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Review Article

ANALYTICAL METHOD VALIDATION OF GLICLAZIDE RELATED SUBSTANCES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

SABHYATHA T. S.[1*], NARAYANA BABU[1]

*[1]Department of Pharmaceutical Chemistry, M S Ramaiah University of Applied Sciences, Bengaluru 560054, Karnataka, India
Email: gowdasabhyatha@gmail.com

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ABSTRACT

The present study was undertaken with the objective of method validation of a rapid, simple, cost-effective HPLC method for the determination of related substances of Gliclazide. A simple, rapid, and specific method for analysis of gliclazide by a sensitive high-performance liquid chromatographic method is described. Validation of the method is carried out by USP and ICH guidelines. The method was validated for parameters like accuracy, precision, linearity, specificity, robustness, and system suitability. These proposed methods are suitable for the determination of title drugs in quality control laboratories in the pharmaceutical industries. The mobile phase used for the chromatographic runs consisted of (450 ml) of acetonitrile and (550 ml) of water. The separation was achieved on the LiChroCART Supers her RP-8 column (250 mm × 4.0 mm, 5 µm), using isocratic mode. Drug peaks were well separated and were detected by a UV detector at 235 nm, the method was linear at the concentration range. Gliclazide limit of detection (LOD) and limit of quantification (LOQ) was 0.003 and 0.01, while LOD and LOQ for Impurity-F were 0.003 and 0.01, respectively. The presented validated method is rapid, economic, simple, accurate, sensitive, robust, specific, and linear. It can be used for routine analysis of gliclazide.

Keywords: Method validation, Relative standard deviation, System suitability, Limit of detection, Limit of quantitation, Impurity-F, Gliclazide, RS-HPLC

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INTRODUCTION

The Pharmaceutical industry refers to a group of companies that make ethical and over-the-counter medications. And it finds, develops, manufactures, and markets pharmaceuticals or pharmaceutical drugs for use as medications to be given to patients (or self-administered) in order to cure, vaccinate, or relieve symptoms.

- The rising paths of research in the pharmaceutical sectors have resulted in the introduction of unique and competent formulations to the market.

- Some of these dose types are quite potent, while others are contaminated. Because pharmaceutical product quality is so important, such advances necessitated the development of accurate, simple, and responsive chemical analysis procedures. Because they deal with human life, medicines, unlike other consumer goods, cannot and do not have a second quality. Only a department dedicated to quality assurance and quality control can guarantee quality.

- The major goal of a related substance test is to keep contaminants from causing degradation.

High-performance liquid chromatography is a widely utilized analytical technology in the pharmaceutical sector. It's a tool for determining the composition of drug-related materials. The results might be qualitative, indicating which chemicals are present in the sample, or quantitative, indicating the exact levels of compounds in the HPLC.

The technique of high-performance liquid chromatography is so-called because of its improved performance when compared to classical column chromatography. Advances in column technology, high-pressure pumping system and sensitive detectors have transformed liquid column chromatography into a high-speed, efficient, accurate and highly resolved method of separation.

Application of HPLC

- One of the most common applications of HPLC is in the pharmaceutical manufacturing process. HPLC is a precise and accurate method of determining product purity. As a result, it can assist pharmaceutical companies in developing the purest goods possible.

- HPLC lends itself to the examination of nutrients in the blood and other medical samples since it can separate components from mixtures.

- HPLC can also be used to detect drug residues in urine.

- HPLC is frequently used to examine biological samples from persons who already have a diagnosis.

- Pesticides, preservatives, artificial flavourings, and colorants can all be identified and quantified using HPLC.

Analytical method validation

Method validation is defined as (ICH) establishing documented evidence that provides a high degree of assurance that a specific activity will consistently produce a desired result or product that meets its predetermined specifications and quality characteristics.

A method's validation is the process by which a method is tested by the developer or user for its dependability, accuracy, and precision in serving its intended purpose.

The resulting data is included in the methods validation package submitted to CDER.

Methods should be repeatable by other analysts, on similar facilities, on different days or locations, and throughout the drug's life cycle. All parameters that are normally clarified during the validation process are accuracy, precision, specificity, linearity, range, and robustness. A validation report that includes all of the experimental conditions, as well as all of the statistics, should be created.

Steps followed for validation procedures

- Establishment of proposed protocols or parameters for validations
- Conduct of experimental studies
- Evaluation of analytical results

- Statistical evaluation
- Preparation of report

Typical validation characteristics which should be considered are listed below

- Specificity
- Precision
- System precision
- Method precision
- Intermediate precision
- Accuracy
- Linearity
- Range
- Robustness
- Limit of detection
- Limit of quantification

Furthermore, revalidation may be necessary in the following circumstances:

- Changes in the synthesis of the drug substances.
- Changes in the composition of the finished product.
- Changes in the analytical procedure.

The extent of revalidation required is determined by the nature of the changes.

Other changes may also necessitate validation.

The parameters for method validation as defined by the ICH guidelines are summarized below.

Specificity

Specificity is the ability to assess the analyte unequivocally in the presence of components that are expected to be present. Impurities, degradants, matrix, and so on are examples of these.

An individual analytical procedure's lack of specificity may be compensated for by another supporting analytical procedure (s).

Accuracy

The accuracy of an analytical procedure expresses the degree of agreement between the value accepted as a true conventional value or an accepted reference value and the value discovered.

This is sometimes termed trueness.

Precision

The closeness of agreement (degree of scatter) between a set of measurements acquired from multiple sampling of the same homogeneous sample under the stipulated conditions is expressed by the precision of an analytical method condition.

There are three levels of accuracy: repeatability, intermediate precision, and reproducibility.

- Precision should be investigated with true, homogeneous samples. If obtaining a homogeneous sample is not possible, artificially generated samples or a sample solution may be studied.
- The variance, standard deviation, or coefficient of variation of a sequence of data is commonly used to express the precision of an analytical technique.

➢ **Repeatability:** The term "repeatability" refers to the precision of a measurement made under the same operating conditions over a short period of time. Intra-assay precision is another synonym for repeatability.

➢ **Intermediate precision:** Variations in laboratories express intermediate precision: various days, different analyzers, different equipment, and so on.

➢ **Reproducibility:** Reproducibility refers to the consistency of results across laboratories (collaborative studies, usually used to standardize methodology).

Linearity

The capacity of an analytical process to produce test results that are directly proportional to the concentration (quantity) of analyte in the sample (within a specific range) is known as linearity.

Range

The range of an analytical technique is the distance between the sample's upper and lower analyte concentrations (amounts) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Robustness

The robustness of an analytical procedure is a measure of its ability to remain unaffected by modest but deliberate changes in method parameters, and it indicates its reliability in routine use.

Related substances

- A related substance is one that is structurally similar to a drug substance. These substances could be identified or unidentified degradation products or impurities resulting from a manufacturing process or during material storage.
- Related substances can be divided into three categories.

➢ **Process impurities:** These will have resulted from the reactions that were used to create the final substance. These could be organic substances that were used in the reactions or impurities produced during the reactions. These can be both organic and inorganic in nature.

➢ **Residual solvents:** All organic reactions will have used organic solvents as a reaction medium. There may be residual amounts of these left over from the reactions.

➢ **Degradation products:** After the final product is created, it may begin to degrade, resulting in smaller sub-molecules. These are degradation products that would be tracked as part of a stability study.

All of them are classified as "Related Substances." They're all linked to the main complex in some fashion.

Even in small concentrations, impurity has a significant impact on the safety, efficacy, and stability of pharmaceutical products.

Gliclazide

It was first patented in 1996 and received FDA approval for medical use in 1972. It is marketed under the brand name Diamicron and is used to treat type 2 diabetes when dietary changes, exercise, and weight loss are insufficient.

It primarily operates by boosting insulin release

Gliclazide, 1-(4 methylbenzenesulphonyl) 3-(3 azabicylco [3.3.0] octyl) urea (I), is a type II diabetes medication that belongs to the second generation of sulphonylureas. It is used to treat non-insulin-dependent diabetic mellitus (NIDDM)

Gliclazide may be suitable for use in diabetic patients with renal impairment, as well as in older people whose diminished renal function may increase the risk of hypoglycaemia while taking some sulphonylureas due to its short-acting nature.

Adverse effects

- Hypoglycaemia
- Low blood sugar, vomiting, abdominal pain, rash, and liver problems are some of the symptoms. It is not advised to use this

product if you have serious kidney or liver problems or if you are pregnant.

Overdose

Overdosing on gliclazide can result in severe hypoglycaemia, necessitating immediate glucose IV treatment and monitoring.

Molecular structure of gliclazide

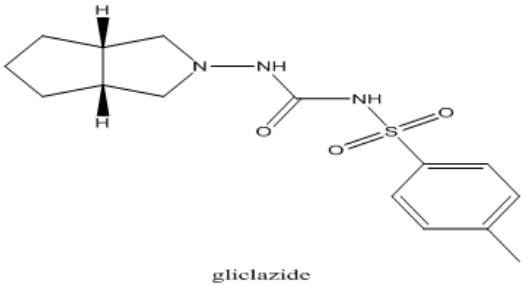

Fig. 1: Structure of gliclazide

Chemical Name: 1-(hexahydrocyclopenta [c] pyrrole-2 (1H)-yl)-3-(4-methylphenyl) sulfonyl urea

Molecular Formula: $C_{15}H_{21}N_3O_3S$

Molecular Weight: 323.4

Physical Form: A white or almost-white powder

Melting Point: 180 ° to 182 °C

PKa: 14.09 (strongest acidic) and 9.67 (strongest basic)

Category: sulfonylurea

Available dosage forms: Injection, Tablet, and Capsule

Route of administration: Oral route

Mechanism of action

Gliclazide binds to the sulfonylurea receptor on-cells (SUR1). The ATP-sensitive potassium channels K are therefore blocked as a result of this interaction (ATP). The binding causes the channels to close, resulting in a reduction in potassium outflow and depolarization of the cells. Calmodulin activation results from the opening of voltage-dependent calcium channels in the cell, which leads to exocytosis of insulin-containing secretory granules.

K (ATP) channels are important in the stimulus–secretion coupling of cells; they are not required for glycaemic regulation.

Sulfonylurea receptor 1 (SUR1) blockers have been revealed to have cardiac ischemia protective effects in recent research.

Insulinotropic activity of sulfonylurea medications is mediated through inhibition of K (ATP) channels in the pancreas. However, these channels are also found in cardiac and vascular smooth muscle, suggesting that they may have negative cardiovascular consequences.

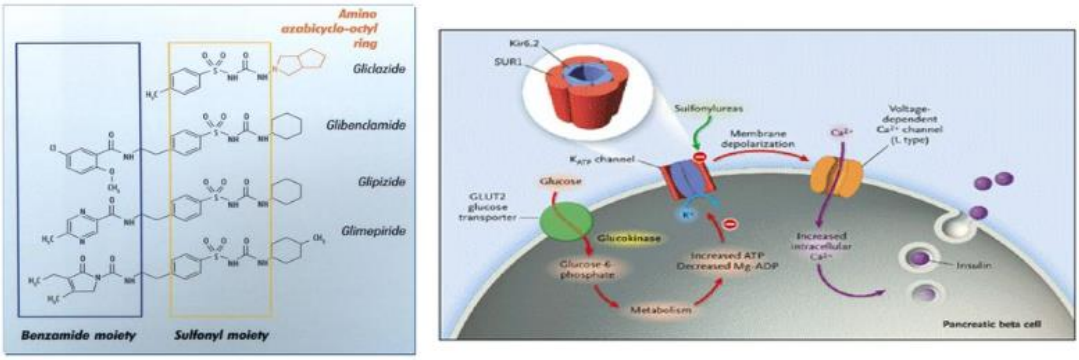

Fig. 2: SUR1 K_{ATP} channels contains two high-affinity binding sites, one accepts sulfonyl moiety and one that accept benzamide moiety, Gliclazide and the non-SU glinides, only binds to sulfonyl moiety

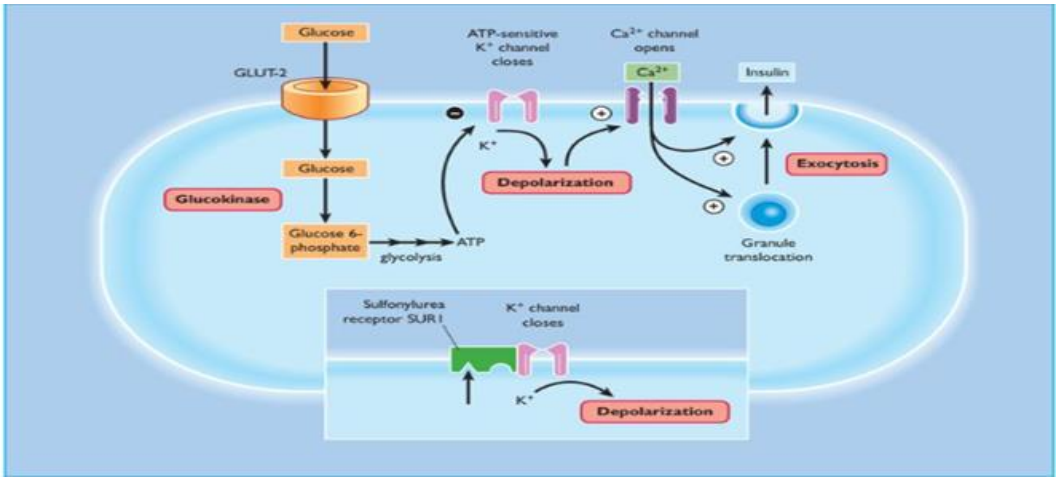

Fig. 3: Sulfonylureas act on the pancreatic insulin secretion. They bind to the cytosolic surface of the sulfonylurea receptor 1 (SUR1), causing closure of ATP-sensitive Kr 6.2 potassium channels, depolarizing the plasma membrane, opening calcium channels, and activating calcium-dependent signalling proteins that control insulin exocytosis

MATERIALS AND METHODS

Instrumentation

HPLC instrument specifications

HPLC: Waters: e2695 with HPLC with PDA detector

Columns: LiChroCART Supersher RP-8

Software: Empower-2 software

Other equipment/instruments

Analytical Balances: Mettler Toledo and XP-205

Ultra micro balance: Mettler Toledo and UMX-2

Chemicals and materials

The present work describes a validated reverse phase RP-HPLC method for the estimation of type 2 anti-diabetic drug Gliclazide in dosage form.

Chromatography was performed on LiChroCART Supersher RP-8 column, (250 mm × 4.0 mm, 5 µm) with the mobile phase composed of water (550 ml) and acetonitrile (450 ml). The flow rate was 0.9 ml/min, the Injection volume was 20 µl, and the DAD/VWD detection is at 235 nm where column temperature was 45˚C and sampler temperature was 5˚C. The retention time of Gliclazide is 11.6 min and the total Elution time was 35 min.

The detector response was found to be linear with regression coefficient (r^2)1.000. The method was validated according to ICH guidelines. The parameters like system suitability, specificity, Matrix interference, system precision, method precision, intermediate precision, accuracy, linearity, range, and robustness were performed for the validation of this method. % RSD for validation parameters for Gliclazide were found to be less than 2%.

Experimental

Method

Instrument: Reverse phase Liquid chromatography with Isocratic elution having PDA detector.

Preparation of blank solution: Filled 50 ml volumetric flask halfway with 23 ml acetonitrile and diluted to volume with water. To combine, give it a good shake.

Table 1: Chemicals/reagents

S. No.	Chemicals/Reagents	Manufacturer	Grade
01	Trifluoroacetic acid	Spectrochem	AR
02	Triethylamine	Merck	AR
03	Acetonitrile	Merck	HPLC
04	Milli-Q water	In-house	N\A

Table 2: Impurity/Standard/Sample

S. No.	Name of the impurity/standard/sample	Purity/Assay
01	Impurity-F standard	99.83
02	Impurity-B standard	99.90
03	Gliclazide Reference standard	99.79
04	Gliclazide sample	99.91

Table 3: Instrument

S. No.	Name of the instrument	Make and model	Instrument ID
01	HPLC with PDA detector	Waters e2695 with Empower-2 software	QC/HLC/099
02	HPLC	Waters e2695 with Empower	QC/HLC/100
03	HPLC	Waters e2695 with Empower	QC/HLC/113
04	Analytical balance	Mettler ToledoandXP-205	QC/ABL/006
05	Ultra micro balance	Mettler ToledoandUMX-2	QC/ABL/007

Table 4: Column

S. No.	Name of the column	Dimensions	Column ID
01	LiChroCART Supersher RP	250 mm × 4.0 mm, 4.0 µm	HLC/COL/144
02	LiChroCART Supersher RP	250 mm × 4.0 mm, 4.0 µm	ML15/ARD/lCC-167

Note: Inject a standard solution, a reference solution, and a sample solution that have all been newly produced.

Preparation of Mobile Phase: To 550 ml of water, add 450 ml of acetonitrile. Then 1 ml of triethylamine and 1 ml of trifluoroacetic acid added. Shake vigorously to combine. To degas, filter the solution and sonicate it.

Preparation of Diluent: In a 45:55 (percent v/v) ratio, combined acetonitrile and water. Shaked well to combine, and then sonicate to degas.

Preparation of standard solution: Weighed approximately 50 mg of Gliclazide reference/working standard accurately and transferred to a 50 ml volumetric flask. Added 23 ml of acetonitrile, dissolve, and diluted with water to volume. Shake vigorously to combine.

Reference solution (a): 1 ml of the standard solution was transferred to a 100 ml volumetric flask and diluted to volume with the solvent mixture. Shake vigorously to combine.

Transferred 5 ml of the above solution to a 50 ml volumetric flask and diluted with the solvent mixture to volume. Shake vigorously to combine.

Reference solution (b): Weighed approximately 5 mg of Gliclazide reference/working standard and 15 mg of Gliclazide impurity-F CRS into a 50 ml volumetric flask.

Add 23 ml of acetonitrile and dilute with water to volume. Shake vigorously to combine.

1 ml of the above solution is transferred to a 20 ml volumetric flask and diluted to volume with the solvent mixture.

Reference solution (c)

Weighed 5 mg of gliclazide impurity-F CRS accurately and transferred to a 50 ml volumetric flask. Added 25 ml of acetonitrile and diluted with water to volume. Shake vigorously to combine.

Fill a 100 ml volumetric flask halfway with the solvent mixture and added 1 ml of the above solution.

Preparation of sample solution

Weighed approximately 50 mg of sample and transferred to a 50 ml volumetric flask. Added 23 ml of acetonitrile and diluted with water to volume. Shake vigorously to combine.

Chromatographic conditions

Stainless steel tubing, a variable wavelength UV detector, an injector, and a data processor are all included in this liquid chromatography system.

Table 5: Chromatographic conditions

Column	LiChroCART Supersher RP-8, (250 mm × 4.0 mm, 5 µm)
Flow rate	0.9 ml/minute
Wavelength	235 ŋm
Injection volume	20 µl
Run time	35 min
Retention time	About 11.6 min
Elution mode	Isocratic

Procedure

Equilibrate the column with mobile phase Inject Blank (Diluent) (2 Injection), Standard solution (5 Injections), Sample solution (1 injection) and Reference solution (b) solution-Bracketing* (1 injection) (Inject intermittent reference solution-b for every three batches) check for the system suitability parameters.

System suitability

- The reference solution (b) form between two sample solution peak as per USP resolution should not be more than 1.8
- The retention time (RT) and relative retention time (RRT) of the impurities are tabulated as below:

Table 6: System suitability

Name of the component	RT (min)	RRT
Impurity-F	~10.8	~0.93
GLU	~11.6	1.00

Calculation

Calculate the content of Impurity-F from reference solution (c) (%w/w) by using the following formula:

$$= \frac{A_1}{A_{S1}} \times \frac{W_{S1}}{50} \times \frac{1}{100} \times \frac{50}{W_T} \times P_1$$

Calculate the Unspecified impurity (%w/w) by using the formula:

$$= \frac{A_{US1}}{A_{S2}} \times \frac{W_{S2}}{50} \times \frac{1}{100} \times \frac{5}{50} \times \frac{50}{W_T} \times P_2$$

Calculate the sum of Impurities other than Impurity-F (% w/w) by using the formula:

$$= \frac{A_{OTI}}{A_{S2}} \times \frac{W_{S2}}{50} \times \frac{1}{100} \times \frac{5}{50} \times \frac{50}{W_T} \times P_2$$

Where,

A_1: Area of Impurity-F peak in the sample solution

A_{S1}: Average area of Impurity-F peak in duplicate injections of reference solution (c)

W_{S1}: Weight of Impurity-F reference/working standard in reference solution (c) (mg)

W_T: Weight of sample in mg

P_1: Purity of Impurity-F reference/working standard

A_{S2}: Average area of Gliclazide peak in duplicate injections of reference solution (a)

W_{S2}: Weight of Impurity-F reference/working standard in reference solution (a) (mg)

A_{USI}: Area of unspecified impurity in the sample solution

A_{OTI}: Area of Gliclazide reference/working standard

P_2: Purity of Gliclazide reference/working standard

Acceptance criteria: Not less than 1.8

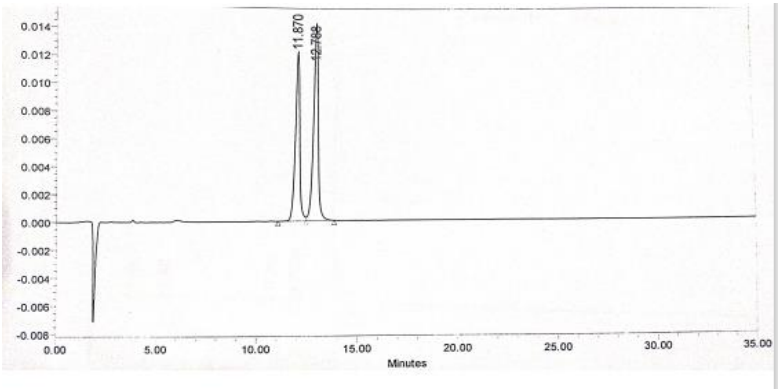

Fig. 4: Typical chromatogram of reference solution (b) in the test for related substances

Table 7: Typical chromatogram of reference solution

	Peak name	RT	Height (µV)	Area	% Area	Int type	RT ratio	USP resolution	USP plate count	USP tailing
1	IMP-F	11.870	11435	191225	44.47	BB	0.93		15474	1.10
2	GLU	12.788	13330	238788	55.53	BB		2.27	15998	1.06
Sum				430012.9						

Table 8: Injection profile

Name of the solution	No. of injection
Blank	2
Reference solution (b)	1
Reference solution (a)	2
Reference solution (c)	2
Sample solution	1
Reference solution (b) solution-Bracketing	1

System suitability

To verify that the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set.

Acceptance criteria

From reference solution (b), resolution between two solvents peaks should not be less than 1.8

Specificity

Specificity is the ability of an analytical method to assess the analyte unequivocally in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components.

Impurity-F standard stock solution: Prepared by using 10 mg of impurity-F reference standard and 45 ml of acetonitrile transferred to 100 ml volumetric flask, diluted with Milli-Q water.

Impurity-F standard solution (0.1%) or reference solution: 1.0 ml of Impurity-F standard stock solution transferred into 100 ml volumetric flask and diluted by using diluent.

Impurity-B standard stock solution: In a 100 ml volumetric flask, weighed around 20 mg of gliclazide impurity-B reference standard, dissolved in 45 ml acetonitrile, and diluted using Milli-Q water.

Fill a 50 ml volumetric flask with 1 ml of this solution and diluted volume with diluent.

Impurity-B standard solution: In a 50 ml volumetric flask, transferred 1 ml of impurity-B standard stock solution, diluted with diluent.

Gliclazide standard stock solution: In a 100 ml volumetric flask, transferred 10 mg of Gliclazide reference standard, 45 ml of acetonitrile and diluted with Milli-Q water.

Gliclazide standard solution (0.1%) reference solution (a): In a 100 ml of volumetric flask transferred 1 ml of Gliclazide standard stock solution, diluted by using diluent.

Spiked solution: Weighed almost 100 mg of Gliclazide as standard transferred in a 100 ml of the volumetric flask, 1 ml of impurity-F standard stock solution and 2 ml of impurity-B standard stock solution was added, 45 ml of acetonitrile was used to dissolve above solution and diluted using Milli-Q water.

Procedure: Equilibrate the column and mobile phase for 30 min. Followed by injection sequence

Table 9: Injection profile

S. No.	Name of the injection	No. of injection
1	Blank	1
2	Impurity-F standard solution	1
3	Impurity-B standard solution	1
4	Gliclazide standard solution	1
5	Spiked solution (Impurities spiked with the main compound)	1

Acceptance criteria

- At the retention durations of the identified contaminants and gliclazide peak in the spiked solution, there should be no interference from the blank.
- For the spiked solution's Impurity-F, Impurity-B, and Gliclazide peaks, the purity threshold should be greater than the purity angle.
- Individual known contaminants and gliclazide retention times should be comparable to the standard retention period with the spiked solution.

Limit of detection (LOD)

The lower concentration of an analyte in a sample that can be reliably detected with a given probability is known as the limit of detection (LOD) (typically at 95 percent certainty)

Note: If any of the impurities aren't detected at the 0.003 percent level, bring them up to the detection level

Procedure: Equilibrate the column and mobile phase for 30 min. Followed by injection sequence

Table 10: Injection profile

S. No.	Name of the injection	No. of injection
1	Blank	1
2	Reference solution-(b)	1
3	Blank	1
4	LOD solutions	3
5	Reference solution-(b)	1

Acceptance criteria

- It must meet the system suitability requirements.
- Raw data of LOD concentration was reported.

Limit of quantitation (loq)

The lowest amount of analyte in a sample that can be quantitatively measured with sufficient precision and accuracy is the quantitation limit of a particular analytical process. The quantitation limit is a parameter of quantitative tests for low quantities of chemicals in sample matrices, and it is used to determine contaminants and/or degradation products in particular.

Prepare a concentration of all contaminants at 0.01 percent or three times the LOD concentration in relation to the test concentration.

Procedure: Equilibrate the column and mobile phase for 30 min. Followed by injection sequence

Note: For the purposes of LOQ accuracy, accuracy level-1 findings will be considered

Table 11: Injection profile

S. No.	Name of the injection	No. of injection
1	Blank	1
2	Reference solution-(b)	1
3	Blank	3
4	LOD solutions	6
5	Reference solution-(b)	1

Acceptance criteria

- It must fulfill the system suitability requirements.
- The %RSD for the results of six replicate injections of known impurities and gliclazide should be less than 10 from LOQ solution.

Linearity and range

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Linearity is usually expressed as the regression line calculated according to an established mathematical relationship from the test

results obtained by analysis of standard solution with varying analyte concentrations.

Prepared the linearity solutions from 50% to 120% of working concentration

The range of analytical method is the interval between the upper and lower levels of analyte that shall be demonstrated to be determined with suitable accuracy and linearity.

Derived the specified range from linearity and accuracy studies.

Linearity standard stock solution: in a 100 ml of volumetric flask transferred accurately weighed 10 mg of impurity-F, 10 mg of gliclazide and dissolved in 45 ml of acetonitrile, diluted using Milli-Q water.

Linearity Level-1 preparation (LOQ): Use the LOQ solution or LOQ results in this manner.

Linearity Level-2 preparation (50%): in the 100 ml of volumetric flask transferred 0.5 ml of linearity standard stock solution, dissolve and make up the volume using diluent.

Linearity Level-3 preparation (80%): in the 100 ml of volumetric flask transferred 0.8 ml of linearity standard stock solution, dissolve and make up the volume using diluent.

Linearity Level-4 preparation (100%): in the 100 ml of volumetric flask transferred 1 ml of linearity standard stock solution, dissolve and make up the volume using diluent.

Linearity Level-5 preparation (120%): in the 100 ml of volumetric flask transferred 1.2 ml of linearity standard stock solution, dissolve and make up the volume using diluent.

Procedure: Equilibrate the column and mobile phase for 30 min. Followed by injection sequence.

Linearity: First two injections Linearity level-1, Linearity level-5

Reporting the range: LOQ six replicate injections, Level-5

Consider the linearity levels from LOQ to linearity level-5 after determining the limit of detection and limit of quantitation from the linearity levels.

Table 12: Injection profile

S. No.	Name of the injection	No. of injection
1	Blank	1
2	Reference solution-(b)	1
3	Blank	3
4	Linearity level-1	6
5	Linearity level-2	2
6	Linearity level-3	2
7	Linearity level-4	2
8	Linearity level-5	6

For each known impurity calculate the relative response factor by using formula:

$$\text{Relative Response factor (RRF)} = \frac{\text{Slope of impurity curve}}{\text{Slope of standard curve}}$$

Acceptance criteria

- It must fulfil the system suitability requirements.
- Impurity-F and Gliclazide linearity correlation co-efficient should be NLT 0.98
- %RSD results of linearity level-5 solution for gliclazide and impurity-F should be less than 5

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value (Standard value).

Performed accuracy in different levels by spiking known quantity of API into placebo Sample at 50%, 100%, and 120% with respect to the sample concentration. Analysed these samples in triplicate for each level. From the results, calculated the % recovery

Impurities were spiked into the test solution with a concentration of accuracy level-1 (LOQ), accuracy level-2 (50%), accuracy level-3 (100%) and accuracy level-4 (120%).

Note: for method precision can be use 100% level spiked with known impurities (six preparations of accuracy level-3)

Accuracy impurity stock solution: Prepared by using 10 mg of impurity-F reference standard and 45 ml of acetonitrile transferred to 100 ml volumetric flask, diluted with Milli-Q water.

Test solution preparation (as such): In a 50 ml of the volumetric flask, add accurately weighed 50 mg of test sample dissolved in 23 ml of acetonitrile, diluted with Milli-Q water.

Standard solution: In a 50 ml of the volumetric flask, add accurately weighed 50 mg of gliclazide dissolved in 23 ml of acetonitrile, diluted with Milli-Q water.

Reference solution-(a): In 100 ml of the volumetric flask, transferred 1 ml of standard stock solution, diluted with diluent. Then take 10 ml of above solution transferred into 100 ml of the volumetric flask, diluted with diluent.

Reference solution-(b): in 50 ml of volumetric flask dissolved the 5 mg gliclazide reference solution and 15 mg of gliclazide impurity-F reference standard. Then add 23 ml of acetonitrile, diluted with Milli-Q water. Then take 1 ml of above solution into 20 ml of volumetric flask, diluted with diluent.

Reference solution-(c): 10 mg of gliclazide taken in a 100 ml of volumetric flask, dissolved it by using 45 ml of acetonitrile, diluted with Milli-Q water. Then take 1 ml of above solution transferred into 100 ml of volumetric flask, diluted with diluent.

Accuracy level-1 preparation: in 100 ml of volumetric flask added 100 mg of test sample, respected to concentration which equal to LOQ level spike the impurity-F, dilute and make up the volume using diluent.

Accuracy level-2 preparation: in 100 ml of volumetric flask add 100 mg of test sample, 0.8 ml of accuracy stock solution dissolved by using 45 ml of acetonitrile and diluted by Milli-Q water.

Accuracy level-3 preparation: in 100 ml of volumetric flask add 100 mg of test sample, 1 ml of accuracy stock solution dissolved by using 45 ml of acetonitrile and diluted by Milli-Q water.

Accuracy level-4 preparation: in 100 ml of volumetric flask add 100 mg of test sample, 1.2 ml of accuracy stock solution dissolved by using 45 ml of acetonitrile and diluted by Milli-Q water.

Procedure: Equilibrate the column and mobile phase for 30 min. Followed by injection sequence.

Accuracy calculation

Amount added in accuracy level (A) (%):

$$\frac{\text{Wt. of impurity taken in accuracy level (mg)} \times \text{Diluent amount (ml)} \times 100}{\text{Diluent volume (ml)} \times \text{Diluent volume (ml)} \times \text{Wt.of sample taken (mg)}} \times 100$$

Impurity content in accuracy level (B) (%)

$$\frac{\text{Area of Imp.in accuracy level} \times \text{Wt. of Imp. standard(mg)} \times \text{Dilution of impurity(ml)} \times \text{vol. taken for sam.prep}}{\text{Avg.area of reference area} \times 100 \times \text{volume diluted for standard preparation(ml)} \times \text{Wt.of sample taken(mg)}} \times \frac{1}{\text{RRF}}$$

Impurity content in as such test sample injected in accuracy level(c)

$$\% \text{ Recovery} = \frac{B - C}{A} \times 100$$

Table 13: Injection profile

S. No.	Name of the injection	No. of injection
1	Blank	1
2	Reference solution-(b)	1
3	Blank	1
4	Reference solution-(a)	2
5	Reference solution-(c)	2
6	Test sample as such	1
7	Blank	1
8	Accuracy level-1 (3 preparations)	each 1
9	Accuracy level-2 (3 preparations)	each 1
10	Accuracy level-3 (6 preparations)	each 1
11	Accuracy level-4 (3 preparations)	each 1
12	Reference solution-(b)	1

Acceptance criteria

- It must fulfil the system suitability requirements.
- Impurity-F accuracy level (1) (at LOQ) % recovery should be from 70% to 130%.
- Impurity-F accuracy level (2), (3), and (4) % recovery should be from 80% to 120%.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of series of measurements.

System precision: System precision is a check by using standard chemical substance to ensure that the analytical system is working properly. Measure the retention time, area response of six determinations and calculate the relative standard deviation.

Standard solution: in a 100 ml of volumetric flask 10 mg of Gliclazide standard was weighed and dissolved in 45 ml of acetonitrile and diluted with Milli-Q water.

Reference solution-(a): In a 100 ml of the volumetric flask, transferred 1 ml of standard solution and diluted with water.

Procedure: Equilibrate the column and mobile phase for 30 min. Followed by injection sequence

Table 14: Injection profile

S. No.	Name of the injection	No. of injections
1	Blank	1
2	Reference solution-(b)	1
3	Blank	1
4	Reference solution-(a)	6

Acceptance criteria

- It must fulfil the system suitability requirements.
- The percent RSD for gliclazide area response from six replicate injections should not exceed 5.0 percent.

Method precision

In method precision, a homogeneous sample of a single batch should be analyzed six times. This indicates whether a method is giving consistent results of a single batch.

Impurity stock solution: In a 100 ml of volumetric flask, transferred 10 mg of impurity-F reference standard then added 45 ml of acetonitrile was diluted with Milli-Q water.

Spiked preparation: In a 100 ml of volumetric flask transferred 100 mg of gliclazide then 1 ml of impurity stock solution, 45 ml of acetonitrile was added and diluted using Milli-Q water.

Note: For method precision, data from six accuracy level-3 preparations will be used.

Acceptance criteria

It must fulfil the system suitability requirements.

The %RSD for the results of Six Preparations of Impurity-F should be NMT 10.0 percent and sum of impurities should be NMT 15%.

Intermediate precision (Ruggedness)

Precision carried out on multiple days, with different analysts, different equipment, and different columns is referred to as intermediate precision.

Impurity stock solution: In a 100 ml of volumetric flask transferred 10 mg of impurity-F reference standard then added 45 ml of acetonitrile diluted with Milli-Q water.

Spiked preparation: In a 100 ml of volumetric flask transferred 100 mg of gliclazide then 1 ml of impurity stock solution, 45 ml of acetonitrile was added, and diluted using Milli-Q water.

Procedure: Equilibrate the column and mobile phase for 30 min. Followed by injection sequence

Table 15: Injection profile

S. No.	Name of the injection	No. of injections
1	Blank	1
2	Reference solution-(b)	1
3	Blank	1
4	Reference solution-(a)	2
5	Reference solution-(c)	2
6	Spiked preparation	6 prep
7	Reference solution-(b)	1

Acceptance criteria

- It must fulfill the system suitability requirements.
- The %RSD for the results of impurity-F from six spiked sample preparation should be NMT 10.0
- The cumulative %RSD for the results of impurity-F from both method and intermediate precision should be NMT 10.0

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Condition (1): Alter the mobile phase flow±10%

a) Alter the flow condition 0.81 ml instead of 0.9 ml.

b) Alter the flow condition 0.99 ml instead of 0.9 ml

Condition (2): Alter the acetonitrile composition in the mobile phase±5%

a) Alter the acetonitrile composition 427.5 ml instead of 450 ml.

b) Alter the acetonitrile composition 427.5 ml instead of 450 ml.

Procedure: Equilibrate the column and mobile phase for 30 min. Followed by injection sequence

Table 16: Injection profile

S. No.	Name of the injection	No. of injections
1	Blank	1
2	Reference solution-(b)	1

Acceptance criteria

- It must fulfil the system suitability requirements.

Mobile phase stability

Prepare the needed quantity of mobile phase for the entire investigation. Keep the mobile phase at room temperature and examine it at the beginning, 24 h, and 48 h. Observe and document what you see on a daily basis. If any particles or turbidity are found in the mobile phase, report them and stop the investigation.

Procedure: Equilibrate the column and mobile phase for 30 min. Followed by injection sequence

Table 17: Injection profile

S. No.	Name of the injection	No. of injections
1	Blank	1
2	Reference solution-(b)	1
3	Blank	1
4	Reference solution-(a)	2
5	Reference solution-(c)	2
6	Test sample	1
7	Reference solution-(b)	1

Note: system suitability and test solutions should be prepared each day freshly

Acceptance criteria

- At each interval of the mobile phase stability study, there should be no turbidity or particles in the mobile phase.
- At each interval, it must fulfil the system suitability requirements.
- The difference in impurity-F values between the first and subsequent interval days must not exceed 0.03 percent.

Solution stability

Standard solution was prepared as per the test method and injected at different time intervals up to 36 h at room temperature.

Note: 8th h at room temperature sample was unstable, sample should be prepared freshly keep sample at 5 °C and restart the analysis.

Procedure: Equilibrate the column and mobile phase for 30 min. Followed by injection sequence

Table 18: Injection profile

S. No.	Name of the injection	No. of injections
1	Blank	1
2	Reference solution-(b)	1
3	Blank	1
4	Reference solution-(a)	2
5	Reference solution-(c)	2
6	Test sample (initial)	1
7	Reference solution-(b)	1
8	Test sample (4th h)	1
9	Reference solution-(b)	1
10	Test sample (8th h)	1
11	Reference solution-(b)	1
12	Test sample (16th h)	1
13	Reference solution-(b)	1
14	Test sample (24th h)	1
15	Reference solution-(b)	1
16	Test sample (36th h)	1
17	Reference solution-(b)	1

Note: report same if impurities or degradants extraneous peak is observed in sample and standard chromatogram

Acceptance criteria

- It must fulfil the system suitability requirements.
- The difference in impurity-F value between the initial and each interval studies should be NMT 0.03%.
- The difference in any unspecified value between the initial and each interval studies should be NMT 0.03%.
- The difference in total impurities value between the initial and each interval studies should be NMT 0.03%.

RESULTS AND DISCUSSION

Chromatography

The present study was undertaken with an objective of method validation a rapid, simple, cost-effective HPLC method for the determination of related substances of Gliclazide.

Chromatography was performed on LiChroCART Supersher RP-8 column, (250 mm × 4.0 mm, 5 µm) with the mobile phase composed of water (550 ml) and acetonitrile (450 ml). The flow rate was 0.9 ml/min, the Injection volume was 20 µl, and the DAD/VWD detection is at 235 nm where column temperature was 45 °C and sampler temperature was 5 °C. The retention time of Gliclazide is 11.6 min and the total Elution time was 35 min.

Validation parameters

System suitability

By injecting blank and reference standard-(b) system suitability was performed.

Acceptance Criteria: From reference solution (b), resolution between two solvents peaks should not be less than 1.8

Table 19: Results of system suitability

System suitability	Result	Acceptance criteria
Resolution between the Impurity-F and Gliclazide	2.34	Not less than 1.8

Specificity studies

Retention times of impurities were confirmed by injecting blank, individual impurities, gliclazide standard and spiked solution specificity were performed. By using PDA detector peak purity can be identified.

Precision

System precision

The system precision is a check by using standard chemical substances to ensure that the analytical system is working properly. Measure the retention time, area response of six determinations and calculate the relative standard deviation.

Injected six replicates of gliclazide standard solution (0.1%) to perform the system precision.

Acceptance criteria

- It must fulfill the system suitability requirements.

The percent RSD for gliclazide area response from six replicate injections should not exceed 5.0 percent.

Table 20: Acceptance criteria

S. No.	Results	Acceptance criteria
1	No interference is observed at the retention times of known impurities and gliclazide due to blank.	No interference should be observed at the retention times of known impurities and gliclazide.
2	The retention times of the individual known impurities and gliclazide are comparable with that of the spiked solution.	The retention times of the individual known impurities and gliclazide should be comparable with that of the spiked solution.
3	Purity angle is less than the purity threshold for impurity-F and gliclazide in spiked solution.	Purity angle should be less than the purity threshold for impurity-F and gliclazide in spiked solution.

Table 21: Peak purity and RRT of components in spiked solution

S. No.	Name of the component	Peak purity		Retention time	Relative retention time
		Purity angle	Purity threshold		
1	Impurity-F	18.96	21.66	11.457	0.93
2	Gliclazide	3.68	21.82	12.337	---

Table 22: Injection results

Injection No.	Area of standard
1	47842
2	47732
3	47940
4	47785
5	47627
6	47255
Mean	47697
%RSD	0.50
Acceptance criteria for % RSD	Not more than 10

Table 23: Injection results

Preparation no.	Impurity-F % w/w
1	0.1037
2	0.1036
3	0.1043
4	0.1036
5	0.1035
6	0.1034
Mean	0.1037
%RSD	0.31
Acceptance criteria for % RSD	Not more than 10

Method precision

In method precision, a homogeneous sample of a single batch should be analyzed six times. This indicates whether a method is giving consistent results of a single batch.

Injecting six spiked sample preparation methods, precision was performed.

Acceptance Criteria

- It must fulfill the system suitability requirements.
- The %RSD for the results of Six Preparations of Impurity-F should be NMT 10.0 percent and the sum of impurities should be NMT 15%.

Linearity

Recorded the area response at each level and calculated the correlation coefficient (R), slope, y-intercept, % of y-intercept at 100% specification limit.

Plotted a graph with concentration (in ppm) on x-axis and areas response on y-axis.

Acceptance criteria

- It must fulfil the system suitability requirements.
- Impurity-F and Gliclazide linearity correlation co-efficient should be NLT 0.98
- %RSD results of linearity level-5 solution for gliclazide and impurity-F should be less than 5

Table 24: Linearity–impurity-F

Concentration (% w/w)	Area of injection-1	Area of injection-2	Average area
0.0103	1443	1457	1450
0.0515	7366	7407	7387
0.0824	11760	11669	11715
0.1030	14728	14607	14668
0.1236	17620	17719	17670
Correlation Co-efficient (R)			0.99998
y-intercept			-11.97
%y-intercept			-0.08
Acceptance criteria for Correlation Co-efficient (R)			Not less than 0.98

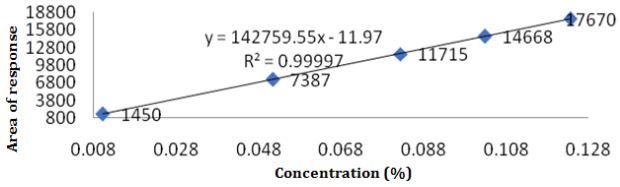

Fig. 5: Area of response

Table 25: Linearity–gliclazide

Concentration (% w/w)	Area of injection-1	Area of injection-2	Average area
0.0101	4765	4793	4779
0.0504	23767	23821	23794
0.0806	38327	38285	38306
0.1008	47564	47662	47613
0.1209	57041	57082	57062
Correlation Co-efficient (R)			0.99998
y-intercept			54.89
%y-intercept			0.12
Acceptance criteria for Correlation Co-efficient (R)			Not less than 0.98

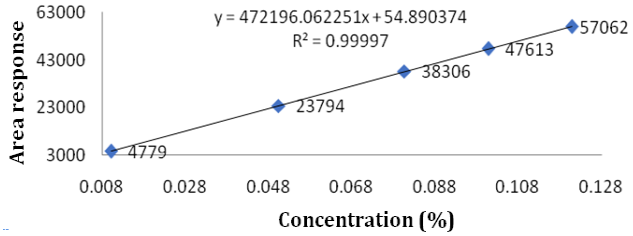

Fig. 6: Area response

Limit of detection and limit of quantitation

By injecting 0.003% concentration of all known impurities and drug substances limit of detection was determined.

Limit of quantitation was determined three times higher than limit of detection level, performed with precision and accuracy.

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value (Standard value).

Performed accuracy in different levels by spiking known quantity of API into placebo Sample at 50%, 100%, and 120% with respect to the sample concentration. Analysed these samples in triplicate for each level. From the results, calculated the % recovery.

Acceptance criteria

- It must fulfil the system suitability requirements.
- Impurity-F accuracy level (1) (at LOQ) % recovery should be from 70% to 130%.
- Impurity-F accuracy level (2), (3), and (4) % recovery should be from 80% to 120%

Table 26: Limit of detection area results (LOD)

Injection no.	Impurity-F (0.003%)	Gliclazide (0.003%)
1	399	1529
2	398	1630
3	485	1600

Table 27: Precision at limit of quantitation level (LOQ)

Injection no.	Area of Impurity-F (0.01% w/w)	Area of gliclazide (0.01% w/w)
1	1443	4765
2	1457	4793
3	1497	4770
4	1437	4756
5	1495	4716
6	1460	4739
Mean	1465	4756
%RSD	1.75	0.56
Acceptance criteria for %RSD	Not more than 10	

Table 28: Accuracy at limit of quantitation level (LOQ)

Level	Name of the component	Amount recovered (%w/w)	Amount recovered (%w/w)	% of recovery	Range (%)	Acceptance criteria
LOQ	Impurity-F	0.0104	0.0101	97.12	97.12-104.81	Between 70% and 130%
		0.0104	0.0109	104.81		
		0.0104	0.0109	104.81		

Table 29: Accuracy results for impurity-F

Level	Amount added (%w/w)	Amount recovered (%w/w)	% of recovery	Range (%)	Acceptance criteria
80%	0.0828	0.0820	99.03	98.43-99.88	
	0.0828	0.0827	99.88		
	0.0828	0.0815	98.43		
100%	0.1035	0.1037	100.19	100.10-100.77	Between 80 and 120%
	0.1035	0.1036	100.10		
	0.1035	0.1043	100.77		
120%	0.1243	0.1230	98.95	98.39-98.95	
	0.1243	0.1227	98.71		
	0.1243	0.1223	98.39		

Range

The range of the analytical method is the interval between the upper and lower levels of analyte that shall be demonstrated to be determined with a suitable accuracy and linearity.

Derived the specified range from linearity and accuracy studies.

Acceptance criteria

- It must fulfil the system suitability requirements.
- Impurity-F and Gliclazide linearity correlation co-efficient should be NLT 0.98
- %RSD results of linearity level-5 solution for gliclazide and impurity-F should be less than 5

Intermediate precision (Ruggedness)

Analysing 6 preparations of same batch from different analyst, day, column and different instrument ruggedness was performed.

Table 30: Precision at lower and upper level

Injection no	Linearity level-1 (Area at LOQ level)		Linearity level-5 (Area at 120% level)	
	Impurity-F	Gliclazide	Impurity-F	Gliclazide
1	1443	4765	17620	57041
2	1457	4793	17719	57082
3	1497	4770	17636	57190
4	1437	4756	17782	57182
5	1495	4716	17781	57061
6	1460	4739	17699	57160
Mean	1465	4756	17706	57119
%RSD	1.75	0.56	0.39	0.11
Acceptance criteria for %RSD	Not more than 10			

Table 31: % of the recovery for lower and upper levels:

Accuracy level	Impurity-F	Acceptance criteria
LOQ % (Lower)	97.12-104.81	Between 70% and 130%
120% (Upper)	98.39-98.95	Between 80% and 120%

Table 32: Ruggedness

Preparation	Impurity-F results (Spiked samples)	
	Impurity-F (% w/w)	
	Analyst-1	Analyst-2
Preparation-01	0.1037	0.1076
Preparation-02	0.1036	0.1048
Preparation-03	0.1043	0.1067
Preparation-04	0.1036	0.1051
Preparation-05	0.1035	0.1045
Preparation-06	0.1034	0.1039
Mean	0.1037	0.1054
%RSD	0.31	1.34
Cumulative mean	0.1046	
Cumulative % RSD	1.24	
Acceptance criteria for % RSD	Not more than 10	

Table 33: Robustness

S. No.	Robustness condition	Actual conditions	Altered conditions	Resolution between impurity-F and gliclazide
1	Flow rate	0.90 ml/min (±10%)	0.81 ml/min	2.32
			0.99 ml/min	2.39
2	Acetonitrile composition in mobile phase	450 ml (±5%)	427.5 ml	2.54
			472.5 ml	2.08
Acceptance criteria for resolution between the Impurity-F and Gliclazide			Not more than 1.8	

Robustness

The standard conditions of column oven temperature, Buffer pH, mobile phase proportion of Organic solvents and flow rate are varied and the results for those parameters are shown below.

Acceptance criteria

- It must fulfil the system suitability requirements.

Mobile phase stability

Prepared sufficient quantity of mobile phase undergo analyzed at initial, after 24th hr and after 48th hr at room temperature.

Acceptance criteria

- At each interval of the mobile phase stability study, there should be no turbidity or particles in the mobile phase.
- At each interval, it must fulfil the system suitability requirements.
- The difference in impurity-F values between the first and subsequent interval days must not exceed 0.03 percent.

Solution stability

By analysing the reference solution-(b) and test solution at initial, 4th, 8th, 16th, 24th, and 36th hrs at room temperature, stability studies are performed.

Acceptance criteria

- It must fulfil the system suitability requirements.
- The difference in impurity-F value between initial and each interval studies should be NMT 0.03%.
- The difference in any unspecified value between initial and each interval studies should be NMT 0.03%.

The difference in total impurities value between initial and each interval studies should be NMT 0.03%.

Table 34: Mobile phase stability

Day	Resolution (Between the impurity-F and gliclazide)	Turbidity/Particles	Unspecified impurity (% w/w)	Sum of impurities (Other than impurity-F) (% w/w)
Initial	2.14	Not observed	0.01	0.02
After 24th h	2.19	Not observed	0.01	0.02
After 48th h	2.19	Not observed	0.01	0.02
Acceptance criteria	NLT 1.80	Should not show any turbidity/particles	NMT+0.03%	NMT+0.1%

Table 35: Solution stability

Day	Resolution (Between the Impurity-F and Gliclazide)	Unspecified impurity (% w/w)	Sum of impurities (Other than impurity-F) (% w/w)
Initial	2.10	0.01	0.04
After 4th h	2.08	0.03	0.05
After 8th h	2.10	0.05	0.07
After 16th h	2.14	0.14	0.16
Acceptance criteria	NLT 1.80	NMT+0.03%	NMT+0.1%

Table 36: Retention and relative retention time

S. No.	Name	Impurity-F	Gliclazide
1	Retention time	About 12	About 13
2	Relative retention time	About 0.93	1.0

The limit of detection, limit of quantitation and relative response factor for Impurity-F and Gliclazide as mentioned below:

Table 37: Limit of detection, limit of quantitation and relative response factor

S. No.	Name	Impurity-F	Gliclazide
1	Limit of detection (% w/w)	0.003	0.003
2	Limit of quantitation (% w/w)	0.01	0.01
3	Relative response factor	0.30	1.00

The prepared mobile phase can be used for 3 d at room temperature from the date of preparation. The test solution is unstable. So freshly prepared test solution be used for regular analysis

Method recommendations

- Based on the method validation study, the following recommendations can be incorporated in the standard test procedure of related substances by HPLC.
- The retention times and relative retention times of Impurity-F and Gliclazide was mentioned below.

CONCLUSION

The proposed research describes a validated High-Performance Liquid Chromatographic (HPLC) method for estimating gliclazide for injection. The peaks of the active drug, known degradants, and related substances were well resolved by the developed analytical method. The method was validated and found to be simple, sensitive, accurate and precise.

The proposed method can be used for the determination of related substances of Gliclazide. From the above experimental data and results, the developed HPLC method is having the following advantages.

- There is no interference or co-elution of blank, impurities in quantifying Gliclazide.
- The standard and sample preparation requires less time.
- No tedious extraction procedures were involved in the analysis of the drug.
- Mobile phase was stable up to 48th hrs; standard solution and sample solution was not stable from the time of preparation when stored at ambient temperature (25 °C).
- Hence, the chromatographic method validated for determination of related substances is said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control departments in industries and approved testing laboratories.
- The method stands validated and can be used for routine analysis to determine the related substances in gliclazide which complies with acceptance criteria of the analytical parameters such as system suitability, specificity, precision, linearity, accuracy, range, intermediate precision (Ruggedness), robustness, solution stability and mobile phase stability.

ABBREVIATIONS

RSD: Relative Standard Deviation, SST: System suitability, NMT: Not more than, NLT: Not less than, LOD: Limit of detection, LOQ: Limit of quantitation, Impurity-B: 2-nitroso-octahydrocyclopenta[*c*]pyrrole, Impurity-F: 1-(hexahydrocyclopenta[*c*]pyrrole-2(1H)-yl)-3-[(2-methyl phenyl)sulfonyl]urea

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Lal B, Kapoor D, Jaimini M. A review on analytical method validation and its regulatory perspectives. J Drug Delivery Ther. 2019 Mar 14;9(2):501-6. doi: 10.22270/jddt.v9i2.2403.
2. https://en.wikipedia.org/. [Last accessed on 06 Jul 2022]
3. https://asq.org/quality-resources/quality-assurance-vs-control. [Last accessed on 06 Jul 2022].
4. https://www.pharmatutor.org./pharma-analysis.
5. http://www.oswego.edu/~kadima/CHE425/chapter1_all_one%20slide.
6. http://che1.lf1.cuni.cz/html/Chromatography.pdf.
7. Coskun O. Separation tecniques: chromatography. North Clin Istanbul. 2022. doi: 10.14744/nci.2016.32757.
8. McPolin O. An introduction to HPLC for pharmaceutical analysis. Warren point, Northern Ireland: Mourne Training Services (MTS); 2009.
9. Lloyd RS, Joseph J, Kirkland JL, Glejesh. Pract HPLC method dev pharmaceutical. Trans J Chem. 1996;20:269-75.
10. Instrumental methods of analysis, Willard Merritt Dean settle. 7th ed. Vol. 580; 1988.

11. Today C. What are the most common applications of HPLC? Chromatography Today. 2022.
12. Asian guidelines for validation of analytical procedures adopted from ICH guidelines. 27th Oct 1994.
13. ICH Harmonised tripartite Guideline, Validation of analytical procedure: text and Methodology; Q2(R1), Current Step 4 version Parent Guideline dated; Oct 1994.
14. Yang W, Yang X, Shi F, Liao Z, Liang Y, Yu L. Qualitative and quantitative assessment of related substances in the compound ketoconazole and clobetasol propionate cream by HPLC-TOF-MS and HPLC. J Pharm Anal. 2019;9(3):156-62. doi: 10.1016/j.jpha.2018.08.006, PMID 31297292.
15. Rouini MR, Mohajer A, Tahami MH. A simple and sensitive HPLC method for determination of gliclazide in human serum. J Chromatogr B Anal Technol Biomed Life Sci. 2003;785(2):383-6. doi: 10.1016/s1570-0232(02)00951-0, PMID 12554153.
16. Gosar A, Shaikh T, Joglekar A, Mahadik N. Development of analytical method for determination of p-toluenesulfonylurea and its impurities in gliclazide drug substances by using HPLC. J Pharmacol Res Dev. 2021;3(1):16-20. doi: 10.46610/JPRD.2021.v03i01.004.
17. Al Mahmud MA, Bhadra S, Haque A, Al Mamun ME, Haider SS. Development and validation of HPLC method for simultaneous determination of gliclazide and enalapril maleate in tablet dosage form. Dhaka Univ J Pharm Sci. 2015;13(1):51-6. doi: 10.3329/dujps.v13i1.21859.
18. Hossain MK, Sultan MZ, Rahman A, Uddin MG, Chowdhury AA, Rashid MA. RP-HPLC method development and validation for quantitative analysis of metformin and gliclazide in pharmaceutical formulations. J Sci Res. 2013;6(1):161-8. doi: 10.3329/jsr.v6i1.15963.
19. Nirupa G, Tripathi UM. RP-HPLC analytical method development and validation for simultaneous estimation of three drugs: glimepiride, pioglitazone, and metformin and its pharmaceutical dosage forms. J Chem. 2013;2013:1-8. doi: 10.1155/2013/726235.
20. Balaji N, Sultana S. Sensitive determination of related substances in pioglitazone hydrochloride by HPLC. Int J App Pharm. 2017;9(2):34. doi: 10.22159/ijap.2017v9i2.16828.
21. Rathinavel G, Uma Nath U, Valarmathy J, Samueljoshua L, Thanuja CS, Ganesh M. RP-HPLC method for the simultaneous estimation of rosiglitazone and gliclazide in tablets. E-Journal of Chemistry. 2009;6(4):1188-92. doi: 10.1155/2009/298379.
22. Ahmed AB, Bordoloi R, Bhattacharya K. Development and validation of a RP-UHPLC method for estimation of gliclazide loaded microsphere. Res J Pharm Technol. 2022:773-8. doi: 10.52711/0974-360X.2022.00129.
23. Monzon CM, Teglia CM, Delfino MR, Goicoechea HC. Chemometric optimization and validation of a novel dispersive liquid-liquid microextraction–HPLC method for gliclazide, glibenclamide and glimepiride quantitation in serum samples. Microchem J. 2016;127:113-9. doi: 10.1016/j.microc.2016.02.011.
24. Kimura M, Kobayashi K, Hata M, Matsuoka A, Kitamura H, Kimura Y. Determination of gliclazide in human serum by high-performance liquid chromatography using an anion-exchange resin. J Chromatogr. 1980;183(4):467-73. doi: 10.1016/s0378-4347(00)81589-3, PMID 7430298.
25. Sebaiy M, Eladl S, Baraka M, Hassan A. Rapid RP-HPLC method for simultaneous estimation of some antidiabetics; metformin, gliclazide and glimepiride in tablets. Egypt J Chem. 2018. doi: 10.21608/ejchem.2018.4394.1388.
26. Charles BG, Ravenscroft PJ. Measurement of gliclazide in plasma by radial compression reversed-phase liquid chromatography. Clin Chem. 1984;30(11):1789-91. doi: 10.1093/clinchem/30.11.1789, PMID 6488522.
27. Sasongko L, Pratiwi GK, Leo M, Adiwidjaja J. Simultaneous HPLC assay of gliclazide and ciprofloxacin in plasma and its implementation for pharmacokinetic study in rats. J Chromatogr Sci. 2021;59(4):338-46. doi: 10.1093/chromsci/bmaa111, PMID 33401303.
28. Lakshmi KS, Rajesh T. Separation and quantification of eight antidiabetic drugs on a high-performance liquid chromatography: its application to human plasma assay. ISRN Pharm. 2011;2011:1-7. doi: 10.5402/2011/521353.
29. Rouini MR, Mohajer A, Tahami MH. A simple and sensitive HPLC method for the determination of gliclazide in human serum. J Chromatogr B Analyt Technol Biomed Life Sci. 2003;785(2):383-6. doi: 10.1016/s1570-0232(02)00951-0, PMID 12554153.