Biological Forum – An International Journal **15(5): 786-792(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Analytical Method Development and Validation of Gliclazide using RP-HPLC from Pharmaceutical Dosage Form

Shweta H. Shahare[1]*, Dipti G. Phadtare[1], Hitesh V. Shahare[2], Yunus N. Ansari[2], Bhavesh B. Amrute[2], Sagar S. Vidhate[2] and Sachin N. Kapse[3]

[1]Department of Pharmaceutics,
KCTs, R.G. Sapkal Institute of Pharmacy, Nashik (Maharashtra), India.
[2]Department of Pharmaceutical Chemistry,
SNJBs Shriman Sureshdada Jain College of Pharmacy (Maharashtra), India.
[3]Department of Pharmaceutical Chemistry,
Matoshri College of Pharmacy, Eklahare, Nashik (Maharashtra), India.

(Corresponding author: Shweta H. Shahare)*
(Received: 08 March 2023; Revised: 18 April 2023; Accepted: 21 April 2023; Published: 20 May 2023)
(Published by Research Trend)

ABSTRACT: Elevated sugar levels brought on by a lack of insulin are a defining feature of diabetes mellitus. The sulfonylurea class of insulin secretagogues, which includes gliclazide, is a second-generation hypoglycemic drug that works by boosting insulin secretion from beta cells in the pancreatic Islet of Langerhans. Developing a selective method for accurate measurement in pharmaceutical dosage forms is challenging due to potential interference from excipients. This study gives valuable insights into optimizing chromatographic parameters, including mobile phase composition, and pH, to enhance the separation, resolution, and peak symmetry of Gliclazide. The procedure was designed utilizing a 4.6 mm × 250 mm, 5 Hypersil OSD C18 column at 25 °C, 1.0 mL/min flow rate, 20 L volume, and a run time of 15 minutes at a wavelength of 228 nm with phosphate buffer: Acetonitrile (10:90 v/v) maintained at pH3 as the mobile phase. System appropriateness, specificity, precision (system and technique), accuracy, linearity, ruggedness, robustness, the limit of detection (LOD), and the limit of quantitation (LOQ) were all evaluated for the developed method. The validated approach can be utilized for the regular analysis of Gliclazide from pharmaceutical dosage forms because it was discovered to be quick, accurate, sensitive, and precise.

Keywords: Gliclazide, Diabetes, Insulin, Validation, RP-HPLC.

INTRODUCTION

Diabetes, often known as diabetes mellitus, is a metabolic condition marked by elevated blood sugar levels. The hormone Insulin regulates the body's blood sugar levels, however, under some conditions, the body's ability to produce or use insulin results in increased sugar levels. Gliclazide (1-(3-azabicyclo [3.3.0] oct- 3-yl)) - 1-(hexahydrocyclopenta [c]pyrrol-2 (1H)-yl) or 3-olylsulfonylurea Three (p-tolylsulfonyl) Type-II diabetes mellitus is treated with urea, an oral hypoglycemic medication (Gilroy *et al.,* 2015; Kumar *et al.,* 2013). It is a second-generation hypoglycemic medication that is a member of the sulfonylurea class of insulin secretagogues. These drugs function by boosting the production of insulin from the beta cells in the pancreatic Islet of Langerhans. Additionally, it raises the peripheral body's sensitivity to insulin. By potentiating insulin release, it improves insulin dynamics as a whole (Nazief *et al.,* 2020). It is heavily metabolized, and only 4% of the total drug clearance comes from renal clearance. An azabicyclo-octyl group in the molecule confers distinct properties on the basic sulphonylurea moiety (Yang *et al.,* 2018). Additionally, there is a reduction in hepatic glucose production and an increase in glucose clearance without any changes to the insulin receptors. This suggests that insulin action may be affected post-receptor by the activation of muscle glycogen synthase and hepatic fructose-2,6-bisphosphatasemoiety (Schernthaner *et al.,* 2003). While reducing platelet adhesion, aggregation, and hyperactivity, gliclazide enhances fibrinolysis. These benefits, which are thought to be independent of gliclazide's hypoglycemic efficacy, may help avoid the onset of diabetic microangiopathy. Gliclazide undergoes significant liver metabolism, and its byproducts are excreted in both urine and feces (Yngen *et al.,* 2005).

In India, gliclazide 30, 60, and 80 mg tablets are marketed as AZUKON MR, CLAZ OD tab, and CLAZIN tab, while DIAMICRON is the brand name used in the USA. Diabetic patients frequently take gliclazide tablets to regulate their blood sugar levels. New analytical techniques for isolating Gliclazide from pharmaceutical dosage forms are urgently required given the widespread use and significance of this dosage form in clinical research. A thorough literature search revealed that numerous HPLC procedures have been discussed in the literature for Gliclazide and the combination of

Gliclazide with other medications, either in pharmaceutical goods or biological samples (Soni *et al.*, 2012; Gedawy *et al.*, 2019; Raul *et al.*, 2016). Similarly, to this, analytical techniques utilizing UV spectroscopy are also mentioned in the literature for the estimation of gliclazide (Dadhania *et al.*, 2011). Despite the existence of these stated methods, a quick, straightforward, repeatable approach for the estimate of gliclazide from pharmaceutical dosage forms still has to be developed. To produce the most precise and accurate approach for developing and validating Gliclazide utilizing the RP HPLC technology, the current inquiry was carried out (Kumar *et al.*, 2012).

MATERIAL AND METHODS

Chemicals and Reagents. Dr. Reddy's Laboratories in Hyderabad, India provided gift samples of the working standard and pure Gliclazide. The CLAZ OD tablets (Franco Indian Pharmaceuticals PVT Ltd) were bought from a neighborhood pharmacy. Other analytical reagents were employed for an analytical grade, including acetonitrile (HPLC grade), potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate, methanol (HPLC grade), and orthophosphoric acid. Merck (India) provided the high-purity Milli-Q water that was purchased. All of the other compounds were AR grade.

HPLC Method Development:

Chromatographic conditions and instrument. HPLC [Schimadzu Model LC-2030 PLUS (IND)] with a PDA detector made up of a Thermo - Hypersil ODS, C18, 4.6 mm x 250 mm, 5 m column was used to develop and validate the analytical technique. The autosampler and column temperatures were kept at 10°C and 25°C, respectively. With mobile phase (gradient mode) consisting of phosphate buffer: acetonitrile (10:90 v/v) maintained at pH 3, the flow rate of 1.0 mL/min, injection volume of 20 L with a run time of 15 minutes, and wavelength of 228 nm were optimized. Table 1 displays the various mobile stages and tried run times. Other tools utilized in the validation, such as the pH meter from Mettler Toledo, the ultra sonicator from PCI-Analytics, and the electronic balance from Mettler Toledo, were calibrated.

Preparation of buffer solution. In 550 ml of the distilled water, 0.299 g of disodium hydrogen phosphate and 1.625 g of potassium dihydrogen phosphate were weighed, added, and continuously stirred until they completely dissolved. A 0.45 m millipore membrane filter was used to filter the buffer solution.

Preparation of mobile phase. As the mobile phase, diluent, and blank solution, the buffer and acetonitrile solution was filtered and degassed in the proportion of (10:90% v/v). Ortho-phosphoric acid was added to the mobile phase to bring the pH level down to 3.

Preparation of stock and standard solutions. In a 100 mL calibrated volumetric flask, 10 mg of Gliclazide that had been precisely weighed was dissolved in adequate amounts of the mobile phase. To dissolve the medication, the solution was sonicated for 10 minutes. The final volume of 100 mL was then adjusted by adding the mobile phase. The resulting solution, which contained 100 g/mL, was regarded as a stock solution. To obtain a final concentration ranging from 10 to 70 g/mL, 0.1, 0.2, 0.3, 0.4, and 0.5, 0.6, and 0.7 mL solutions were transferred from this stock solution into a 10 ml volumetric flask.

Preparation of sample solution. About 20 undamaged Gliclazide tablets were carefully weighed, crushed, and thoroughly combined. Accurately weighed powder corresponding to 10 mg of Gliclazide was put into a 100 mL volumetric flask, and sufficient diluents were then added. Using the same diluents, the solution was sonicated for 20 minutes before being diluted to 100 mL. To achieve the appropriate Gliclazide concentration, additional dilutions were prepared using this solution, which was used as a stock solution.

Method Validation. According to ICH guidelines, the developed analytical method underwent tests for system appropriateness, specificity, precision (system and method), accuracy, linearity, ruggedness, the limit of detection (LOD), and limit of quantitation (LOQ). (Yang *et al.*, 2018).

System suitability study. Two separate HPLC systems were used in the research of system-to-system variability, which was carried out under comparable circumstances at various times. Five samples were created, and each one underwent the prescribed analysis. For this test, the chromatographic criteria, such as the trailing factor, retention duration, theoretical plate, and %RSD, were taken into consideration. These chosen parameters were applied to the technique to verify repeatability and resolution. The system suitability study's acceptance criteria were chosen based on the list below.

The percentage RSD for the primary peak retention periods from five replicate injections of each standard solution should not be more than 1.0%. The RSD should not exceed 2.0% for the peak area responses of the primary peak from 5 duplicate injections of each standard Solution. The tailing factor (T) for the Gliclazide peaks should be NMT 2.0, and the number of theoretical plates (N) for the Gliclazide peaks should not be less than 2000.

Linearity. From the stock solution, solutions of 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mL were placed into 10 ml volumetric flasks, diluted with mobile phase to obtain a final concentration ranging from 20 to 70 g/mL, and then injected (20 L) in triplicate at a flow rate of 1.0 mL/min for 15 minutes. At a wavelength of 228 nanometers, the samples were examined. Between the obtained peak area and concentration, the calibration curve was plotted. The y-intercept percentage should be±2.0 and the correlation coefficient must be at least 0.9990.

Precision. Precision was carried out to confirm the method's repeatability. The precision of the system and approach was evaluated.

System Precision. As per procedure, five injections of the Gliclazide standard solution were given into the body. Peak area RSD should be less than 2.0%.

Method precision. Six separate injections of the Gliclazide standard solution were made using a single according to the test technique. Gliclazide should assay at a minimum of 90.0% and a maximum of 110.0%.

Specificity. By injecting reference and test solutions of the Gliclazide, the specificity of the analytical method

was evaluated. The chromatogram of the samples was examined for interference peaks. Standard and test chromatograms should have nearly comparable retention times.

LOD/ LOQ. The lowest concentration that the system can detect was determined to be the limit of detection, and the lowest amount that can be precisely and reliably quantified was determined to be the limit of quantification (LOQ). LOD and LOQ were determined from the linearity data using the following formula.

$LOD=3.3\sigma/S$

σ = standard deviation of the response

S=slope of the calibration curve of the analyte.

$LOQ=10\sigma/S$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

Accuracy (recovery). According to the test method, the assay was carried out in triplicate using an equivalent concentration of gliclazide at 50%, 100%, and 150% of the labeled amount. According to their strengths, the accuracy was assessed against the conventional answer. Gliclazide's typical recovery rate was calculated. The mean percentage of Gliclazide recovery at each spike level should be between 90 and 110 percent.

Robustness. Intentional flow rate fluctuation served as proof of the developed method's robustness. At flow rates of 0.8 mL/min, 1.0 mL/min, and 1.2 mL/min, the standard solution was prepared in accordance with the test method and added to the HPLC system. The parameters for system appropriateness were assessed. Gliclazide standards should have a tailing factor of NMT 2.0 for variation ingress.

Ruggedness. A study on system-to-system variability was done on various HPLC systems, under comparable circumstances, at various dates. Each of the six samples was produced and examined using the test technique. Gliclazide's percentage relative standard deviation throughout the six sample preparations shouldn't exceed 2.0%. Gliclazide's test should range from 90.0% to 110.0%.

RESULT AND DISCUSSION

Method development and optimized chromatographic conditions. The choice of stationary phase is a very important step in the creation of analytical methods, and it mostly depends on the molecular weight and solubility of the molecule. Based on prior research suggesting that reverse-phase chromatography would be the most effective method for studying gliclazide, the C18 column was chosen for this investigation. To obtain the drug's symmetric peak with a shorter run time, several concentrations of the buffer and acetonitrile were researched and optimized.

Acetonitrile. Buffer at a ratio of 90:10 (v/v) produced excellent symmetrical peaks with proper separation and resolution. Gliclazide's retention period was measured at 9.534 minutes. Table 2 lists the different mobility phases that were explored along with observations. Table 3 lists the ideal chromatographic circumstances. Fig. 1 displays the chromatograms of the standard and test solutions.

System Suitability. Five replicate injections into the HPLC system produced findings that were seen to be within acceptable bounds. With 0.03% RSD (accepted threshold NMT 1%) and being found to be properly retained and separated at 9.530 min, the gliclazide showed good repeatability of the replicate injections. The trailing factor, which satisfied the acceptance criterion of NMT 2%, was found to be 0.907, indicating excellent peak symmetry. Additionally, it was discovered that the number of theoretical plates was greater than 6000 (approval criteria NLT 2000), demonstrating high column efficiency. Additionally, the major peak's peak area responses were discovered to be 3351663 with 0.02% RSD (accepted criteria NMT 2%). Data from the System Suitability Study are shown in Table 4.

Linearity. Between the concentration range of 20 to 70 g/mL, the Gliclazide calibration curve was shown to be linear. Fig. 2 presents the graphic representation. The regression coefficient (R2) was found to be 0.999, with an acceptable limit of 0.999. Table 5 displays the concentration range and its area response.

Specificity. Both the reference and test solutions' Gliclazide retention times showed no interference. It was discovered that the retention times of the standard and test solutions were identical and satisfied the specificity test's acceptance requirements. The retention times of the test and standard solutions were 9.528 and 9.527 minutes, respectively.

LOD/ LOQ. Gliclazide's LOD was determined to be 0.029997 g/mL, while its LOQ was determined to be 0.090900 g/mL. The developed method's goal is to quantify gliclazide, hence these values should be viewed as the limit of the method's sensitivity. The method demonstrated high LOD and LOQ sensitivity.

Robustness. By changing the flow rate from 0.8 mL to 1.2 mL, the robustness of the approach was investigated, and it was found to be within acceptable bounds. All other peaks were separated from gliclazide, and their retention durations were similar to those reported for mobile phase flow rates of 1.0 ml/min. No substantial changes were produced when the chromatographic conditions were slightly altered, showing that the approach is resistant to tiny, purposeful variations in flow rate. Table 6 presents the outcomes. Less than 1% RSD was discovered, which demonstrated the method's robustness.

Ruggedness. Comparing the outcomes from the two separate HPLC systems demonstrates how robust the assay test procedure is against system-to-system variability. In terms of % relative standard deviation, ruggedness was also expressed, and statistical analysis revealed no appreciable differences between outcomes from the two alternative approaches. The percent assay of Gliclazide was determined to be 100 and 100.4% (Acceptance criteria: between 90.0% - 110.0%), while the percent relative standard deviation of Gliclazide from the six sample preparations was found to be 0.03% (Acceptance criteria: NMT 2.0%). Table 7 displays information from two different systems.

Accuracy (Recovery). The proposed method's validity and accuracy were demonstrated by how well the measured values of the analyte matched the claimed theoretical concentrations at various levels. More than 99% of the gliclazide was recovered from the spiked excipients (acceptance criteria: 90.0% and not more than

110.0%). The Gliclazide recovery results are shown in Table 8.

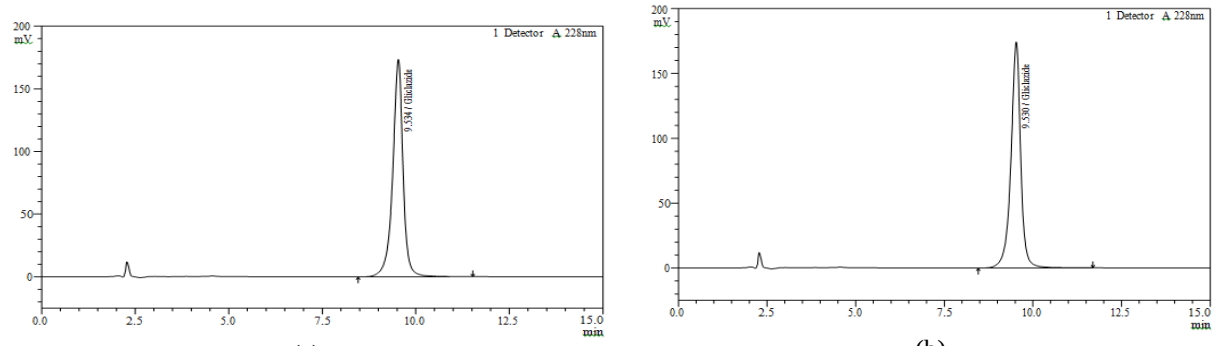
Precision. After six injections of the Gliclazide standard solution, it was discovered that the peak areas and retention times were repeatable with 0.00% and 0.01% RSD, respectively (acceptance criteria NMT 2%).

These observations demonstrated the remarkable system repeatability and precision. Table 9 displays the system precision repeatability data.

Six samples were independently injected to test the method's accuracy, and it was discovered that the peak area and Gliclazide assay both fell within the permitted range. Gliclazide's assay resulted in a result of 100.14% (acceptance criteria: between 90.0to 110.0%), demonstrating a good level of method precision and repeatability. Table 10 displays the procedure precision repeatability statistics.

Table 1: Mobile phases tried at a different time interval.

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



(a) (b)

Fig. 1. Chromatogram of Gliclazide(a) standard and (b) test solution.

Table 2: Composition of mobile phases and observations.

Sr. No.	Acetonitrile %	Phosphate Buffer %	Observation
1.	50	50	The peak was splited & 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 3: Optimized chromatographic conditions.

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

Table 4: Data of system suitability study.

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	…	…

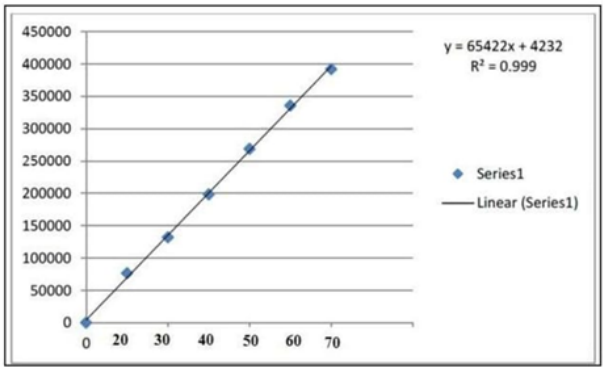


Fig. 2. Linearity of Gliclazide.

Table 5: The concentration range and area response of Gliclazide.

Concentration (ppm)	Area	Statistical Analysis
0	0	Linearity Equation=Y=65422x–4232 Correlation Coefficient (R^2) =0.999
20	76425	
30	131952	
40	198503	
50	268753	
60	335704	
70	392155	

Table 6: Data for Effect of variation in flow rate.

	Std Area	Tailing factor		Std Area	Tailing factor		Std Area	Tailing factor
Flow 0.8 ml	2682319	0.943	Flow 1.0 ml	3351542	0.906	Flow 1.2 ml	4023479	1.002
	2684236	0.944		3352145	0.906		4026485	1.004
	2685264	0.945		3354682	0.906		4024586	1.005
	2682456	0.943		3352564	0.906		4026482	1.002
	2683541	0.944		3353148	0.907		4023568	1.002
Avg	2683563	0.944	Avg	3352816	0.906	Avg	4024920	1.003
SD	1236.911	0.001	SD	1196.767	0.000	SD	1492.063	0.001
%RSD	0.05	0.09	%RSD	0.04	0.05	%RSD	0.04	0.14

Table 7: Data of peak area and % assay from system 1 and system 2.

		System 1		System 2	
	Injection	Peak area	%Assay	Peak area	%Assay
50 ppm	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	728862	100.06	3359482	100.21
Statistical Analysis	**Mean**	**758424**	**100.00**	**3357040**	**100.14**
	SD	**233.539**	**-**	**3061.539**	**0.091**
	%RSD	**0.03**	**0.03**	**0.09**	**0.09**

Table 8: Recovery data of the Gliclazide.

Concentration (%) spiked level	Area	Amount Added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery (Mean)
50% Sample1	5052345	300	301	100.33	99.97%
50% Sample2	5031196	300	300	99.91	
50% Sample3	5018775	300	299	99.67	
100% Sample1	6747650	600	603	100.50	100.71%
100% Sample2	6781221	600	606	101.00	
100% Sample3	6756043	600	604	100.63	
150% Sample1	8392600	900	900	100.00	100.07%
150% Sample2	8395957	900	900	100.04	
150% Sample3	8406028	900	901	100.16	

Table 9: Data of Repeatability (System precision).

Concentration	Injection	RT	Peak area
60 ppm	1	9.528	3352425
	2	9.530	3352187
	3	9.528	3352441
	4	9.528	3352127
	5	9.528	3352247
Statistical Analysis	**Mean**	**9.528**	**3352285**
	SD	**0.001**	**141.375**
	%RSD	**0.01**	**0.00**

Table 10: Data of Repeatability (Method precision).

Concentration	Injection	Peak area of Gliclazide	% Assay
50 ppm	1	3355786	100.10
	2	3353478	100.04
	3	3358248	100.18
	4	3354124	100.05
	5	3361124	100.26
	6	3359482	100.21
Statistical Analysis	**Mean**	**3357040**	**100.14**
	SD	**3061.539**	**0.091**
	%RSD	**0.09**	**0.09**

CONCLUSIONS

In conclusion, the development and validation of a reverse phase RP-HPLC method for the estimation of Gliclazide in pharmaceutical dosage form have been successfully achieved. This method provides a simple and accurate alternative to existing methods. The method demonstrated high accuracy and sensitivity in the estimation of Gliclazide, which is an important type 2 anti-diabetic drug (Sabhyatha *et al.*, 2022). The method developed uses HPLC Schimadzu Model LC-2030 PLUS (IND)with a PDA detector made up of a Thermo - Hypersil ODS, C18, 4.6 mm × 250 mm. This method can be used for the analysis of both Gliclazide in raw materials and finished products. The reverse phase RP-HPLC method developed in this study offers a simple and effective means of estimating Gliclazide in pharmaceutical dosage form with high accuracy and sensitivity. Moreover, this method presents an affordable and less complex alternative to traditional methods. Furthermore, the method developed can be used for the analysis of both Gliclazide in raw materials and finished products.

FUTURE SCOPE

The newly developed method will be useful and suitable for estimation of the Gliclazide drug in a pharmaceutical dosage form.

Acknowledgment. We authors are thankful to respective College of Pharmacy for providing research facilities towards research work.
Conflict of Interest. None.

REFERENCES

Dadhania, K. P., Nadpara, P. A. and Agrawal, Y.K. (2011). Development and validation of spectrophotometric method for simultaneous estimation of gliclazide and metformin hydrochloride in bulk and tablet dosage form by simultaneous equation method. *Int J Pharm Sci Res.*, *2*(6), 1559-1563.

Gedawy, A., Al-Salami, H. and Dass, C. R. (2019). Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, Metformin and Gliclazide. *J Food Drug Anal.*, *27*(1), 315-22.

Gilroy, C. A., Luginbuhl, K. M. and Chilkoti, A. (2015). Controlled release of biologics for the treatment of type 2 diabetes. *J Control Release*, *240*(28), 151-164.

International Conference on Harmonization, ICH Guidelines, Validation of analytical procedures technical requirements for registration of pharmaceuticals for human use: Text and Methodology Q2 (R1), International Conference on Harmonization: Geneva, Switzerland. (2005)

Kumar, B. V., Patnaik, A. K., Raul, S. K. and Rao, N. N. (2013). A RP-HPLC Method Development and Validation for the Estimation of Gliclazide in bulk and Pharmaceutical Dosage Forms. *J Appl Pharm Sci.*, *3*(4), 59-62.

Kumar, S. D. and Kumar, D. H. (2012). Importance of RP-HPLC in analytical method development: a review. *Int. J. Pharm Sci Res*., *3*(12), 4626-33.

Nazief, A. M., Hassaan, P. S., Khalifa, H. M., Sokar, M. S. and El-Kamel, A. H. (2020). Lipid-based gliclazide nanoparticles for treatment of diabetes: formulation, pharmacokinetics, pharmacodynamics and subacute toxicity study. *Int J Nanomedicine*, 1129-1148.

Raul, S. K., Spandana, B., Sameera, P. and Vikitha, V. (2016). UV Spectrophotometric Method Development and Validation for the Estimation of Gliclazide in Bulk and Pharmaceutical Dosage Form. *Asian J. Pharm. Anal.*, *6*(3), 143-146.

Sabhyatha, and Babu, N. (2022). Analytical method validation of gliclazide related substances by high performance liquid chromatography method. *International Journal of Current Pharmaceutical Research*, 36–41.

Schernthaner, G. (2003). Gliclazide modified release: a critical review of pharmacodynamic, metabolic, and vasoprotective effects. *Metabolism*, *52*(8), 29-34.

Soni, L. K., Narsinghani, T., & Jain, M. (2012). Development and validation of RP-HPLC method for simultaneous estimation of metformin hydrochloride and repaglinide in tablet dosage form. *Journal of liquid chromatography & related technologies*, *35*(3), 385-392.

Yang, F., Xiong, X., Liu, Y., Zhang, H., Huang, S., Xiong, Y., Hu, X. and Xia, C. (2018). CYP2C9 and OATP1B1 genetic polymorphisms affect the metabolism and transport of Glimepiride and Gliclazide. *SciRep*, *8*(1), 1-9.

Yngen, M. (2005). Platelet function in diabetes mellitus: Relationships to hyperglycaemia, antidiabetic treatment and microangiopathy. Department of Medicine; Karolinska Uni., Stockholm, Sweden, 2005.

How to cite this article: Shweta H. Shahare, Dipti G. Phadtare, Hitesh V. Shahare, Yunus N. Ansari, Bhavesh B. Amrute, Sagar S. Vidhate and Sachin N. Kapse (2023). Analytical Method Development and Validation of Gliclazide using RP-HPLC from Pharmaceutical Dosage Form. *Biological Forum – An International Journal*, *15*(5): 786-792.