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Original Article

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

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ABSTRACT

Objective: 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.

Methods: 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.

Results: 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 Supersher 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.

Conclusion: 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 [1].

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 [2].

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 high speed, efficient, accurate and highly resolved method of separation [2].

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 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 [2].

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 [3].

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 [4].

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 [5]. 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 [6].

Molecular structure of gliclazide

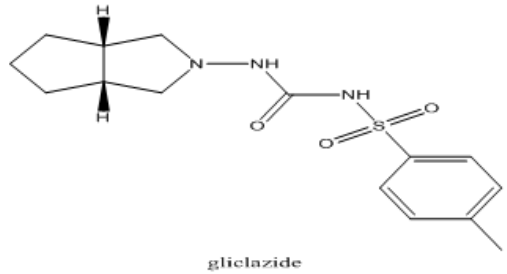

Fig. 1: Structure of gliclazide

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 [7]

Mechanism of action

Gliclazide binds to the sulfonyl urea 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 [8].

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 [9].

MATERIALS AND METHODS

Instrumentation

The present work describes a validated reverse phase RP-HPLC method for the estimation of type 2 anti-diabetic drug Gliclazide in dosage form [10]. Used Waters: e2695 with HPLC with PDA detector by using Empower-2 software, 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, 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, robustness were performed for the validation of this method. % RSD for validation parameters for Gliclazide were found to be less than 2%.

Standard and sample preparation

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.

Validation of developed method

Validation of method is carried out in accordance with USP and ICH guidelines for the assay of active ingredients. The method was validated for parameters like accuracy, precision, linearity, specificity, robustness and system suitability. These proposed methods are suitable for the estimation of title drugs in quality control laboratories in pharmaceutical industries [11].

RESULTS AND DISCUSSION

System suitability

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

Discussion

From the above results, it was concluded that the system suitability parameters were found to be within the limits.

Specificity studies

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

Discussion

From the above data, this can conclude blank (diluent) were not interfered with that the retention times of main peak and impurity peaks were not interfered with each other.

Table 1: Results of system suitability

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

Table 2: Results of specificity

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

Table 3: 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	---

Linearity and range

Table 4: 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

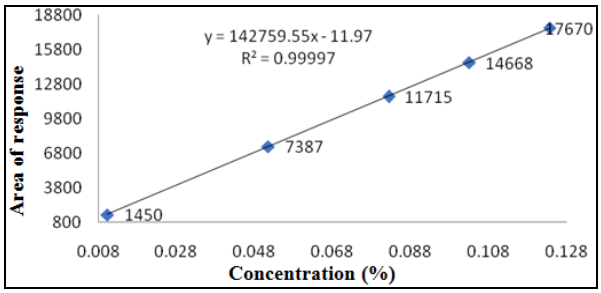

Fig. 2: Linearity of impurity-F

Table 5: 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

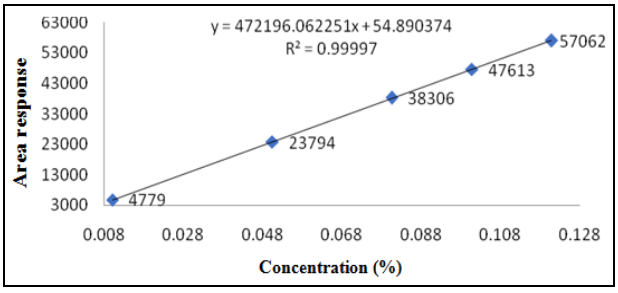

Fig. 3: Linearity of gliclazide

Table 6: 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			

Discussion

From the statistical treatment of the linearity data, it was clear that the response of Gliclazide was linear between 50 to 120% of working concentration. The Correlation Coefficient (R) should be NLT 0.98 respectively.

Discussion

From the above results, it was concluded that the range of the method is from 50% to120% of working concentration.

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 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.

Discussion

From the above results, it was concluded that the recovery is well within the limit. Hence the method is accurate.

Precision

System precision

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

Table 7: % of 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 8: 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 9: 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 10: 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 11: 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		
	0.0828	0.0827	99.88	98.43-99.88	
	0.0828	0.0815	98.43		
100%	0.1035	0.1037	100.19		
	0.1035	0.1036	100.10	100.10-100.77	Between 80 and 120%
	0.1035	0.1043	100.77		
120%	0.1243	0.1230	98.95		
	0.1243	0.1227	98.71	98.39-98.95	
	0.1243	0.1223	98.39		

Table 12: 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 13: 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 method precision was performed.

Discussion

From the above results, it was concluded that the method is precise.

Intermediate precision (Ruggedness)

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

Table 14: 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	

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.

Mobile phase stability

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

Discussion

Above results indicate from the date of preparation mobile phase is stable for 3 d.

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.

Table 15: 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	

Table 16: 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 HR	2.19	Not observed	0.01	0.02
After 48th HR	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 17: 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%

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.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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