm J*, 21(2), 2017, 338-444.
5. Kashyap T, Honey K, Priyanka V, Mihir J. Novel RP-HPLC method for simultaneous determination of dapagliflozin and teneligliptin in tablet formulation and identification of degradation products by LC-MS/MS, *Futur J Pharm Sci*, 10(1), 2024, 154.
6. Hoffmann A, Zollinger M. Reversed-phase HPLC based assay for selective and sensitive endotoxin quantification - Part II, *J Chromatogr A*, 1717, 2024, 464657.
7. Dabrowska M, Hubicka U. Identification and determination of gliclazide and its impurities in various medicines by thin-layer chromatography and densitometry, *JPC – Journal of Planar Chromatography – Modern TLC*, 14(3), 2001, 183-187.
8. Ohtsuki T, Huang Y, Kamiya A, *et al*. Development of an HPLC method using relative molar sensitivity for the measurement of blood concentrations of nine pharmaceutical compounds, *J Pharm Health Care Sci*, 10(1), 2024, 35.
9. Pop C E, Miu B A, Németh D, *et al*. Bisphenol A analysis and quantification inconsistencies via HPLC-UV: A systematic review with technical notes, *Discover Applied Sciences*, 6(171), 2024, 1-19.
10. Al-Hakkani M F, Hassan M H A. Rapidly, sensitive quantitative assessment of thiopental via forced stability indicating validated RP-HPLC method and its in-use stability activities, *Sci Rep*, 13, 2023, 10294.

11. Gedawy A, Al-Salami H, Dass C R. Development and validation of a new analytical HPLC method for simultaneous determination of the anti-diabetic drugs, metformin and gliclazide, *J Food Drug Anal*, 27(1), 2019, 315-322.
12. Shahare S H. Analytical method development and validation of gliclazide using RP-HPLC from pharmaceutical dosage form, *Biol Forum-An Inter Jour*, 15(5), 2023, 786-792.
13. Kumar Shisodia V, Kumar N, Kumar B, *et al*. Method development and validation of gliclazide for different pharmaceutical formulation and its network, *Pharmacology Study*, 44(5), 2023, 1415-1425.
14. Bolla A S, Priefer R. Blood glucose monitoring- an overview of current and future non-invasive devices, *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 14(5), 2020, 739-751.
15. Reddy S P, Chandra J S, Yoganand K S, *et al*. Optimization and validation of RP-HPLC stability indicating method for determination of gliclazide in pure and bulk drug, 2022.
16. Hanwate R, Chavan S. Method development and validation for estimation of Gliclazide in bulk and tablet form by UV Spectrophotometer, *Journal of Drug Delivery and Therapeutics*, 14(7), 2024, 6-9.
17. Singh H P, Vashistha K. Stability indicating analytical method development and validation for assay of gliclazide in tablet dosage form by using reverse phase high performance liquid chromatography, *The Pharmaceutical and Chemical Journal*, 11(2), 2024, 110-120.
18. Patidar V, Nagle S. Analytical method development and optimization on high performance liquid chromatography for related substances test of gliclazide drug as active pharmaceutical ingredient, *International Journal of Pharmaceutical Sciences and Medicine*, 9(5), 2024, 91-113.
19. Bhattacharya K, Mathew J. Development and validation of stability-indicating UPLC method for the determination of gliclazide and its impurities in pharmaceutical dosage forms, *Futur J Pharm Sci*, 7, 2021.
20. Rao P V, Babu S M. Development and validation of RP-HPLC method for simultaneous estimation of gliclazide and metformin in pure and tablet dosage form, *J Chromatogr Sci*, 52(9), 2014, 1038-1042.
21. Al-Shami N, Naseef H, Moqadi R, Kanaze F. HPLC method development and validation for the determination of apixaban and clopidogrel in novel fixed-dose combination tablets, *J Chem*, 2024.
22. Pop C E, Miu B A, Németh D, *et al*. Bisphenol A analysis and quantification inconsistencies via HPLC-UV: A systematic review with technical notes, *Discover Applied Sciences*, 6, 2024.
23. Kumar B V V R, Patnaik A K, Raul S K, Rao N N. A RP-HPLC method development and validation for the estimation of gliclazide in bulk and pharmaceutical dosage forms, *J Appl Pharm Sci*, 3(4), 2013, 059-062.
24. Hoffmann A, Zollinger M, Pacios K, *et al*. Reversed-phase HPLC based assay for selective and sensitive endotoxin quantification - part II, *J Chromatogr A*, 1717, 2024, 464657.
25. Ramadan H S, Belal F, Roshdy A, Salim M M. Deduction of full factorial design of HPLC technique for the simultaneous analysis of meloxicam and esomeprazole in their laboratory prepared tablets, *Sci Rep*, 15(1), 2025, 12922.

Please cite this article in press as: Meena Yadav *et al*. Analytical method development and validation using HPLC for routine quality control of Gliclazide in pharmaceutical preparations, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 13(1), 2025, 11-20.