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Analytical Method Development and Validation by RP-HPLC for Quantitative Determination of Glimepiride in pharmaceutical formulations

Neeru Sharma[1], Varsha Rani[1], Nikita Kaushik[2], Rajat Arora[3], Meena Yadav[1*]

1.Department of Chemistry, School of Physical Sciences, Starex University, Gurugram-122413, Haryana, India

2.School of Pharmaceutical Sciences, Starex University, Gurugram-122413, Haryana, India

3.Department of Chemistry, Deenbandhu Chhotu Ram University of Science and Technology, Murthal-131039, Haryana, India

ABSTRACT

Current study has developed two precise and direct RP-HPLC approaches for quantitative investigation of glimepiride (GLM) in both mass and pharmaceutical formulations. Glimepiride was analyzed using the RP-HPLC method with C-18 stationary phase and mobile phase of methanol and phosphate buffer (PBS) at pH 4.0 in equivalent volume ratio. The location was established at 239 nm wavelength, and the adaptable stage was extracted at a rate of 0.5 mL/mi. The retention time was observed at 2.470 minutes. Present approach was authenticated in terms of linearity, accurateness, precision, system applicability, limit of detection (LOD), limit of quantification (LOQ), robustness, and ruggedness. It has been demonstrated that the suggested approach is appropriate for monotonous examination of glimepiride in dose and bulk forms, yielding precise results. This method was employed to determine a compound's concentration in commercial pharmaceutical dosage forms. In comparison to alternative chromatographic techniques, this method is more direct, precise, and reproducible, rendering it a superior choice for routine quality control.

Keywords: RP-HPLC, Glimepiride, Validation, Chromatography, Pharmaceutical formulation

*Corresponding Author Email: meenuyadav333@gmail.com

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INTRODUCTION

HPLC is the most prevalent and versatile chromatographic technique. In a real division process during the fluid phase, which involves transfer between the stationary phase (a solid sorbent contained within a segment) and the mobile phase (a flowing liquid) sample is distinguished into its individual components [1, 2]. To generate a chromatogram, a web-based identifier verifies that each isolated component in the flowing segment is centrally positioned [3]. High-Performance Liquid Chromatography (HPLC) is predominant and utmost informative advancement for quantitative analysis of biomolecules, polymers, and other substances in pharmaceuticals [4, 5].

HPLC restricts solubility within enclosed sections containing minute particles under elevated pressure to attain optimal separations. This system can distinguish and identify species across various natural, inorganic, and organic materials [6]. The purpose of the research is to establish an RP-HPLC procedure for quantification of glimepiride (GLM) in mass and pharmaceutical formulations [7, 8].

As an oral hypoglycaemic medication, GLM, a powerful prime III-generation sulfonylurea derivative, is frequently used to treat non-insulin-dependent type II diabetic mellitus [9]. Its chemical formula is {p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamide) ethyl] phenyl) sulfonyl}-3-(trans-4-methylcyclohexyl) urea[10, 11].

Comparable to other sulfonylureas, Glimepiride functions as an insulin excretion that lowers body fluid glucose by stimulating extra-pancreatic upshots (expanding peripheral tissue perceptivity to insulin) and stimulating insulin secretions from functioning pancreatic beta cells [7, 12]. Glimepiride may attach to ATP-sensitive potassium network receptors on the surface of pancreatic beta cells, dropping potassium conductance through the membrane and depolarizing it, which in turn encourages the influx of calcium ions thru voltage-sensitive calcium networks [13]. Insulin release is triggered by this rise in intracellular calcium ion concentration.

For medication of non-insulin reliant on (type II) diabetic mellitus, it can be used along metformin, thiazolidinediones, alpha-glucosidase scavengers, and insulin [14]. The gastrointestinal tract fully absorbs it after oral dosing. Toxic consequences include severe hypoglycaemia reactions that result in coma, seizures, or other neurological damage. Aplastic and hemolytic anemias, cholestatic jaundice, agranulocytosis, rashes, widespread hypersensitivity reactions, and nausea and vomiting are other side effects of sulfonylureas. [11, 15, 16].

A thorough review of the literature showed that many different approaches are being documented for qualitative and quantitative analysis of GLM in biological samples plasma, serum, and urine and in pharmaceutical formulations containing single drug as well as in combination with other

drugs [9, 11]. Methods have also been developed for the estimation of GLM in combination with other drugs simultaneously in pharmaceutical formulations by RP-HPLC techniques [12, 17]. According to the literature review, HPLC techniques have been applied most frequently for GLM analysis [18, 19].

Due to their time-consuming nature, high detection limits, usage of excess organic diluters, laborious sample formulation, costly equipment, besides lengthy chromatographical run times, the majority of the older techniques are not optimal [20]. Dissolution studies have been a vital tool in the pharmaceutical industry in recent years due to the fact that a drug must first be solubilized to be captivated and made accessible to the systemic movement [21,22]. As a result, dissolution studies are utilized not only to examine consistency of drug release from solid dosage forms from batch to batch but also in several critical stages of formulation development for screening and appropriate evaluation of various formulations. Furthermore, the successful characterisation of the in vivo behaviour of medications has been made possible by the data gathered from in vitro dissolution experiments [23, 24]. The designed and verified approach can be used as a means for pharmaceutical dosage form excellence control since it is quick, repeatable, has a straightforward moveable phase, uncomplicated sample preparation processes, enhanced sensitivity, and brief chromatographical run time. In current study, we developed a sensitive and straightforward RP-HPLC process to quantify glimepiride in mass medications and drug plans.

MATERIALS AND METHOD

Chemicals and Reagents

Standard reference samples for Glimepiride were acquired from the commercial marketplace of India (Reddy's laboratory). Remaining samples were procured from retail drug market as pharmaceutical doses (Cipla Ind). Rest of the chemicals and reagents were obtained from market for HPLC procedure (HPLC grade mark India). All chemicals and substances used were of analytical grade (AR).

Mobile Phases

Glimepiride was assessed in a variety of solvent systems and subsequently introduced as a pure compound into the HPLC system. The ideal circumstances for glimepiride separation were determined through the analysis of a variety of portable stages, such as methanol and water or methanol and a variety of phosphate buffers with pH values of 6, 5.5, and 3.5. In comparison to other mobile phases, methanol, and potassium dihydrogen orthophosphate buffer (pH: 4) produced superior results. Ultimately, it was shown that methanol and phosphate buffer were the most

adaptable stage components. The drug exhibited a moderate retention time, elevated resolution, and sufficient peak regularity in this stage of mobility.

Phosphate buffer preparation

7.0 g of KH_2PO_4 was dissolved in 1000 mL of HPLC-grade water, and the resultant solution was transferred to a 1000 mL volumetric flask. The pH was elevated to 4.0 utilizing orthophosphoric acid [25]. Prepare the mobile phase by mixing 500 mL of the specified buffer with 500 mL of HPLC-grade methanol, then degas the solution for five minutes using an ultrasonic water bath. Traverse a 0.45-channel vacuum filter.

Working Standard Solutions

Approximately 10 mg of glimepiride was measured in a 100 mL volumetric bottle, dissolved in 50 mL of methanol, and subsequently diluted by methanol to achieve required concentration. The subsequent configuration was executed utilizing Whatman channel paper No. 41 [26].

Solution Samples

Twenty tablets were meticulously crushed following precise weighing. Glimepiride tablet powder, comprising 10 mg, was sonicated and subsequently filtered using Whatman channel paper (No. 41) in a 100 mL volumetric flask containing 50 mL of methanol. The channel paper was purified with additional solvent to obtain the filtrate. A comparable dissolvable was utilized to modify filtrate volume in line with the inscription, yielding 100 µg/mL concentration. The resultant configuration was directed utilizing Whatman channel paper [25].

Column and mobile phase Maintenance

The columns were conditioned and eluted with HPLC-grade methanol for 30 minutes at a flow rate of 1 mL per minute before subsequent HPLC analysis. To eliminate any potential remnants from the column's previous operation. The mobile phase, having been filtered and degassed, was subsequently introduced into the pipe [27]. A newly formulated mobile phase was employed to prime distinct channels. Validation of the RP-HPLC methodology. The endorsement of a comprehensive technique is the predominant approach employed in research facility inquiries to verify that the method meets the criteria for the anticipated

Linearity Curve

Various 10 mL volumetric bottles were filled by suitable aliquot part of standard Glimepiride stock solutions (100 mg/mL). The resultant configuration was subsequently diluted to achieve concluding concentrations of 10 µg/mL to 50 µg/mL. The solutions were introduced in the chromatographical apparatus. Now, the peak area was plotted against the applied concentration of Glimepiride to establish the calibration curve for the drug under study.

Precision

Assessments of intraday besides interday variations demonstrated method's accurateness. The intraday tests comprised six repeated infusions of a standard arrangement, and the percent RSD and reaction component of the medication were determined. A chromatogram was observable. During the between-day variability preliminaries, the drug's response component and percent RSD were determined following six consecutive days of standard solution infusion repetitions. The collected data indicated that the developed method was deemed accurate.

Accuracy

The extent to which test results align with the true value is the method's accuracy. After weighing and grinding twenty tablets from each formulation, the precision of the results was assessed. The conventional option strategy, incorporating a defined metric of standard medicine response for specified preparations (50%, 100%, and 150 %), was employed to perform rehabilitation assessments.

LOD and LOQ

As progressively lower concentrations of standard solutions are introduced, Limit of Detection of established strategy is not entirely established by means of synthesized RP-HPLC method. The analyte concentration at which a detectable response can be achieved (signal-to-noise ratio of 3:1) is referred to as the limit of detection (LOD). LOQ analyses were established by adopting three-fold multiplication process based on the strength of the LOD. Both LOD and LOQ values were calculated by adopting equations 1 and 2, respectively:

$$LOD = 3.3\times\sigma/S \quad (1)$$

$$LOQ = 10\times\sigma/S \quad (2)$$

where σ = response standard deviation and S = slope for standardization curve of analyte.

Roughness

Prior to producing the final volume using the versatile stage, 10 mg of glimepiride was measured and diluted in 100 mL volumetric beaker with a transportable stage (50 mL). Subsequently, they underwent sonication for a duration of 30 minutes. A 10 mL volumetric cup was filled with the standard stock solution, which was dispensed in 3 mL increments using a pipette. The required volume was subsequently attained by filling the cup with adaptable stage material [28].

Robustness

To evaluate the efficacy of a strategy, various operational parameters are modified within a practical range, including pH, flow speed, column temperature, infusion volume, and mobile phase conformation [18, 19]. The precise quantitative effect of these alterations remains uncertain. The

chromatograms exhibited no noticeable alterations, indicating the reliability of the RP-HPLC method outlined.

RESULTS AND DISCUSSION

The development of a systematic approach for RP-HPLC drug selection has garnered considerable attention due to its importance in prescription and medication quality control as shown in Table 1. The aim of present study is to establish an RP-HPLC method for analyzing Glimepiride in bulk medicine and drug formulation utilizing widely employed RP-C18 column with UV detection [17]. Every example was pervaded several times within five-minute duration. The maintenance duration was established as 2.470 ± 0.032 minutes as portrayed in Figure 1(a, b). Upon analyzing the centralizations of Glimepiride and their corresponding peak areas for relapse through the least squares method, a strong direct correlation (R^2= 0.9997) was identified amid concentrations of Glimepiride and their individual peak areas within concentration assortment of 10 µg/mL to 50 µg/mL as shown in Table 2. The relapse condition for Glimepiride is defined as Y = 109290X – 17080, with 'X' representing the Glimepiride grouping and 'Y' denoting the apex region as portrayed in Figure 2.

Table 1: Characterization Parameters for Glimepiride

Parameters	HPLC
Standardization range (µg/mL)	10-50
Detectable wavelength (nm)	238.5
Mobile stage {methanol: 0.051 M)- phosphate buffer (PBS)} (1:1 v/v; pH: 4.0)	50:50
Retention period (min)	2.470 ± 0.032
Regression linear equation (Y*)	Y = 109280 X -17081
Slope of linear fit (m)	109292
Intercept of calibration curve (c)	-17081
Correlation coefficient (R^2)	0.9997
LOD (µg/mL)	0.02
LOQ (µg/mL)	0.07

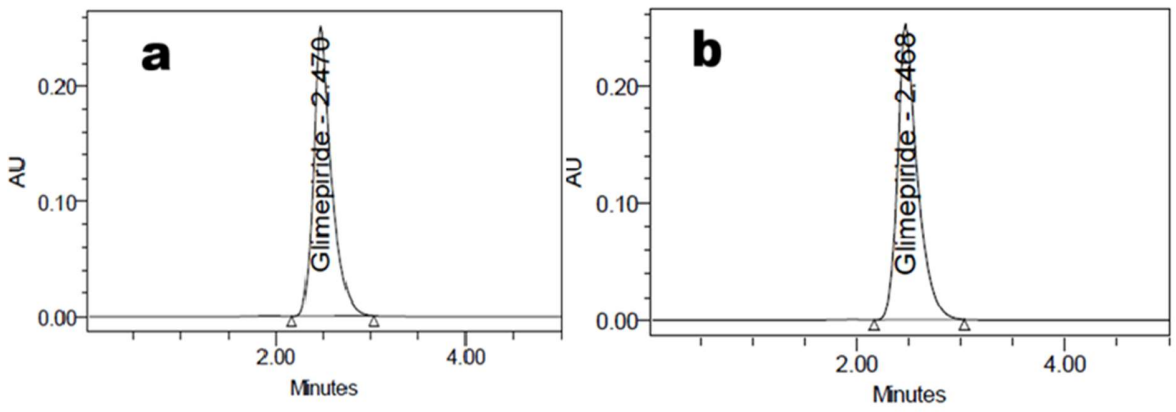

Figure 1: HPLC curve for Glimepiride as (a) Standard and (b) Test at 239 nm

Table 2: Glimepiride- Adjustment - HPLC

S. No.	Concentrations (µg/mL)	Area
1	0	00
2	10	10735
3	20	21574
4	30	32968
5	40	43029
6	50	54803

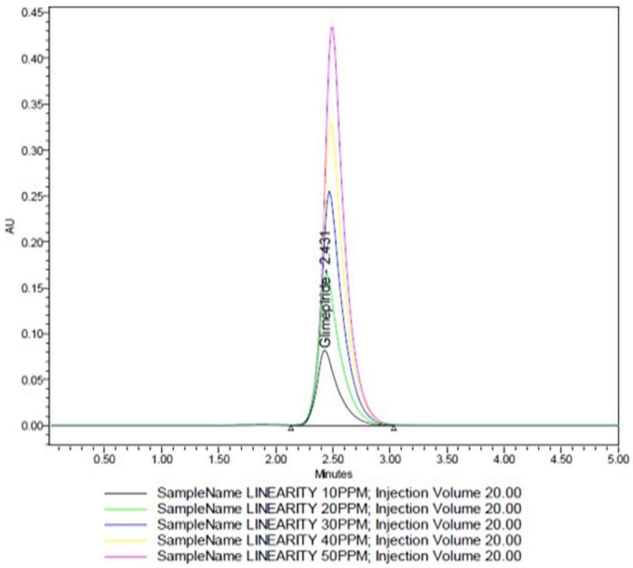

Figure 2: Chromatograms for different drug (GLM) concentrations

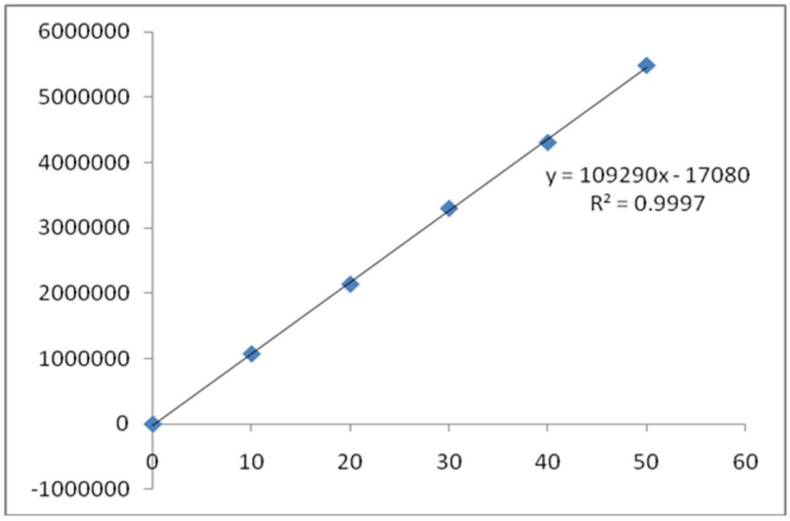

Figure 3: Calibration Curve for different Glimepiride concentrations

Linearity was observed within the concentration range of 10–50 µg/mL as portrayed in **Figure 3.** The relationship coefficient (R^2) was calculated to be 0.9997. The mass medication was

administered accurately. The percent RSD for Accuracy both within and between days was 0.20 and 0.086, respectively, following normalization as depicted in Table 3. As each percent RSD measurement fell the process is within two standard deviations. was deemed accurate. This study employed the RP-HPLC method to quantify glimepiride formulations in tablet form. A group of analysts evaluated Glimepiride tablets, each containing 1 mg of the drug. The accuracy percentage was calculated using the mean area. The results typically range from 98% to 102%. Glimepiride assays were performed by multiple analysts on different days. The assay's percentage was obtained to be within acceptable range of 98% to 102% portrayed in Table 4.

Table 3: Precise HPLC results for Glimepiride

S. No.	Concentrations(µg/mL)	Intra day	Inter day
1	30	3305558	3305441
2	30	3292266	3297255
3	30	3293512	3302419
4	30	3285834	3302324
5	30	3296448	3302637
6	30	3298422	3299541
Average	-	3295354	3301621
SD*	-	6606.32	2836.58
%RSD*	-	0.22	0.083

Table 4: Drug recapture for Glimepiride

Level of recovery (%)	Amount of drug-added (µg)	Amount of drug-Recovered (µg) *	% Recovery ± SD*
50	5	4.92	99.3±0.44
100	10	9.98	99.4±0.37
150	15	14.76	98.1±0.48

Table 5 showed chromatograms of the drug arrangement were obtained at various stream rates of 0.4 mL/min, 0.5 mL/min, and 0.6 mL/min, respectively. While maintaining a constant mobile phase composition of methanol and phosphate buffer (pH: 4) in a 1:1 (v/v) ratio. The peaks were acute at flow rate of 0.5 mL/min; however, the remainder of the stream remained excessively elevated to be considered acceptable. Thus, flow rate, 0.5 mL/min was sustained throughout evaluation with Roughness study for Glimepiride depicted in Table 6.

Table 5: **Robustness (At different flow rates)**

S. No.	Flow Rate (mL/min)	System Suitability Results		Retention Time (t_R)
		Plate Count	Tailing	
1	0.40	2812	1.20	2.7360
2	*0.50	2874	1.30	2.4730
3	0.60	2798	1.20	2.2240

Table 6: **Roughness study for Glimepiride**

Samples	Claim At label (mg)	Analyst I		Analyst II	
		Amount found (mg)	% Recovery ± SD**	Amount found (mg)	% Recovery ± SD**
1	1.00	1.00370	100.38±0.0732	1.00270	100.27± 0.09160
2	1.00	1.00250	100.26±0.1146	1.00240	100.24±0.07940

Chromatograms of drug arrangements were acquired by altering the proportions of the portable stage, specifically methanol: phosphate buffer (0.051 M, pH: 4) in ratios of 60:40, 50:50, and 40:60 v/v, while maintaining a constant flow rate of 0.5 mL/min. The pinnacles were newly constructed alongside the portable stage (50:50 v/v methanol: phosphate cradle). The ratio of the portable stage (methanol: phosphate support, 50:50 v/v) was maintained consistently throughout the study.

CONCLUSION

Glimepiride is officially listed as a medication in the British Pharmacopoeia. Glimepiride, a long-acting anti-diabetic agent from the sulfonylurea class, aids in sustaining a more naturally regulated insulin excretion during physical effort. The objectives of present study were justified by developing a novel, streamlined, more accurate, and cost-efficient HPLC technique for identification of Glimepiride as a functional drug in pharmaceutical formulations, free from interference by various constituents in the analyses.

STATEMENTS AND DECLARATIONS

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Credit authorship contribution statement

Credit authorship contribution statement

Neeru Sharma: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Resources; Validation; Roles/Writing - original draft

Varsha Rani: Formal analysis, Data curation, Resources

Nikita Kaushik: Formal analysis, Data curation, Resources

Rajat Arora: Formal analysis, Data curation, Resources

Meena Yadav: Conceptualization; Supervision; Methodology; Formal analysis, Investigation, Validation; Visualization; Writing - review and editing

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