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Development, validation and green assessment of an RP-HPLC method for estimation of gliclazide in bulk and pharmaceutical dosage forms

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Abstract

This article mainly focuses on the development and validation of an easy-to-use, less time-consuming, and sensitive RP-HPLC method for the estimation of Gliclazide present in bulk drug and other pharmaceutical dosage forms. Gliclazide is one of the important second-line sulfonylurea drug candidates most commonly used to treat the Type 2 diabetes mellitus. Chromatographic separation and estimation of Gliclazide are performed by using a Waters C18 column. The mobile phase is Acetonitrile and 0.02 M Disodium Hydrogen Phosphate Buffer in the ratio of 80:20 (v/v), at a flow rate of 0.7 mL/min is used for the separation of Gliclazide. Detection time for Gliclazide is found to be 2.6 minutes. Good separation and a stable baseline indicate that the developed method is reliable and is not influenced by any minor change in chromatographic conditions. The developed chromatographic method complies with ICH guidelines for various parameters such as linearity, accuracy, precision, robustness, sensitivity, and system suitability. Excellent linearity over the concentration range of 10–50 µg/mL with a correlation coefficient of 0.9991. Low %RSD values confirmed the accuracy and precision of the method. By employing various tools such as BAGI, GAPI, AGREEprep, and AGREE metrics, the environmental friendliness of the developed method. Therefore, this RP-HPLC is found to be consistent, cost-effective, and appropriate for routine analysis of Gliclazide in various pharmaceutical formulations.

Keywords: Gliclazide; UV Spectroscopy; Method development; Validation; Estimation

1. Introduction

Gliclazide (GL) is a second-line sulfonylurea drug that is widely used for the treatment of Type 2 Diabetes Mellitus (T2DM). Non-insulin-requiring diabetes mellitus called known as Type 2 diabetes. Gliclazide chemical name is 1-(Hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-[(4-methylphenyl)sulfonyl]urea. Gliclazide is prescribed by a physician as a second-line drug when first-generation anti-diabetic drugs fail to give the expected pharmacological response, i.e., blood sugar level remains high. Gliclazide lowers the blood sugar level by increasing the production of glucose from pancreatic beta cells[1,2]. Pancreatic beta cells, also called the Islet of Langerhans, release insulin into the blood. leading to increased peripheral insulin sensitivity and activity. All these sequential changes resulted in more and more insulin release and enhanced the insulin dynamics.[3] Insulin's half-life is nearly 10 h, and giving 85 to 95% protein binding in plasma[4,5]. Diabetes mellitus is one of the major reasons for fatality across the world. Several antidiabetic drugs are available in the market with different mechanisms of action, and these antidiabetic drugs are extensively used to treat diabetic patients. Gliclazide is one of the commonly used drugs in the treatment of non-insulin-dependent diabetes mellitus. In addition to anti-diabetic activity, gliclazide also has anti-oxidant properties and is used for the protection of damage to blood vessels caused by diabetes. Gliclazide gives double benefits, like reducing the blood sugar level as well as protecting the blood vessels from diabetic complications and damage[6]. Gliclazide is an effective oral hypoglycaemic drug. Gliclazide has several advantages over the other drugs of the same class, such as lower side effects and very few chances of hypoglycaemia i,e it does not reduce the blood sugar level below the normal level, it is also has the advantage

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of long-term use without any potential health risk. Because of gliclazide having its good ability to manage sugar levels, efficiency, and good patient tolerance, it is often considered as a first-line drug in the treatment of Type 2 Diabetes Mellitus (T2DM)[7].

Even considering various advantages of gliclazide it has low water solubility and few clinical complications[8].

Analytical chemistry involves various branches such as chemical analysis, separation techniques, and the use of sophisticated instruments. Analytical chemistry is helpful in identifying constituents present in a drug sample and estimating the quantity with accuracy. Analytical methods have very high importance in the field of pharmaceutical drug manufacturing because they are used in pharmaceutical drug development, QC and QA. Analytical methods also ensure compliance with the various regulatory protocols, leading to safe and effective drug products. A quality drug product manufacturing is an important aim of pharmaceutical analysis. Besides all the different approaches used in analytical chemistry, instrumental methods have great importance. All the separation and detection techniques, like spectroscopic methods, chromatographic separation techniques, and electrochemical analysis, give rapid, delicate, and precise outcomes[9].

Various methods, including capillary electrophoresis (CE) and HPLC methods, are described in the literature for the determination of GL in various biological specimens such as blood, plasma and urine, which is helpful in pharmacokinetic studies of drugs as well as therapeutic drug monitoring that is important to[10–13]. In previous work on Gliclazide, is having shorter retention time and a complex procedure for analysis. In the current method of analysis, conditions are optimized, and a reliable method for the analysis of gliclazide is developed successfully. The developed method is a liquid-liquid extraction procedure it gives high selectivity, that gives this method a suitable choice for accurate quantification in various biological samples, such as blood and serum. Considering the handy nature of this developed method and the repeatability or reproducibility of the results, this method is well-suited for day-to-day analysis.

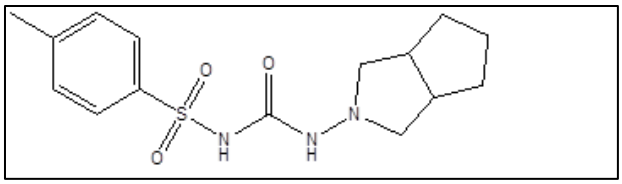

Figure 1 Gliclazide (GL)

2. Materials and method

2.1. Instrumental And Analytical Conditions

For the method development, an RP-HPLC system of Waters Alliance 2696 model equipped with Waters 2996 dual UV-visible absorbance detector is used. Waters Alliance 2696 system equipped with a C18 column with dimensions of 4.6 × 150 mm diameter used for analysis and detection of Gliclazide. The analyte sample is monitored at 228 nm, and different concentrations of the analyte were analyzed. An single mobile phase combination of acetonitrile and 0.02 M disodium hydrogen phosphate in the quantity of 80:20 (v/v) is used for the sample analysis while maintaining the flow rate at 0.7 ml/min for a total run time of 6 min. Before use the mobile phase is passed through a 0.4 µm membrane filter and then sonicated for about 10 minutes to remove any dissolved air to ensure accuracy and reliability of results.

2.2. Reagents And Chemicals

Gliclazide drug was sourced from SWAPNROOP DRUGS & PHARMACEUTICALS (Aurangabad-431154, Maharashtra, India). Tablets were purchased from a local pharmacy, manufactured by Cadila Pharmaceuticals (Glyloc). Ultra-pure water was obtained from a (catalyst) system. HPLC-grade acetonitrile was obtained from DIPA CHEMICAL INDUSTRIES, 42. MIDC CHIKALTHANA, AURANGABAD 431 006 (MS) INDIA. All other chemicals used were A grade. The optimum chromatographic conditions were summarized in table-9.

2.3. Preparation Of Mobile Phase

For the preparation of the mobile phase, disodium hydrogen phosphate (31.2 mg) and 100 mL of HPLC water are mixed. The above solution is subjected to sonication to remove any dissolved air. The mobile phase is prepared by mixing 80 ml of ACN and 20 ml of buffer (0.02M). This mobile phase is again sonicated to remove dissolved air.

2.4. Preparation Of Standard Solution

Accurately weigh 10 mg of Gliclazide and 10 ml of ACN added to a 10 ml volumetric flask. Flask is subjected to sonication for 5 min to ensure complete breakdown of the drug. Volume is made up with the acetonitrile (ACN) to prepare a 1000 µg/ml stock solution. After that, 0.1 ml of prepared stock solution is withdrawn using a 1 mL pipette and added to another 10 ml volumetric flask, filling up to the mark to get a 100 µg/ml stock solution. To make the 20 µg/mL concentration solution again, accurately 0.2 ml volume of the solution is taken from the second stock solution of 100 µg/mL, and pipette out carefully. This final 20 µg/mL solution is further used for analysis.

2.5. Method validation

Method validation is performed to check the suitability of the developed method for its applications and compliance with regulatory guidelines of the International Council for Harmonization (ICH). Various performance parameters are checked while developing the analytical method to ensure the reliability and accuracy of the developed method. Parameters like linearity, precision, accuracy, specificity, robustness, and system suitability are monitored carefully. The limit of detection (LOD) and limit of quantification (LOQ) parameters are employed to assess the sensitivity of the developed method. Further evaluation of this method conformed method is accurate, reliable, and also gives reproducible results in routine drug analysis in the pharmaceutical industry.

2.5.1. Linearity

Different concentrations of Gliclazide solution, like 10, 20, 30, 40, and 50µg/mL, were obtained using the serial dilution method. The carefully prepared test solutions of different concentrations are injected to the HPLC system by setting up the injection volume of 10 µL by maintaining a flow rate of 0.7 mL/min. Eluted compounds are detected at 228 nm wavelength. The chromatograms obtained are shown in Table no: 1. A graph of linearity is obtained by peak area against the five varying volumes of Gliclazide, a calibration curve is obtained. The calibration curve showed an satisfactory linear graph over the concentration range with a correlation coefficient of 0.9991 (Fig.2). The value of the correlation coefficient 0.9991 indicates excellent linearity of the developed analytical method.

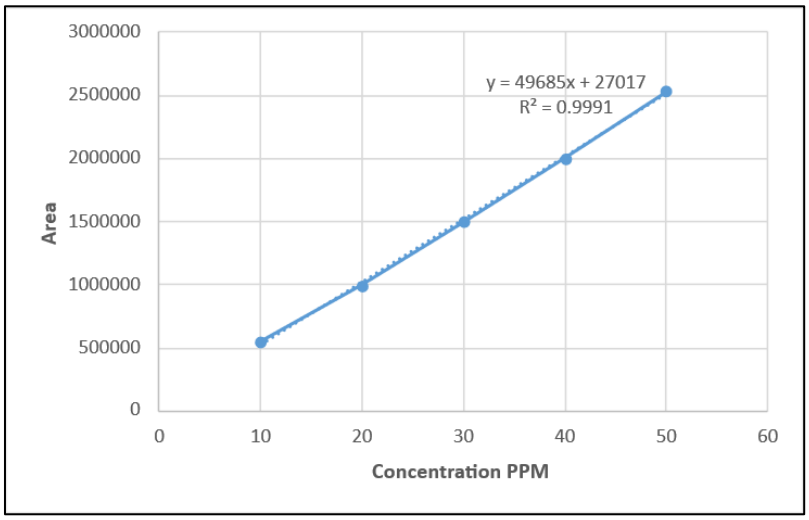

Figure 2 Linearity curve of Gliclazide

Table 1 Linearity parameter for Gliclazide

Conc.(µg/ mL)	Area
10	551982
20	998718
30	1499885
40	2003314
50	2533933

2.5.2. Precision

An intraday precision study is performed by injecting a 10 µg/mL sample of Gliclazide solution six times on the same day to test the repeatability of the present method. The %RSD is determined based on the results obtained, which is 1.155, as shown in Table 2. The low %RSD value, determining the developed analytical method, gives repeatable and consistent results and has good precision and accuracy for routine industrial use.

Table 2 Precision Parameter Of Gliclazide

Repeatability (10ug/ml)			
Injection	**Area**	**Conc Found**	**% Assay**
1	544660	10.29847199	102.98
2	541481	10.23418698	102.34
3	541362	10.23178059	102.32
4	543285	10.27066705	102.71
5	541503	10.23463186	102.35
6	527528	9.952032612	99.52
Mean	539969.8333	10.204	102.036
S.D.	6234.734	0.126	1.261
% RSD	1.155	1.236	1.236

2.5.3. Accuracy

For the accuracy study of Gliclazide; is accurately weighed and mixed with excipients at the three different three volumes namely 80, 100, and 120 µg/mL. Three samples are prepared at each time. Recovery percentage is shown in Table 3.

Table 3 Accuracy Parameter For Gliclazide

Observation Table for Accuracy								
Conc. Taken as 18 ppm (80%)			**Conc. Taken as 20 ppm (100%)**			**Conc. Taken as 22 ppm (120%)**		
Area	**Conc found**	**% Recovery**	**Area**	**Conc found**	**% Recovery**	**Area**	**Conc found**	**% Recovery**
940851	18.31	101.72	1048258	20.48	102.41	1123302	22.00	100.00
947101	18.44	102.43	1052369	20.57	102.83	1125915	22.05	100.24
946161	18.42	102.32	1049147	20.50	102.50	1108907	21.71	98.68
Mean Conc	18.388	102.16	Mean Conc	20.52	102.58	Mean Conc	21.92	99.64
SD of Conc	0.0681	0.3786	SD of Conc	0.0437	0.2187	SD of Conc	0.1852	0.8419
%RSD	0.371	0.371	%RSD	0.213	0.213	%RSD	0.845	0.845

2.5.4. Robustness

Robustness of the developed was evaluated by intentionally varying the two main parameters like the detection wavelength and flow rate. The method is checked by making slight changes in chromatographic parameters such as flow rate and detection wavelength. These slight changes are not reflected in the results of the analysis, as the results obtained by the changed conditions and optimized conditions do not differ significantly. Statistical analysis of these results showed that any variation does not affect or produce any significant difference in the results. Robustness confirms that any minor changes in experimental conditions do not influence results and provide steady and reliable

results. Robustness of the developed method was confirmed by statistical data presented in the following Tables 4,5,6, and 7**.**

Table 4 Robustness parameter for Gliclazide (By using Flow rate 0.6 ml/min)

0.6 ml/Min			
RT	**Area**	**Theoretical Plates**	**Tailing factor**
2.983	675750	2550	1.738
2.995	656771	2561	1.758
3.018	652732	2537	1.774
2.999	661751	2549.33	1.76
0.0178	12290.54	12.014	0.0180
0.5931	1.8573	0.471	1.027

Table 5 Robustness parameter for Gliclazide (Flow rate 0.8 ml/min)

0.8 ml/Min			
	Area	**Theoretical Plates**	**Tailing factor**
2.305	530408	2244	1.742
2.299	534656	2273	1.76
2.309	542568	2143	1.75
2.304	535877	2220.00	1.75
0.0050	6171.32	68.242	0.0090
0.2184	1.1516	3.074	0.515

Table 6 Robustness parameter for Gliclazide (Wavelength 226nm)

226nm			
RT	**Area**	**Theoretical Plates**	**Tailing factor**
2.648	605701	2371	1.75374
2.656	609227	2471	1.742746
2.667	605653	2414	1.741376
2.657	606860	2419	1.75
0.0095	2049.73	50.163	0.0068
0.3590	0.3378	2.074	0.388

Table 7 Robustness parameter for Gliclazide (Wavelength 230nm)

230nm			
RT	**Area**	**Theoretical Plates**	**Tailing factor**
2.685	576079	2448	1.740815
2.674	581360	2430	1.773536
2.683	582927	2391	1.791622
2.681	580122.000	2423.00	1.77
0.006	3587.932	29.138	0.0258
0.219	0.618	1.203	1.456

2.5.5. Ruggedness

For testing the ruggedness of the developed method, inter-day analysis of the gliclazide sample is performed. Six sample injections were given for six consecutive days by different analysts. Ruggedness can also be expressed in terms of percentage relative standard deviation, and the results obtained from ruggedness analysis do not show any major difference between the results are observed by different persons.

2.5.6. Limit of Detection (LOD)And Limit of Quantitation (LOQ)

Standard deviation (σ) and the slope (S) of the calibration curve and formula for LOD = 3.3σ/S and formula for LOQ =10σ/S are used to determine LOD and LOQ. Analysis of Gliclazide for determination of LOD and LOQ values by using relative formulae found to be 0.9304 and 2.8193µg/mL. Table 9.

Table 8 Evaluation data for LOD & LOQ of Gliclazide

Gliclazide	
LOD	0.9304
LOQ	2.8193

Signal-to-noise ratio found during the study shows that the limit of detection (LOD) for Gliclazide is found 0.1 µg/mL and the limit of quantitation (LOQ) is 0.5 µg/mL. LOD and LOQ values show that even a very minute concentration of gliclazide can be detected and measured accurately when the sample is subjected to chromatographic separation. LOD and LOQ values determine the sensitivity of the developed method and rationalize the gliclazide estimation in the sample. Very low values obtained during the method development demonstrate the method is significantly sensitive and best suited for accurate detection of drug concentration even at very low quantities.

2.6. System Suitability Parameter

System suitability tests is performed by preparing a fresh standard solution sample of gliclazide. All the functioning of the chromatography system is checked before running the sample to avoid any instrumental errors during the sample run. All the precautions were ensured by running the test sample six times at an interval of 6 minutes, and the results were recorded. Standard deviation and other system suitability parameters are then calculated from these replicate injections to assess the reliability of the method. System suitability parameters and related values are given in Table 8. These results confirm that the developed method of RP-HPLC is useful in day-to-day testing and analysis of the Gliclazide sample.

Table 9 System Suitability for Gliclazide

Repeatability (10ug/ml)			
Injection	**Area**	**Conc Found**	**% Assay**
1	544660	10.29847199	102.98
2	541481	10.23418698	102.34
3	541362	10.23178059	102.32
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Mean	539969.8333	10.204	102.036
S.D.	6234.734	0.126	1.261
% RSD	1.155	1.236	1.236

3. Results and discussion

Selection of the stationary phase totally depends on what kind of sample we are testing, i.e the nature of the solvent and also depends on the molecular weight and solubility of the drug in the solvent. Separation and detection of Gliclazide is performed by using a C18 column, and results were obtained. The polarity of the mobile phase affects the movement of the drug through the column; because of the polarity hindrance, the mobile phase is optimized carefully to get sharp and symmetric peaks in a very short time period. After analyzing and testing various combinations of mobile phase based on the trial-and-error method, good separation with a sharp peak was obtained by using the solvent mixture of 80:20 (v/v) combination of acetonitrile and buffer. A good retention time of 2.6 minutes was recorded by using optimized conditions. A good peak resolution and a stable baseline show that the method is suitable for analysis.

The developed analytical method is accurate and reliable, as confirmed by the results obtained, such as a percentage relative standard deviation (%RSD) is less than 2%. Proper performance of the chromatographic system is confirmed by system suitability parameters obtained through analysis. As presented in Table 10. A sample test run chromatogram is presented in fig. 3 . Sharpness of the obtained chromatogram indicates that the developed method is useful in daily sample testing in a pharmaceutical drug manufacturing facility.

Table 10 Developed Chromatographic Conditions

Parameters	**Method**
Stationary phase (column)	Waters C18 column (4.6 × 150 mm)
Mobile Phase	Acetonitrile: Phosphate Buffer (80:20 v/v).
Flow rate (mL/min)	0.7 ml/min
Run time (minutes)	6 min
Column temperature (°C)	25 °C
Volume of injection loop (µl)	10 µl
Detection wavelength (nm)	228 nm
Drugs Rtmin)	2.6

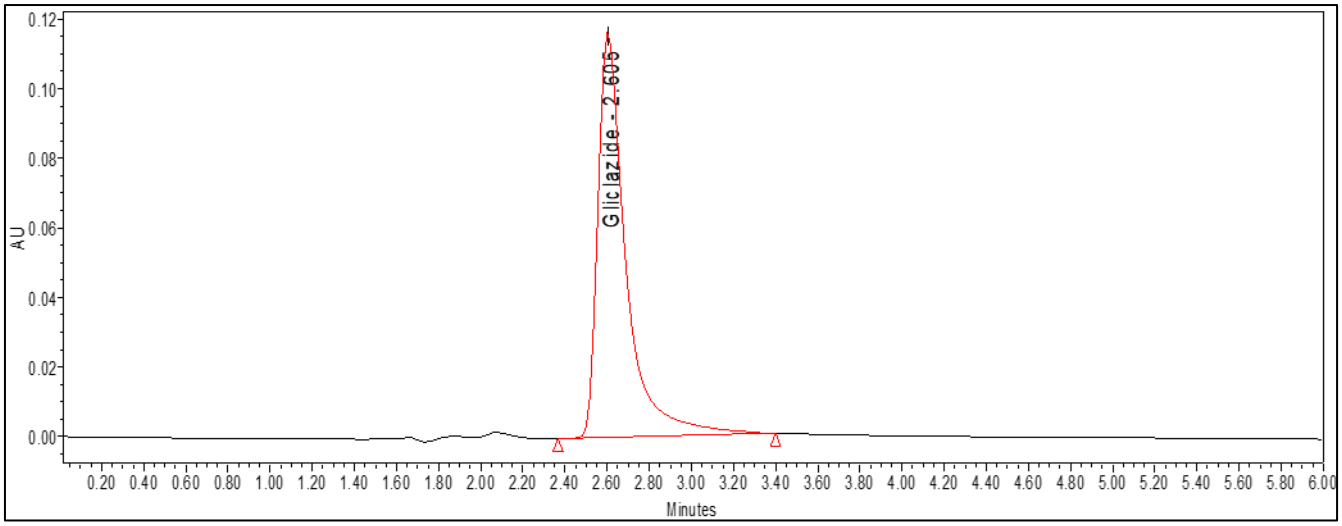


Figure 3 Standard Chromatogram of Gliclazide

3.1. Green assessment

Greenness assessment is a chemical process, analytical technique, or pharmaceutical practice's eco-friendliness testing parameter. Green assessment ensures the very minimal environmental impact. It also avoids the harm caused by hazardous analytical solvents like TFA and other toxic solvents.

3.1.1. Blue Applicability Grade Index (BAGI)

The overall score of 77.5 obtained through the BAGI tool shows that the developed analytical method follows the principles of White Analytical Chemistry (WAC), the method is performed by considering various criteria of WAC. Criteria given by the WAC include the type of analysis used, which sample preparation procedure to be used, and also choice and utilization of various materials and reagents, the quantity of sample required for the drug analysis. BAGI results obtained show that the developed method is having heigh reliability and efficiency as well as compliance with sustainable and environmental friendly analytical practices.

3.1.2. Green Analytical Procedure Index (GAPI)

The GAPI analysis showed eight green sections for reagent and solvent health hazard, safety hazards, instrument energy consumption, and occupational hazards, sample collection, sample preservation, transportation, and storage. The other five test parameters, like waste generation, indicating moderate environmental impact, type of method used, additional sample treatment steps, and the quantity of reagents and solvents required, are marked yellow in the GAPI analysis. Only one waste treatment was marked red. The red-marked parameter tells us that waste management needs to be corrected and resolved. The overall result of the GAPI analysis considers the developed method is environment friendly. The method gave better results as compared to previously reported methods of analysis. Score of 83 from the GAPI metric showing best suitable uniformity with green chemistry guidelines.

3.1.3. Analytical GREEnness Metric testing methodology, particularly for preparation of various kind of samples:

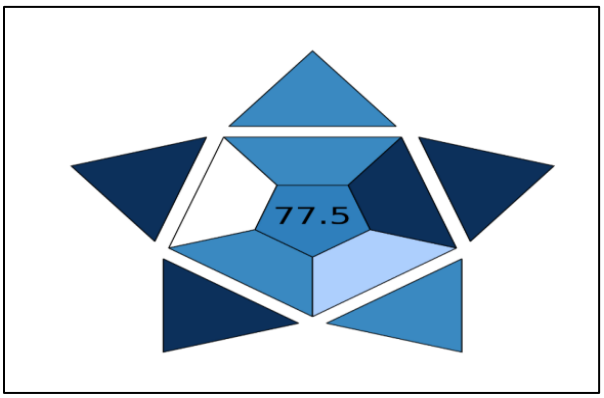
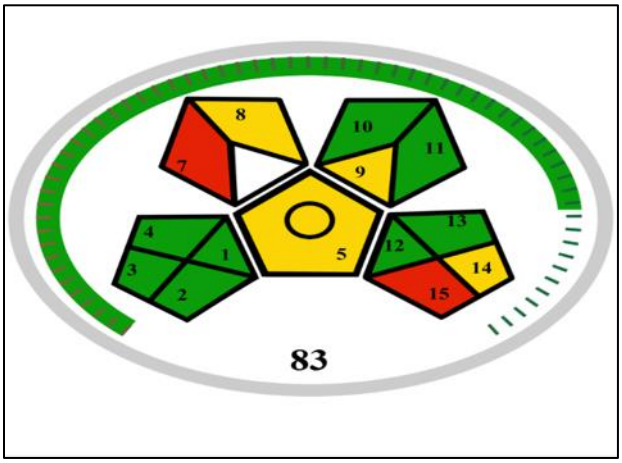
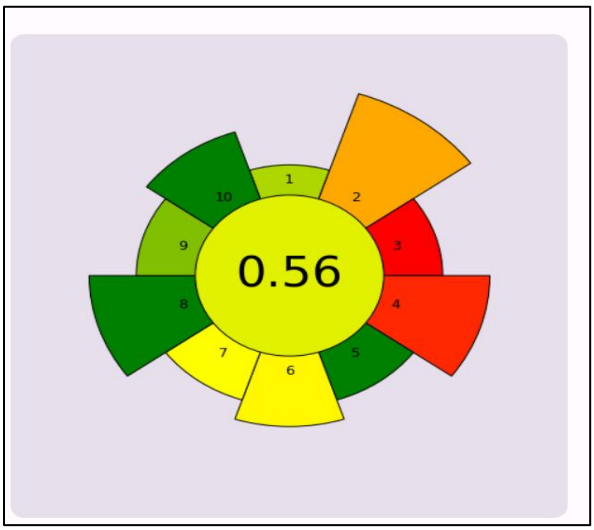
The three dark green sections suggest that the developed method gives the best possible results in terms of sample size, energy consumption, and operator safety. The greener sections indicate that the developed method utilised only a small quantity of sample, required very less energy, and gives a safe working place for laboratory personnel. The light green section refers to sample preparation placement and post-sample preparation configuration, indicating good environmental performance. For waste generation, sample throughput, and integration and automation, moderate scores were generated. The red section shows that use of sustainable, reusable, and renewable materials needs some improvement. The AGREEprep assessment tool gave an overall score of 0.56, showing the developed method is greener and follows majority principle of green analytical chemistry.

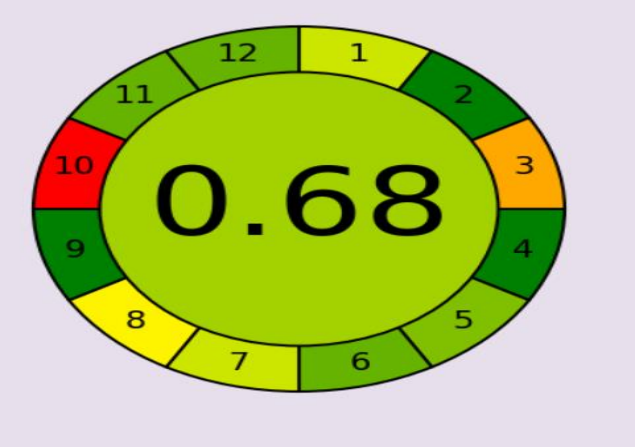
3.1.4. Analytical GREEnness Metric Approach (AGREE)

This evaluation method showed that the various aspects of green chemistry are fulfilled. Sample amount and number of samples, sample preparation steps, derivatization, and energy consumption are represented by green sections that show good environmental performance. Parameters like automation and miniaturization, toxicity, and operator safety

are highlighted with light green colour are also show great compliance with green chemistry principles. Yellow colour ratings are related to sample treatment, waste generation, and analysis throughput, while the orange section represents the device positioning are having moderate environmental impact. The source of reagents is a weak point of this method, and it is also shown by red section. The developed method is eco friendly is confirmed by the score 0.68, and it also confirms that the method follows the principle of green analytical chemistry. AGREE metric showed good compliance with the analysis throughput and toxicity-related parameters.

Table 11 Green assessment results

Name of tool	Pictogram	Score
BAGI		77.5
2) GAPI		83
3) AGREE prep		0.56

4) AGREE		0.68

4. Conclusion

A precise, simple, reliable, and economical chromatographic method for testing of Gliclazide in various pharmaceutical systems is prepared and applied perfectly. Good peak resolution and retention time of 2.6 min confirmed that the developed method has excellent Proportionality, correctness, consistency, robustness, and analytical sensitivity that is comply to ICH rules. A satisfactory low %RSD value showed that the method is consistent and reproducible for routine pharmaceutical analysis of bulk as well as various dosage forms containing Gliclazide. This developed method is successfully applied for analysis of Gliclazide in tablet formulations and produced accurate results. To further assess the eco-friendliness of the method, various tools like BAGI, GAPI, AGREEprep, and AGREE are used, and the results obtained indicate that the method adheres to the principles of green analytical chemistry and does not have any major environmental impact. Hence, this developed method ensures high precision, correctness and analytical reliability; it can be used for day-to-day testing of gliclazide in various pharmaceutical dosage forms

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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