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Development and validation of stability-indicating UPLC method for the determination of gliclazide and its impurities in pharmaceutical dosage forms

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Abstract

Background: For the determination of gliclazide and its three potential impurities quantitatively, the development of a stability-indicating, accurate, simple, and fast, Ultra-Performance Liquid Chromatography (UPLC) method was done.

Results: On Acquity CSH 18 column (50 mm×2.1 mm, 1.7 μ) separation was achieved by the isocratic elution mode using mobile phase (5 mM ammonium acetate buffer of pH 4 and 10% ammonium acetate buffer + 90% acetonitrile, 65/35 v/v). In total, 0.7 mL^{-1} was the chosen flow rate and UV detection was carried out at 227 nm.

Conclusion: By analyzing forced degradation products of the sample, the stability-indicating characteristic of the developed method was proved where the separation of the products of degradation from analyte peak was seen along with spectral purity of gliclazide. Validation of the developed UPLC method was done as per the guidelines of the International Conference on Harmonization in terms of system suitability, precision, accuracy, specificity, sensitivity, linearity, and robustness.

Keywords: Gliclazide, UPLC, Potential impurity, Stability indicating

Background

For treating the non-insulin-dependent or type 2 diabetes mellitus, gliclazide is being used as oral hypoglycemic agent. It is an insulin secretagogue that helps the pancreas to increase the production of insulin and belongs to the second generation sulfonylureas due to the presence of a sulphonamide group in its structure. Gliclazide binds on the sulfonylurea receptors (SUR-1) specifically which are present on the pancreatic beta-cells [1]. Chemically, gliclazide is given as 1-(3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]pyrrol-2-yl)-3-(4-methylphenyl)sulfonylurea [2]. Gliclazide is present in the market with the brand name of Diamicron containing 30, 60, or 80 mg of gliclazide for oral administration in the form of tablets [3]. There is a possibility that decrease in the oxidative stress can improve endothelial functions in vascular diseases thereby having a positive effect on the use of glicazide [4]. Due to its antioxidant properties, intensive glycemic control is observed [5]. Reduction in hyperreactivity of platelet [6], reduced risk of hyperglycemia and cardiovascular safety is also associated with the use of this drug [7].

For the determination of gliclazide and its potential impurities in pharmaceutical dosage forms, analytical method is required to control and monitor the impurities present in the drug-related substances. Several techniques of analysis are hybrid where liquid chromatography (LC) [8], gas chromatography (GC)

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[9], and capillary electrophoresis (CE) [10] are combined to mass spectrometry and are majorly used during reversed-phase liquid chromatography involving a non-polar stationary phase. On doing a literature survey extensively, it was revealed that for the analysis of gliclazide, several high-performance liquid chromatographies (HPLC) were found but to the best of our knowledge, no reported stability-indicating ultra-performance liquid chromatography method for estimating gliclazide is available. Also, there was no such effective analytical method reported which was effective in saving time and money. As analytical studies were done using GC/MSD or LC-MS are highly expensive and fragile in comparison to ultra-performance liquid chromatography (UPLC) and our main focus was on developing an analytical method that is rapid, accurate, reproducible, and cost-effective, UPLC method was chosen for the developing stability-indicating method for determining gliclazide. The developed method was validated as per the Q2 (R1) guidelines of the International Conference on Harmonization (ICH) and USP <1225> compendia procedures [11, 12].

Methods

Materials and reagents

Gliclazide sample and its three impurities, namely, Imp A, Imp C, and Imp F as shown in Fig. 1 were received from Mylan Laboratories, Hyderabad, India. Trifluoro acetic acid of HPLC grade was procured from J.T.Baker, India. Acetonitrile of HPLC grade and hydrochloric acid of AR grade were procured from Rankem, India. Sodium hydroxide of Emplura grade, formic acid of Emparta grade, hydrogen peroxide (30% v/v) of AR grade, and ammonium acetate of Emparta grade were procured from Merck, India. De-ionized water was procured from Millipore, a Milli-Q purification system installed in Mylan Laboratories.

Instrumentation and operating conditions

Autosampler and quaternary gradient pump equipped Waters' Acquity UPLC system with photodiode array detector was used in the development of the stability-indicating method and its validation. Processing of the output signals was performed with the help of Empower software version 2. During the development of the method the UPLC columns used were Acquity CSH-C18 column (50 mm × 2.1 mm × 1.7 μ), Acquity BEH-C18 column (50 mm × 2.1 mm × 1.7 μ), Phenomenex Kinetex-C18 column (50 mm × 2.1 mm × 1.7 μ), and Acquity BEH-C18 column (150 mm × 2.1 mm × 1.7 μ).

Acquity CSH-C18 column (50 mm × 2.1mm × 1.7 μ) thermostated at 45 °C was finally used for separation. The mobile phase was made by mixing 5 mM ammonium acetate buffer of pH 4 and 10% ammonium acetate buffer + 90% acetonitrile in the ratio of 65:35. The flow

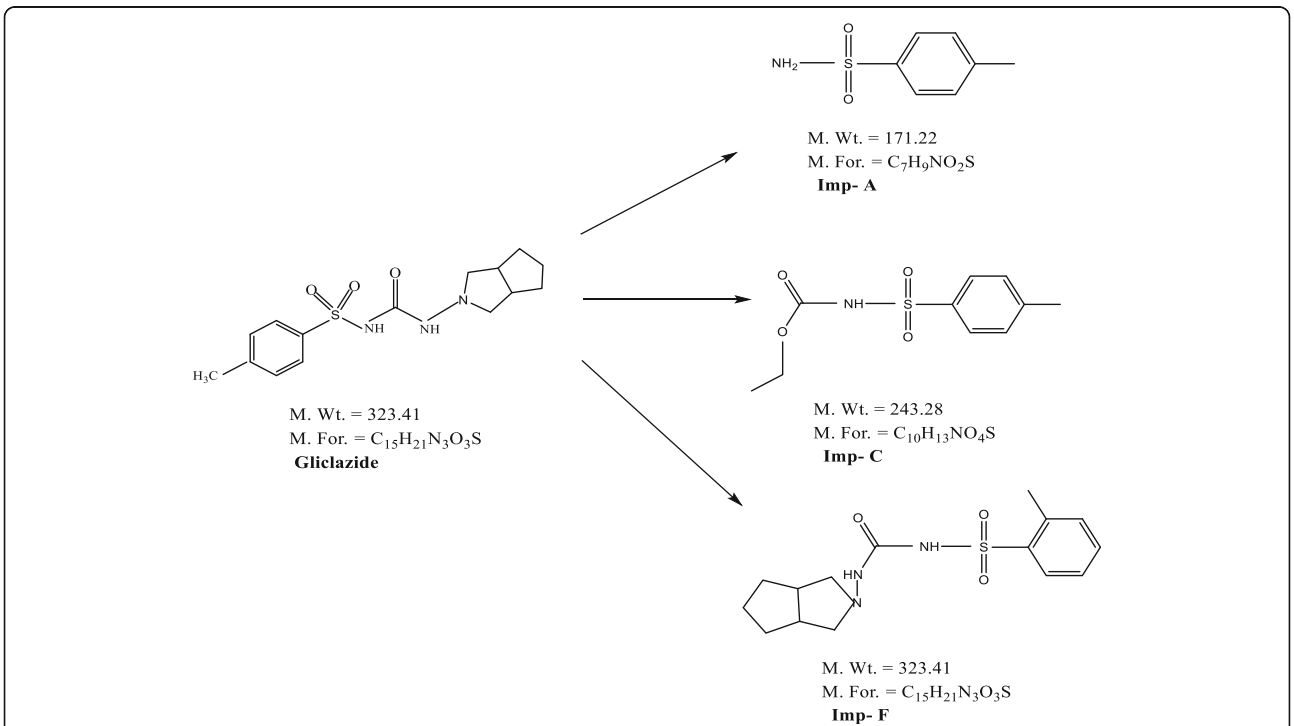

Fig. 1 Degradation-related impurities of gliclazide. **a** Test sample treated with acid. **b** Test sample treated with alkali. **c** Test sample treated with peroxide

Table 1 Forced degradation study results

Stress conditions	% Degradation	Purity angle	Purity threshold	Purity flag
Unstressed condition	0	0.068	0.317	No
Treated with 1 N (5 mL) HCL solution for 45 min on benchtop	5.5	0.088	0.416	No
Treated with 1 N (5mL) NaOH solution for 2 h at 95 °C	5.8	0.056	0.357	No
Treated with 3% (5 mL) H_2O_2 solution for 1 h at benchtop	13.9	0.058	0.308	No
Treated with H_2O (5 mL) for 2 h 90 °C	18.4	0.297	0.314	No
Kept in oven at 105 °C for and 72 h	0.9	0.089	0.408	No
Kept in humidity chamber for 120 h at 75% Relative humidity and 40 °C	0.1	0.102	0.421	No

rate was kept at 0.7 mL^{-1} and injection volume was 2 μL. The data were obtained at 227 nm for 3 min and further, it was processed using the Empower 2 software. Scanning from 200-400 nm using the photodiode array detector was done for forced degradation study analysis and in terms of peak purity, the peak homogeneity was calculated.

Solution preparations

Glicazide is insoluble in water but soluble in acetonitrile. To reduce the organic solvent consumption, different ratios of acetonitrile and ammonium buffer of pH 7.4 was tried to study the solubility and finally acetonitrile and pH 7.4 ammonium acetate buffer in the ratio 70:30 was found to be suitable with good solubility, was fixed as diluent 1 for complete extraction of the drug, acetonitrile and ammonium acetate buffer (pH 7.4) in ratio of 50:50 was fixed as diluent 2 for chromatographic run. Diluent 1 used in the analysis was made by degassing a mixture of 700 mL of acetonitrile with 300 mL of pH 7.4 5 mM ammonium acetate buffer and diluent 2 was prepared by degassing a mixture of 500 volume of acetonitrile with 500 mL of pH 7.4 5 mM ammonium acetate buffer. A standard solution of 480 μg mL^{-1} concentration of gliclazide was prepared by dissolving 60 mg of drug in 50 mL of diluent 1 and then 4 mL of this above solution was diluted using 6 mL of diluent 2. The stock solution of each impurity (Imp-A, Imp-C, and Imp-F) were made at a concentration of 72 μg mL^{-1} using diluent 1. For preparing a standard solution for purpose of quantifying the impurities in accordance with the specified limits (0.15%) of individual impurity, further dilution of these solutions was carried out using diluent 2.

Sample solution of drug formulation was prepared by transferring accurately weighed 10 tablets of gliclazide into a dry 500 mL volumetric flask. Thirty milliliters of acetonitrile was added to the volumetric flask and stirred for 10 min using a magnetic stirrer after placing a magnetic bead inside the volumetric flask for disintegration of tablets. After the disintegration of tablets, 350 mL of diluent 1 was added and stirred for 90 min and the solution was further sonicated for 45 min with occasional shaking at an interval of 5 min. The magnetic bead was removed using a bead retriever and volume was made till the 500 mL mark using diluent 1 in the volumetric flask. A portion of the solution taken out of the volumetric flask was centrifuged at an RPM of 5000 for 10 min. From the centrifuged solution, 4 mL of the solution was pipetted out into a volumetric flask of 10 mL and volume was made up to the mark using diluent 2 to get a sample concentration of 480 μg mL^{-1}.

Analytical procedure

Gliclazide solution having a concentration of 480 μg mL^{-1} was spiked with Imp-C, Imp-A, and Imp-F at a specified level of 0.15 percent and considered as a standard stock solution. Using 2 μL each blank preparation as standard stock solution, under chromatographic conditions, six replicates of standard solution and six replicates of test solutions were injected and chromatograph was obtained. Not less than 2.0 was set as a resolution criterion between gliclazide and its related compound F was set as a parameter of system suitability. During verification of the system precision, not more than 5% RSD was recorded for injecting six replicate injections of all impurities.

Forced degradation study procedure

Based on In-lab developed protocol, a study was conducted to show the effective separation of degradants from gliclazide peak in assay method. Separate portions of blank, placebo, and drug product were treated with 1N HCl (5 mL) solution for 45 h at benchtop, 1N NaOH (5 mL) solution for 2 h at 95 °C, 3% H_2O_2 (5 mL) solution for 1 h at benchtop, treated with H_20 (5 mL) solution for 2 h at 90 °C. After the treatment, the solutions were diluted till the mark to get concentration of 480 μg mL^{-1}. Thermal degradation of solid API was done by keeping the sample in a hot air oven at 105 °C for 72 h. Photolytic degradation of the sample was done by exposing it to a UV source for 7 days and humidity

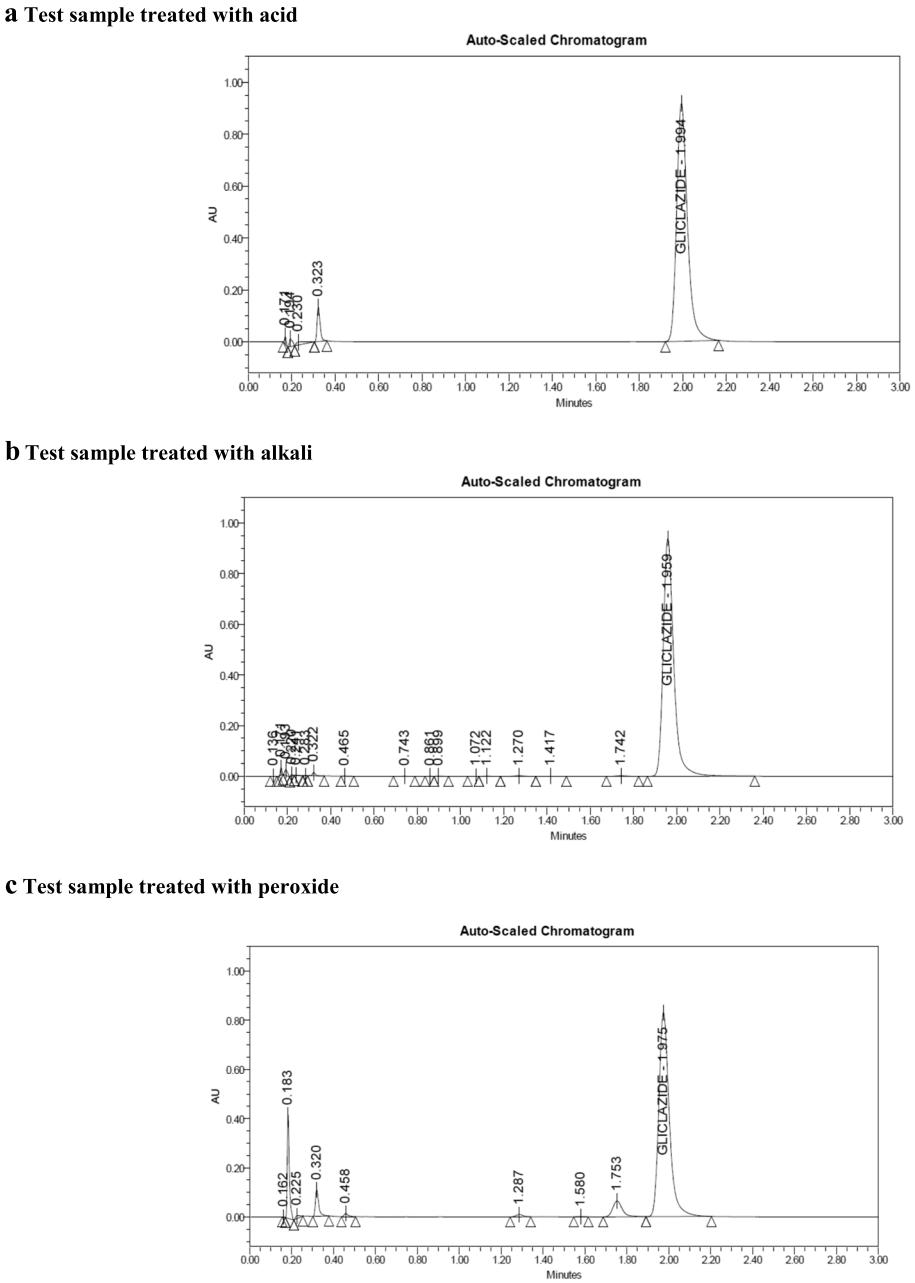

Fig. 2 UPLC chromatograms of forced degradation study. **a** Test sample treated with acid, **b** test sample treated with base, and **c** test sample treated with peroxide

degradation of the sample was done by keeping the sample chamber having 75% relative humidity at 40 °C for 120 h.

Analyses of stressed samples were done as per the test method using a photodiode array detector. Chromatograms obtained were examined for peak purity of peak with the help of Waters Empower 2 software. Standard and sample solutions prepared, using ammonium acetate buffer, were stable during the analysis as gliclazide is insoluble in water and the percentage of buffer used was significantly lower when compared to acetonitrile used as organic solvent. Purity angle was lower than purity threshold without purity flag for gliclazide peak for all forced degradation samples which indicated that in the quantification of the gliclazide in gliclazide tablets, there is no interference from degradants (Table 1).

Calculation of percentage degradation is calculated using the formula: [13]

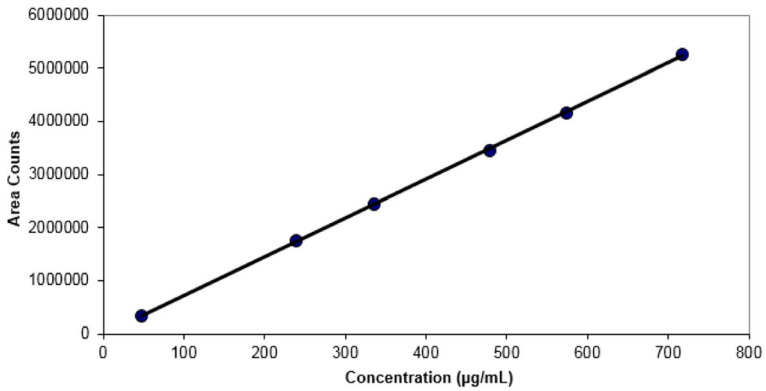

Fig. 3 Linearity of gliclazide. **a** Preparation of blank, **b** Preparation of standard

$$\%\text{degradation} = \frac{\text{Peak area of untreated stock solution - Peak area of treated stock solution}}{\text{Peak area of untreated stock solution}} \text{x } 100$$

Results

Specificity (selectivity)

Well, separation of degradation products and gliclazide in terms of purity angle and purity threshold was revealed from forced degradation studies. In acid, alkali, and peroxide, significant degradation was observed as shown in Fig. 2.

Linearity

Using a plot between concentrations vs area, the linearity of the detector was established. Gliclazide standard was diluted in a series in the range of concentration from 48 to 725 µg/mL from gliclazide stock solution (1000 µg/mL) and analyzed according to a test method. Plotting of graph was done by keeping concentration in µg/mL on *X*-axis and response (area) on *Y*-axis and the slope, intercept, and the correlation coefficient was determined (Fig. 3).

The linearity plot results of gliclazide are given in Table 2.

Precision

System precision

For determining related substances, system precision was performed by analyzing the system suitability mixture six times and gliclazide peak area RSD was found to be 0.84% (Table 3).

Table 2 Linearity plot results of gliclazide

Parameter	Observation
Slope	7228.8621
Intercept	−13415.1160
Correlation	0.999899704
Residual sum of square	3118705907

Method precision

For precision, evaluation of test method was done by doing assay for six separate test preparations of 60 mg strength as per test method. The percentage RSD result of assay from six individual test preparations was found to be within the specified limit (Table 4).

Accuracy (recovery)

A recovery study was done for gliclazide intact tablets in the range of 50 to 150% of the initial assay concentration. Solutions of the samples were made in triplicate at the individual level and according to the test, method analyses were done. Percentage recovery, percentage mean recovery, and percentage RSD were calculated for all the level results obtained were within limit (Table 5).

Solution stability

Benchtop stability of standard preparation and test preparation

Benchtop stability of standard preparation and test preparations was established by conducting a study for 24 h. For studying the standard solution's benchtop stability, similarity factor was calculated. The difference in assay percentage between initial and after 24 h of test preparations is observed to be in the limit. The similarity factor results of the standard were found in between 0.98 and 1.02 which concluded that the standard preparation and test preparations are stable for 24 h in the benchtop.

Benchtop stability study of mobile phase

A 3-day study was conducted for establishing the benchtop stability of the mobile phase. According to test method, system suitability parameters were checked by taking 5 replicate injections of standard solution using the same mobile phase on various days. For 3 days, the results of system suitability were obtained within the

Table 3 Result of system precision

Injection number	Peak area
1	3417135
2	3437162
3	3381424
4	3370534
5	3370306
6	3373095
Average	3391609.333
SD	28534.63979
% RSD	0.84

limits. Haziness was not detected and the solution was found clear during the stability testing period. It was concluded from the tests that the mobile phase was stable for 3 consecutive days on the benchtop.

Robustness

Flow rate variation

For determining the effect of flow rate variation, a study was conducted using five replicate injections of the standard solution. For higher and lower flow rates of 0.6 mL/min and 0.8 mL/min, system suitability parameters were in the acceptable limits from which, it is concluded that the permissible variation in flow rate is in range of 0.6 to 0.8 mL/min which proves that the method is robust for variation of flow rate in the given range.

Column oven temperature variation

For determining the effect of the column oven temperature variation, a study was performed. Using five replicate injections of standard solution, evaluation of system suitability parameters was done at 40 °C and 50 °C column oven temperatures. At both column oven temperatures, results of system suitability were found to be within the limits from which, it can be concluded that the permissible variation in column oven temperature is in range of 40 to 50 °C which proves that the method is robust for variation in the column temperature in the given range.

Variation in pH of mobile phase

For determining the effect of the mobile phase pH variation, a study was conducted. Evaluation of system suitability parameters using five replicate injections of the standard solution was done at pH 3.8 and 4.2. The system suitability results were found to be within the limits at both pH which concluded that the permissible variation in pH is in range of 3.8 to 4.2 which proves that the method is robust for variation of mobile phase pH in the given range.

Variation in composition of mobile phase

For determining the effect of variation in organic composition of mobile phase, a study using five replicate injections of the standard solution was conducted. Preparation of the mobile phase was done by changing the volume of the individual organic component in the mobile phase by absolute ± 10%. Evaluation of system suitability parameters was done using the above mobile phases, and results were found within the acceptable limits for mobile phases which shows that the method is robust for variation in the mobile phase organic composition in the given range.

Application of the method

Analysis of samples of commercial formulations suggested that the developed method is selective and specific for the determination of gliclazide and its potential impurities in pharmaceutical dosage forms.

Discussion

Method development

UPLC chromatographic condition development and its optimization

The primary objective for developing a stability-indicating UPLC method was the detection and determination of the impurities in gliclazide pharmaceutical

Table 4 Result of method precision

Sample number	Injection 1 area	Injection 2 area	Mean area	% Assay
1	3340504	3337686	3339095	97.5
2	3348688	3352607	3350647	97.9
3	3357953	3349257	3353605	97.9
4	3344406	3348032	3346219	97.7
5	3342399	3350527	3346463	97.7
6	3351862	3347991	3349265	97.8
Mean				97.8
SD				0.151657509
%RSD				0.2

Table 5 Result of accuracy study

% Level	Sample no.	% Recovery	Mean % recovery	% RSD
50	1	97.8		
	2	98.2	98.0	0.29
	3	98.0		
100	1	98.8		
	2	98.3	98.3	0.36
	3	97.9		
150	1	97.6		
	2	97.7	97.7	0.07
	3	97.9		

dosage forms and to separate all three potential impurities (Imp-A, Imp-C, and Imp-F) along with its degradation products generated while performing stress studies [14, 15].

Selection of UPLC column

Based on the structure of the drug and functional groups present in them considering their hydrophilicity and lipophilicity, the selection of column is done. Gliclazide is a non-polar drug. So, reverse phase chromatography has been chosen [16]. Generally used columns are C8, cyano, C18, and phenyl columns. Trials were taken in different pH ranges with different columns such as Kinetex C18, Acquity BEH C18 column, and Acquity CSH C18 column. Finally, Acquity CSH C18 (50 mm × 2.1 mm, 1.7 microns) column was selected based on the highest carbon load, better plate count, and better retention time

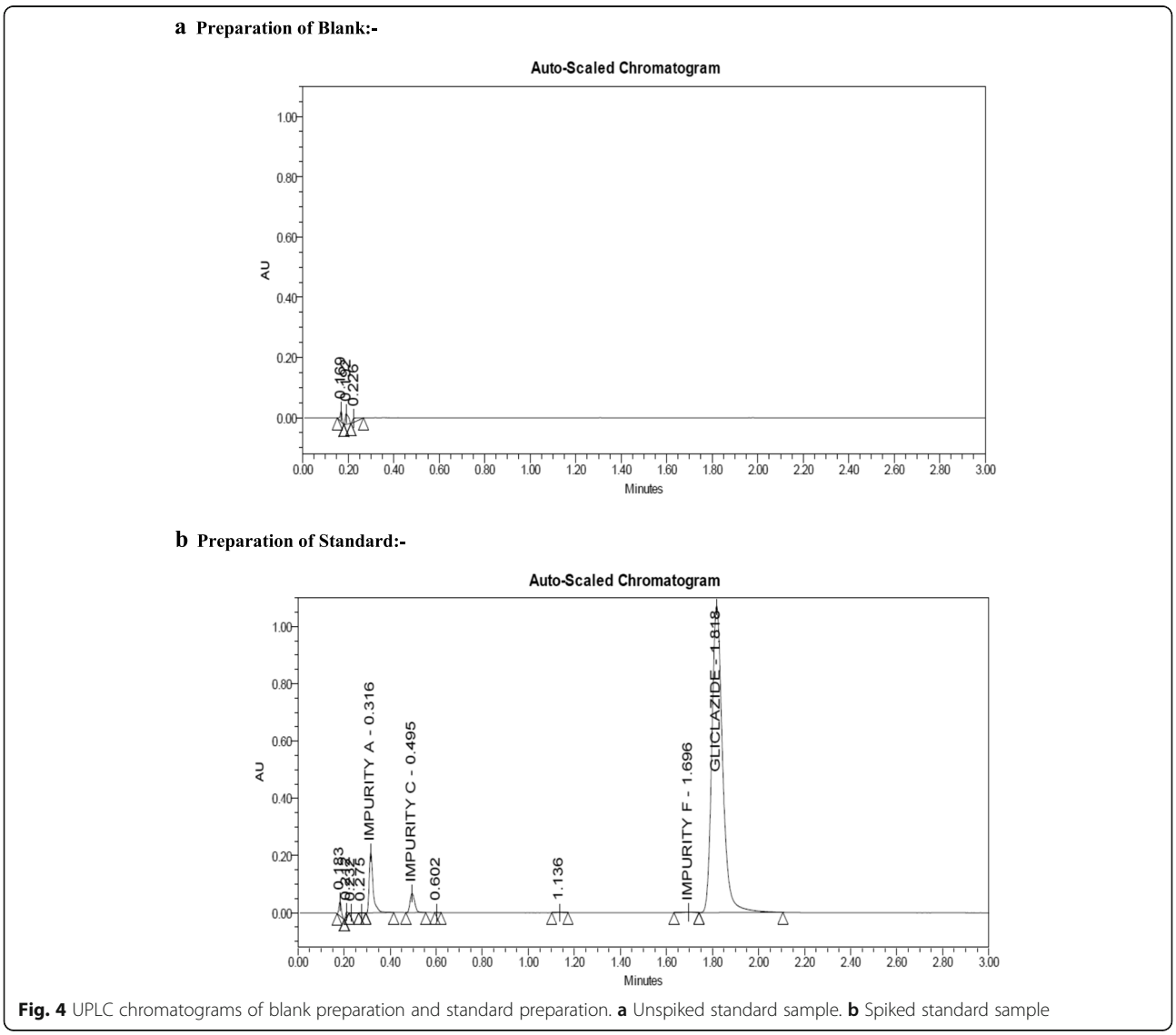

Fig. 4 UPLC chromatograms of blank preparation and standard preparation. **a** Unspiked standard sample. **b** Spiked standard sample

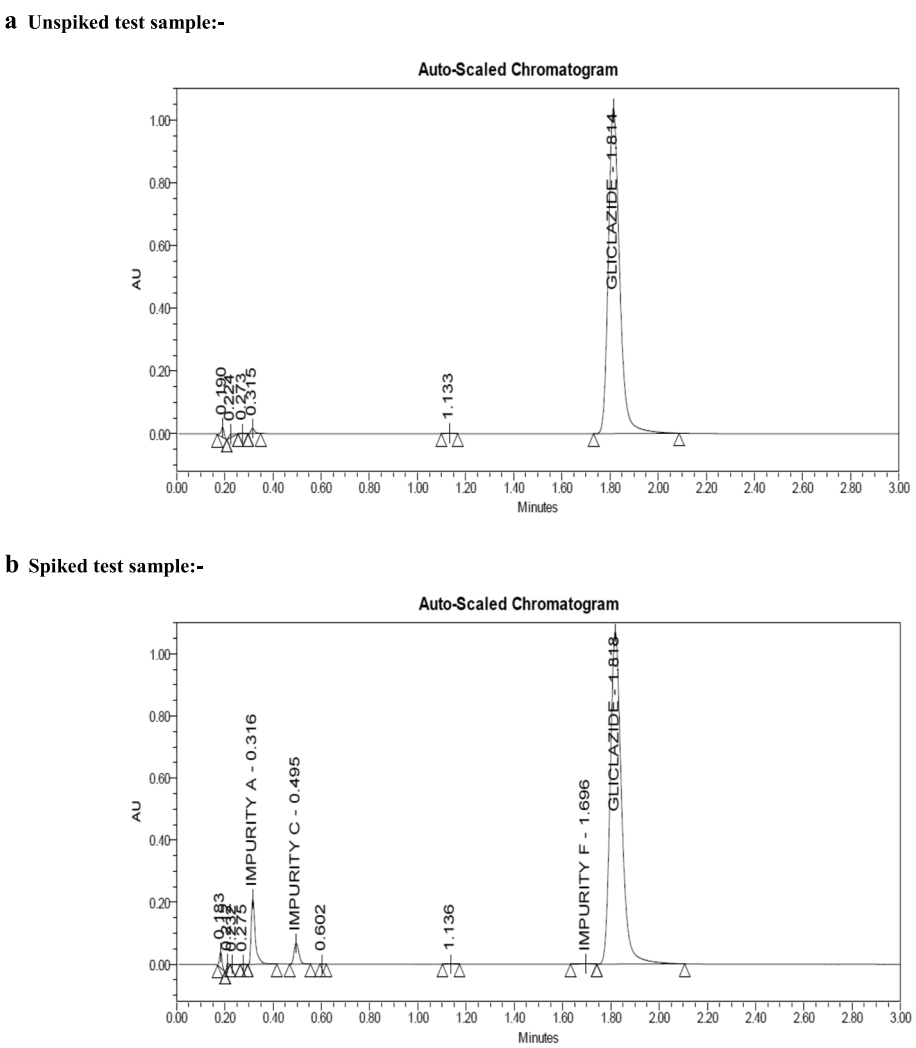

Fig. 5 UPLC chromatograms of test sample preparations. **a** Unspiked test sample. **b** Spiked test sample

with no interference with impurity peaks. As mobile phase pH was nearly 4.0, Acquity CSH C18 (50 mm × 2.1 mm, 1.7 microns) column is used which can be used in pH range 1-11 [17, 18].

Buffer selection

Ammonium acetate which is an organic buffer was selected as buffer having pKa-4.8 and pH range 3.8-5.8. Ammonium acetate pH was adjusted using formic acid which makes the mobile phase compatible with LC-MS. It also improves the resolution. As the stationary phase gets rapidly degraded by inorganic buffers and due to their corrosive nature, they are not considered for this method development. Organic buffers were chosen which were less corrosive than inorganic buffers but gave an equal buffering performance to that of inorganic

Table 6 System suitability study results

System suitability parameters	Observed value	Acceptance criteria
USP plate count	8047	[b]2000
Tailing factor	1.3	[c]2.0
% Relative standard deviation[a]	0.84	[c]2.0

[a]Six replicate injections
[b]Not less then
[c]Not more than

buffers. On the other hand, precipitation problems with organic buffer are less compared to inorganic buffers [19].

Selection of mobile phase

Mobile phase selection is mainly affected by the dissociation constant, drug solubility. Mixtures of solvents are used sometimes for better resolution of the components. Gliclazide is having pKa 5.8. Ninety-nine percent of gliclazide exists in the unionized form at pH lower than 2 units away from pKa and fully ionized at pH above 2 units of pKa. At higher pH 7.0, the main peak of gliclazide was merging with the peak of impurity F. As pH decreased to 4, there was a marked separation between the main peak of gliclazide and the peak of impurity F. Various pH combinations using potassium dihydrogen phosphate and ammonium acetate with acetonitrile were tried but impurity separation was found maximum at pH 4.0. Finally, the mobile phase selected was 65% ammonium acetate buffer of pH 4.0 + 35% mixture of 10% ammonium acetate buffer of pH 4.0 and 90% acetonitrile [20, 21].

Optimization of chromatographic conditions

A wavelength of 227 nm was chosen for the analysis which is the UV maxima of gliclazide. Based on peak height, peak area, and peak shape, the volume of injection can be chosen. Using various injection volumes, trials were taken. Gliclazide has high absorbance at lower microliters so 2 μl was used. Finally, at 2 μl injection volume, a good peak shape with the required peak area was obtained. Retention and degradation of the drug depend on column oven temperature. An elevation in column temperature in certain limit may cause a decrease in the viscosity of the mobile phase which in turn will reduce the retention time of analyte. The separation was good at 45 °C temperature. To get the required resolution with impurities, 45 °C was selected as the temperature of column. The reason behind selecting the flow rate of 0.5 mL/min for the 2.1-mm internal diameter column was that there was a decrease in the resolution between Imp-F and gliclazide to less than 2.0 with an increased flow rate which was not within acceptable limits. Impurity-A, impurity-C, impurity-F, and gliclazide were retained in stationary phase for about 0.316, 0.495, and 1.696 min respectively (Figs. 4 and 5), and the UPLC method developed was found to be very specific for determining gliclazide and its potential impurities in the pharmaceutical dosage forms [22].

Identification of impurities

At desired concentrations of 0.15%, impurities A, C, and F were spiked in gliclazide concerning a test concentration of 480 μg mL^{-1} for confirming the retention times for the chosen method of chromatography. All the impurities were well separated from each other.

System suitability criteria

Based on obtained results in chromatograms, a system suitability test was conducted. USP plate count obtained from the analyte peak was more than 2000 which determined the column efficiency. Tailing factor was found less than 2.0 and in six replicate injections for system suitability, relative standard deviation (RSD) for impurity areas was found less than 5.0%. During validation of the method criteria for system suitability were found within limits. System suitability results are given in Table 6.

Conclusion

A simple, sensitive, stability-indicating, and precise UPLC method for estimation of gliclazide has been developed and validated for determining gliclazide in commercial tablet dosage form of 60 mg. The compound and its impurities were well separated isocratically on a C18 column (Acquity CSH C18, 1.7 μ, 50 × 2.1 mm) using a mobile phase consisting of 65% pH 4.0 5mM ammonium acetate buffer and 35% mixture of 10% buffer and 90% acetonitrile with a flow rate of 0.7 mL/min with UV detection at 227 nm wavelength. The procedure was validated for all compendial and non-compendial parameters in accordance with ICH guidelines. Stress studies concluded that gliclazide undergoes degradation during acid, base, peroxide, and water-based stress studies but is stable to humidity and thermal stress conditions. The performed study demonstrated that the reverse phased liquid chromatography is selective and sensitive for detecting gliclazide and its potential impurities. After taking trials using different C18 columns having different carbon loads and different properties, maximum resolution was found with Acquity CSH C18, 1.7 μ, 50 × 2.1 mm column having high carbon load and less particle size. Validation of the above developed method was done using various validation parameters like system suitability, linearity, precision, specificity, accuracy, solution stability, filter interference, and robustness which were found to be within the acceptance criteria. The developed method is found to be sensitive, precise, accurate, and stability-indicating, resolving all the potential degradation products from the drug.

Abbreviations

ICH: International Conference on Harmonization; SD: Standard deviation; LC-MS: Liquid chromatography-mass spectrometry; RSD: Relative standard deviation; UPLC: Ultra performance liquid chromatography; UV: Ultraviolet-visible

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Authors' contributions
JM carried out the design of analytical method development. KB carried out analytical method development and analytical method validation. KB and JM drafted the manuscript and participated in the design and coordination of the manuscript. JM reviewed the manuscript. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
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Consent for publication
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Competing interests
The authors declare that they have no conflict of interests.

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