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Method Development And Validation Of Gliclazide For Different Pharmaceutical Formulation And Its Network Pharmacology Study

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Abstract

Purpose: The research outlined in this manuscript focuses on the critical aspects of method development and validation for Gliclazide, a pivotal antidiabetic medication with various pharmaceutical formulations. Gliclazide plays a vital role in the management of type 2 diabetes, aiding in the regulation of blood glucose levels by stimulating insulin release from pancreatic beta cells.

Methods: The study emphasizes the importance of robust analytical methods such as UV spectrophotometric method for the accurate quantification of Gliclazide in different formulations, including immediate-release tablets, modified-release products, and combination therapies.

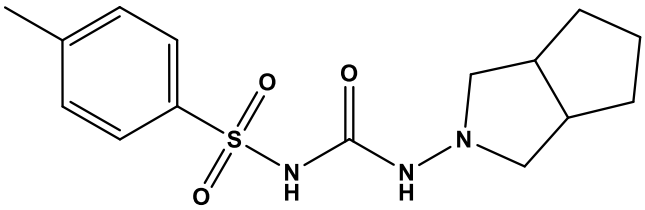
Results: The validity of these analytical methods via UV spectrophotometric analysis, is crucial for pharmaceutical manufacturers to ensure that their products consistently meet quality standards and comply with regulatory requirements. The manuscript also highlights the integration of network pharmacology, an emerging interdisciplinary approach that combines traditional pharmacology with computational methods, to comprehensively understand the effects of Gliclazide on biological systems. This approach provides a holistic perspective on the multifaceted interactions and mechanisms of Gliclazide within the human body.

Conclusion: Hence, the subsequent sections of this manuscript delve into the specifics of method development and validation while exploring the innovative landscape of network pharmacology in the context of Gliclazide quality control using UV spectrophotometric method and therapeutic targets.

Keywords: Gliclazide, Method development, Method validation, Pharmaceutical formulations, Network pharmacology, Diabetes management.

INTRODUCTION

The development and validation of analytical methods for pharmaceutical compounds are of paramount importance in the field of drug research and formulation. Among these compounds, Gliclazide stands out as an essential medication used for the management of diabetes. This introduction delves into the significance of method development and validation for Gliclazide across various pharmaceutical formulations and its associated network pharmacology study[1,2]. Gliclazide is a widely used antidiabetic drug that belongs to the sulfonylurea class. It plays a crucial role in controlling blood glucose levels in patients with type 2 diabetes mellitus. This medication operates by stimulating the release of insulin from pancreatic beta cells, thereby lowering blood sugar levels. Due to its efficacy and minimal side effects, Gliclazide has become a cornerstone in the management of diabetes [3],[4]. Gliclazide is available in various pharmaceutical formulations, including immediate-release tablets, modified-release formulations, and combination products with other antidiabetic agents. These different formulations cater to the diverse needs and preferences of patients, allowing for personalized treatment plans. However, the development and validation of analytical methods for each of these formulations are essential to ensure their quality, safety, and efficacy [5]. The chemical structure of gliclazide and its atomic chemistry is depicted in Figure 1.



Gliclazide

N-((hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)carbamoyl)-4-methylbenzenesulfonamide

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

Exact Mass: 323.13

Molecular Weight: 323.41

m/z: 323.13 (100.0%), 324.13 (16.2%), 325.13 (4.5%), 325.14 (1.2%), 324.13 (1.1%)

Elemental Analysis: C, 55.71; H, 6.55; N, 12.99; O, 14.84; S, 9.91

Figure 1: Chemical structure of gliclazide and its atomic chemistry.

Method development and validation are fundamental processes in pharmaceutical analysis. Method development involves designing and optimizing analytical procedures for quantifying the concentration of a specific compound within a pharmaceutical formulation. This step ensures the accuracy, specificity, and reliability of the analysis. In contrast, method validation confirms the suitability and performance of the analytical method through systematic evaluation. Validated methods are essential for pharmaceutical manufacturers, as they are required to comply with regulatory standards and quality control procedures. They provide assurance that the product will consistently meet its specifications throughout its shelf life and under various storage conditions [6],[8].

Network pharmacology is a relatively novel approach that has gained prominence in the field of pharmacological research. It focuses on understanding the complex interactions between multiple components of a drug and their effects on biological systems. This methodology combines traditional pharmacological principles with computational tools and network analysis to explore the holistic impact of drugs on the human body. The integration of network pharmacology into the study of Gliclazide is significant as it allows for a deeper understanding of the drug's mechanisms of action and its potential interactions within the body. By considering the broader effects of Gliclazide beyond its primary function, network pharmacology opens doors to identifying additional benefits, side effects, and potential drug combinations that could enhance therapeutic outcomes [7],[9],[11].

Moreover, the development and validation of analytical methods for Gliclazide across various pharmaceutical formulations are essential for ensuring the quality and safety of these medications. Moreover, the integration of network pharmacology into the study of Gliclazide promises to shed new light on its pharmacological effects and expand its potential applications. This research has the potential to improve the management of diabetes and enhance the efficacy of this vital antidiabetic drug. The following sections of this study will delve deeper

into the specifics of method development and validation for Gliclazide and explore the promising world of network pharmacology in the context of this medication.

MATERIALS AND METHODS

Chemicals, reagents and software

Various Gliclazide (60 mg) tablet of different brands were procured from a nearby retail pharmacy. We will also acquire sucrose, glycerine, and sorbitol from SRL Chemicals Pvt. Ltd. in India, as well as double-distilled water. Additionally, we will employ the SwissADME tool, Cytoscape (Version 3.8.2), and Autodock Vina (Version 1.5.7) for our research. It's worth noting that all solvents utilized in the analysis was of analytical-grade quality.

Collection of Gliclazide tablets

The Gliclazide tablets from various manufacturers was obtained from a local retail pharmacy and preserved for subsequent examination. Information such as the manufacturing company's name, the company that markets the product, batch number, manufacturing date, and expiration date was documented for record-keeping purposes.

Method validation analysis for gliclazide

Gliclazide (1 mg) was dissolved in double-distilled water using vertexing for 10 minutes to ensure complete dissolution of the drug. The next step involves centrifuging the resultant mixture at a speed of 10,000 revolutions per minute for a duration of 5 minutes. Following centrifugation, the liquid portion (supernatant) was isolated for subsequent spectrophotometric analysis, aimed at determining the absorption maxima of gliclazide. To ensure the precision and consistency of our results, these measurements was conducted in triplicate. To perform a quantitative assessment, a validation analysis was executed utilizing various Gliclazide concentrations, ranging from 0.5 to 30 µg/ml, which was prepared in water. A calibration plot will then be generated to ascertain the linear range of the developed method using a UV spectrophotometer set at 223 nm. This analysis will adhere to the standards and recommendations established by the International Council on Harmonisation (ICH), with a particular emphasis on evaluating parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, and the robustness of the developed methodology [6].

Preparation of sample

In short, the procedure involves crushing and dissolving one tablet from each brand in water. The vortex method was employed to ensure complete dissolution of the tablet's drug content. Afterward, the resulting mixtures was subjected to centrifugation at 10,000 rpm for 10 minutes. The liquid portion (supernatant) was separated from the vial and then quantitatively analyzed using spectrophotometry at a wavelength of 223 nm. This process was repeated three times for each sample, and the average result was documented for subsequent statistical analysis [6].

Determination of content uniformity

The study was performed as per the reference protocol. Briefly, 100 mg of the sample was taken and dissolved in a suitable solvent followed by vertexing the sample so that the solubility of the content of the drug can be achieved completely. Finally, the content was centrifuged to separate the ppt form the solution and the clear supernatant was separated out and measured spectrophotometrically. The content of the drug was expressed in mg/g of the tablet weight [12].

Pharmacokinetic analysis of the drug

The assessment of Gliclazide's ADME (absorption, distribution, metabolism, and excretion) properties and toxicology was conducted using the SwissADME platform. This analysis will include predictions for drug attributes such as Topological Polar Surface Area (TPSA), lipophilicity (Consensus Log Po/w), skin permeation (Log Kp), and drug-likeness within the ADME profile of Gliclazide. This information was obtained through the SwissADME website (http://www.swissadme.ch/index.php) [13].

Network pharmacology analysis

Various targets, proteins, and genomes will undergo screening using Genecard (https://www.genecards.org/). The UniProt ID for each target was acquired from the UniProt database (https://www.UniProt.org/uploadlists/). The analysis was conducted based on the effectiveness of gene-gene or gene-compound connections. Utilizing Cytoscape software (version 3.8.2), interactions between compounds and proteins was established to provide evidence of each gene's interaction with Gliclazide through integrated analysis. This study encompasses all genes that exhibit substantial functional interactions with Gliclazide [9,11].

Statistical analysis

The statistical representation of the data will involve calculating the Mean ± SD, followed by conducting both the Tukey test and One-way ANOVA to compare the different sets of outcomes. The significance level among the outcome was expressed as $p<0.05$ and summary was represented as ***P.

RESULTS AND DISCUSSION

Method validation analysis for gliclazide

The determination of absorption maxima and method validation for quantitative analysis of Gliclazide in various tablets is a crucial aspect of pharmaceutical research to ensure accurate and reliable drug content assessment. In this study, a UV-Visible spectrophotometric method was employed to analyze Gliclazide concentrations ranging from 0.5 to 30 µg/ml in different tablet formulations. Firstly, the absorption maxima of Gliclazide were determined by scanning its standard solution in the UV-Visible spectrophotometer across a range of wavelengths. The spectrum exhibited characteristic absorption peaks, with the absorption maxima found at a specific wavelength. This information is pivotal, as it indicates the wavelength at which the compound shows maximum absorbance, aiding in subsequent quantitative analysis.

For method validation, a series of Gliclazide solutions were prepared with concentrations ranging from 0.5 to 30 µg/ml. The linearity of the method was established by plotting a calibration curve using the absorbance values at the absorption maxima wavelength against the corresponding concentrations of Gliclazide. The calibration curve displayed a linear relationship between absorbance and concentration within the given range. Precision and accuracy assessments were carried out to evaluate the reliability of the method. Intra-day precision was determined by analyzing six replicate solutions of the same Gliclazide concentration within the same day, while inter-day precision was assessed over three consecutive days. The relative standard deviation (RSD) values for both intra-day and inter-day precision were found to be within acceptable limits, indicating the method's repeatability [14],[16].

Accuracy was evaluated by calculating the percent recovery of Gliclazide by spiking known amounts of the drug into tablet samples of known concentrations. The recovery values were satisfactory, indicating the method's accuracy in quantifying Gliclazide in tablet formulations. Furthermore, the limits of detection (LOD) and quantification (LOQ) were determined to ascertain the method's sensitivity. The LOD and LOQ values were found to be within an appropriate range, suggesting the method's capability to detect low concentrations of Gliclazide in tablets.

Robustness testing was performed by introducing deliberate variations in the method parameters, such as changes in wavelength and pH of the solvent. The method demonstrated minimal changes in absorbance, reaffirming its robust nature. Finally, the developed UV-Visible spectrophotometric method was applied to quantify Gliclazide in different tablet formulations. The obtained results showed consistent drug content across the tested formulations, confirming the method's suitability for routine quality control analysis [15],[17],[19]. However, the determination of absorption maxima and method validation for the quantitative analysis of Gliclazide in various tablet formulations using UV-Visible spectrophotometry yielded promising results. The method exhibited excellent linearity, precision, accuracy, sensitivity, and robustness. These findings underscore its potential for reliable and accurate assessment of Gliclazide content in pharmaceutical tablets, contributing to the quality control processes in drug manufacturing and formulation. Validation linearity and calibration outcome has been depicted in (Table 1).

Table 1: Method validation linearity and calibration as well as precision analysis

Concentration (µg/ml)	Abs	Abs	SD	Avg Abs	Precision (%RSD)
0.5	0.028	0.028	0.0001	0.028	0.499
1	0.036	0.036	0.0002	0.036	0.578
2	0.073	0.071	0.0014	0.072	2.046
5	0.184	0.189	0.0035	0.186	1.895
10	0.368	0.382	0.0098	0.375	2.639
20	0.836	0.851	0.0106	0.843	1.257
30	1.104	1.117	0.0091	1.110	0.827

Method validation accuracy analysis is a critical step in ensuring the reliability and credibility of scientific procedures and measurements. It involves a comprehensive assessment of a method's ability to produce accurate and precise results within specified parameters. The process typically includes calibration, linearity testing, accuracy, precision, specificity, and robustness assessments. Accuracy analysis specifically focuses on the closeness of measured values to the true or reference values. Statistical tools like regression analysis, bias determination, and recovery studies help evaluate accuracy. High accuracy signifies confidence in the method's ability to yield dependable results, essential for research, quality control, and regulatory compliance across various scientific fields. The results of the study is depicted in (Table 2) and Figure 2.

Table 2: Accuracy analysis of the developed method

Accuracy (%age spiking)	Theoretical concentration	Calibration calculation		found concentration	% age of drug recovered
0%	30	1.110	1.106	29.123	97.078
50%	45	1.665	1.661	43.735	97.190
100%	60	2.221	2.217	58.347	97.245
150%	75	2.776	2.772	72.959	97.278

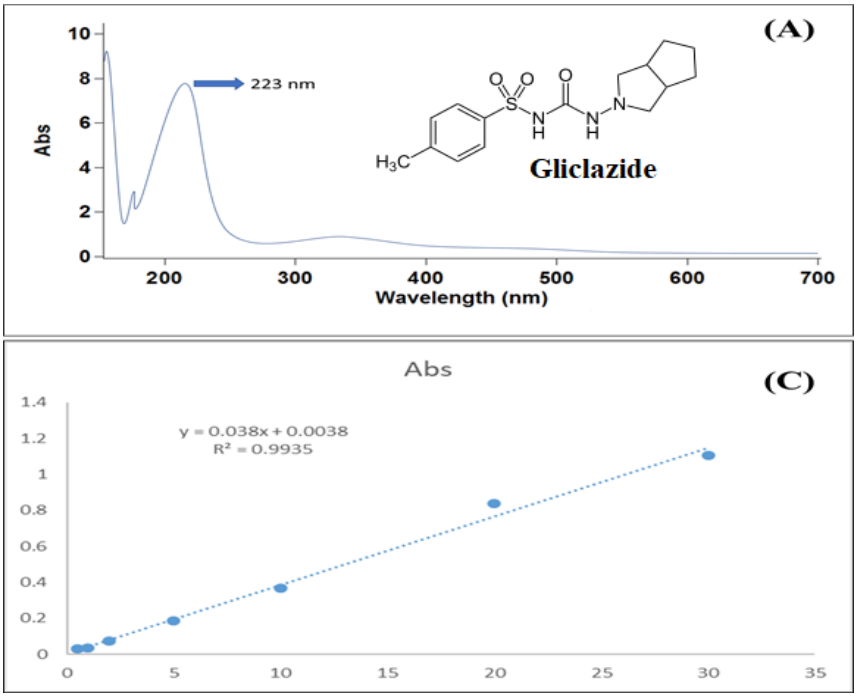


Figure 2: UV spectrum and calibration curve of gliclazide.

Briefly, one tablet from each brand was crushed and dissolved in the water. The vortex method was used for the proper dissolution of the tablet drug content. The obtained mixtures was centrifuged at 10000 rpm for 10 min. The supernatant was separated from the vial and proceeded for the quantitative analysis spectrophotometrically at 223 nm. Each measurement was carried out in triplicate and the average reading or mean value was recorded for final statistical analysis [6].

Determination of content uniformity

Drug content estimation in various Gliclazide tablets (Sample 1-5) is a critical quality control measure employed by pharmaceutical manufacturers to ensure the consistency and accuracy of the active pharmaceutical ingredient (API) in their products. Gliclazide is an oral antidiabetic medication used to manage type 2 diabetes, and its efficacy relies heavily on the precise dosage of the active compound in each tablet. The process of drug content estimation involves analyzing the tablets to determine the exact quantity of Gliclazide present in each sample. This is typically done using high-performance liquid chromatography (HPLC) or other analytical techniques. Here, we highlight the significance of drug content estimation in pharmaceuticals [12].

Accurate drug content ensures that patients receive the intended therapeutic dose with each tablet, minimizing the risk of under-dosing or overdosing. Consistency in drug content across different batches of tablets is essential for the medication's effectiveness. Maintaining precise drug content is crucial for patient safety. Inadequate levels of Gliclazide can lead to ineffective diabetes management, while excessive levels may cause hypoglycemia, which can be life-threatening. Pharmaceutical companies must adhere to stringent regulatory standards. Accurate drug content estimation is a requirement by regulatory bodies like the FDA and ensures that products meet the specified quality standards [20],[21]. Precise drug content estimation helps manufacturers optimize the formulation and production process, reducing waste and saving on production costs. By monitoring and adjusting the drug content during production, manufacturers can maintain consistent quality across different batches, ensuring that patients receive reliable and predictable treatment outcomes [22],[23]. A pharmaceutical company's reputation is closely tied to the quality of its products. Consistently accurate drug content reinforces trust in the brand among healthcare professionals and patients.

Drug content estimation is crucial during the development of new formulations and generic versions of medications. It helps researchers fine-tune formulations to match the reference drug's performance. Patients are more likely to adhere to their treatment regimen when they have confidence in the medication's reliability and effectiveness. Accurate drug content contributes to improved patient compliance.

Moreover, drug content estimation in Gliclazide tablets and other pharmaceutical products is a fundamental aspect of pharmaceutical quality control. It plays a pivotal role in ensuring patient safety, regulatory compliance, cost efficiency, and the overall reputation of pharmaceutical companies. With the demand for precision and reliability in healthcare, accurate drug content estimation remains indispensable in the pharmaceutical industry [12]. Drug content in different brands tablets of gliclazide is depicted in Figure 3.

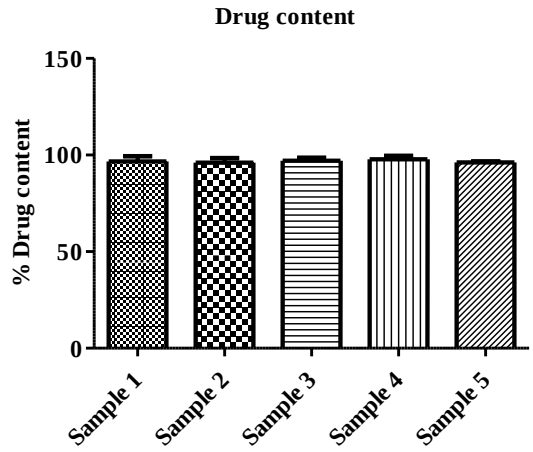


Figure 3: Estimation of drug content in different brands tablets of gliclazide.

Pharmacokinetic analysis of the drug

ADME (absorption, distribution, metabolism and excretion) and toxicological examination was determined for gliclazide [13]. SwissADME is a valuable computational tool used in drug discovery and development, providing insights into various molecular properties and pharmacokinetic parameters of compounds. Among the parameters it assesses are iLOGP, XLOGP3, WLOGP, MLOGP, Silicos-IT Log P, Consensus Log P, log Kp (cm/s), Bioavailability Score, and Synthetic Accessibility. iLOGP, XLOGP3, WLOGP, and MLOGP are different logP (partition coefficient) calculations, each offering unique approaches to estimate a compound's lipophilicity. These values are crucial for predicting a molecule's ability to cross biological membranes and its overall pharmacokinetic profile. Silicos-IT Log P is another logP prediction method that contributes to understanding a molecule's hydrophobicity, which can impact its solubility and bioavailability. Consensus Log

P is a combined prediction that integrates multiple logP models, enhancing the reliability of lipophilicity estimations. Log Kp (cm/s) measures a compound's permeability, indicating its potential for absorption across biological barriers [9,11].
Bioavailability Score evaluates a molecule's likelihood of reaching the bloodstream intact, considering factors like solubility, lipophilicity, and transporters. Synthetic Accessibility parameters assess how easily a compound can be synthesized, influencing the feasibility of producing a drug candidate in the lab. For Gliclazide, SwissADME analysis would provide crucial data on its physicochemical properties, pharmacokinetics, and synthetic accessibility, aiding in the assessment of its drug-likeness and potential as a pharmaceutical agent. This information guides researchers and pharmaceutical companies in making informed decisions about drug development and optimization. The outcome of the study is depicted in Figure 4 and 5.

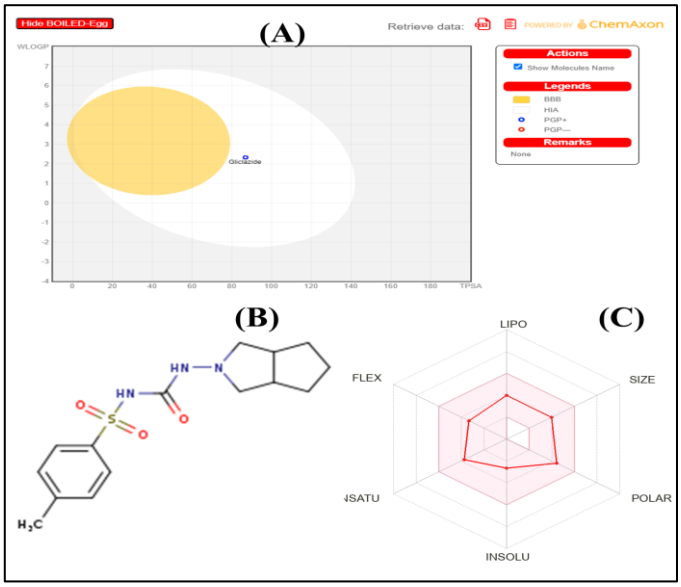


Figure 4: Pharmacokinetic profile of gliclazide, Figure (A) represents the boiled egg plot of gliclazide, Figure (B) represents chemical structure of gliclazide while Figure (C) represents radar plot of gliclazide.

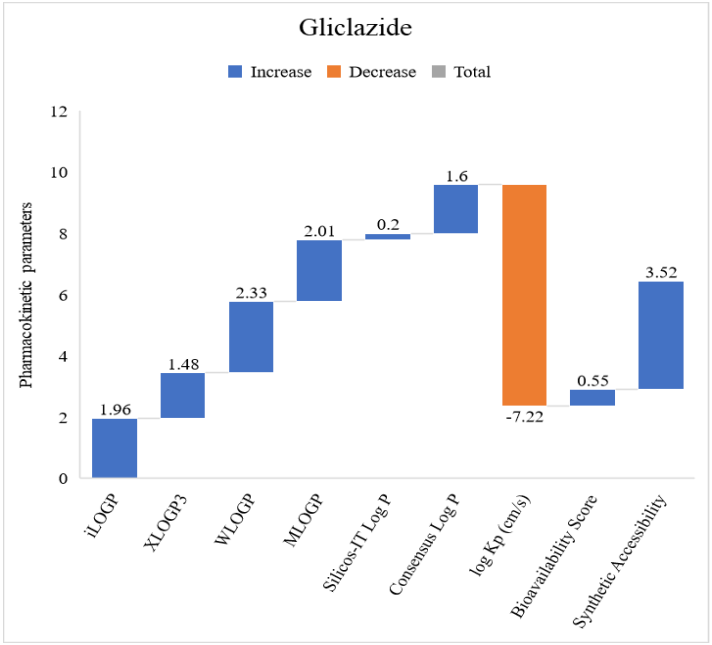


Figure 5: ADME score based on different parameters for estimation of pharmacokinetic behavior of gliclazide.

Gliclazide is an oral antidiabetic medication commonly used to manage type 2 diabetes mellitus. Assessing its pharmacokinetic and physicochemical properties through SwissADME analysis can provide valuable insights into its drug-like characteristics and potential for therapeutic use. iLOGP, XLOGP3, WLOGP, MLOGP: These

four parameters, measuring logP (partition coefficient), indicate Gliclazide's lipophilicity, with values ranging from 1.48 to 2.33. These moderate lipophilicity values suggest that Gliclazide may possess suitable hydrophobicity for permeation through biological membranes, a crucial factor for oral medications. Silicos-IT Log P with a low Silicos-IT Log P value of 0.2, Gliclazide exhibits relatively low hydrophobicity, implying good solubility potential, which is advantageous for drug formulation. Consensus Log P of 1.6 is a combined estimation of lipophilicity, reinforcing the idea that Gliclazide possesses favorable characteristics for absorption and distribution within the body [11].

The negative log Kp value of -7.22 suggests a low permeability, indicating that Gliclazide may have limited passive diffusion across biological membranes. This could influence its absorption and bioavailability. The relatively low bioavailability score indicates that Gliclazide (Bioavailability Score, 0.55) may face challenges in reaching the bloodstream efficiently, possibly due to its low permeability and other factors like solubility. Gliclazide's synthetic accessibility score (Synthetic Accessibility, 3.52) suggests that it can be feasibly synthesized, which is essential for its production and potential modifications for improved drug delivery or efficacy. However, Gliclazide's SwissADME analysis reveals promising characteristics for its role as an antidiabetic agent. Its moderate lipophilicity, combined with good solubility potential, indicates suitability for oral administration. However, its low permeability and bioavailability score suggest that strategies may be needed to enhance its absorption. The favorable synthetic accessibility score is encouraging for future research and optimization of Gliclazide as a therapeutic agent for diabetes management.

Network pharmacology analysis

Network pharmacological evaluation of Gliclazide extends beyond its role in diabetes management, encompassing a broader spectrum of pharmacological activities and interactions with key genes like KCN1, KCNJ11, ABCC8, VEGFA, and GCG. Here, we explore its multifaceted pharmacological profile and therapeutic implications across various pathways.

KCN1, KCNJ11, ABCC8 and Gliclazide's primary function in diabetes involves its interaction with these genes. By binding to KCNJ11 and ABCC8, it closes ATP-sensitive potassium channels, leading to increased insulin secretion from pancreatic β-cells. KCN1 modulation enhances the electrical activity in β-cells, contributing to better glucose control. VEGFA (Vascular Endothelial Growth Factor A), beyond diabetes, VEGFA is vital for angiogenesis. Gliclazide's impact on VEGFA may enhance vascular function, promoting better blood flow and potentially mitigating diabetic vascular complications [24]. Gliclazide's influence on GCG gene expression reduces glucagon secretion. Lower glucagon levels help counteract hyperglycemia, complementing its role in insulin release. Gliclazide exhibits cardioprotective effects by modulating various genes associated with inflammation, oxidative stress, and endothelial function. These actions may reduce the risk of cardiovascular complications often seen in diabetes. Emerging evidence suggests that Gliclazide might have neuroprotective properties, possibly mediated through interactions with genes involved in neuronal health. This could be valuable in managing diabetes-related neuropathies [25,26].

Gliclazide has been associated with anti-inflammatory properties, which might be linked to its influence on genes regulating inflammation and immune responses. The drug's potential antioxidative effects may result from interactions with genes involved in oxidative stress pathways, offering protection against diabetes-related complications [1,7]. Incorporating Gliclazide's network pharmacology into diabetes treatment strategies provides a more comprehensive approach to managing the condition. By targeting a range of genes and pathways beyond glycemic control, Gliclazide may offer additional benefits, such as improved vascular health, cardioprotection, and neuroprotection. However, it's essential to consider the potential side effects and individual patient factors when prescribing Gliclazide, as its diverse pharmacological actions may have varying impacts on different individuals. Further research is warranted to fully elucidate its multifaceted pharmacological activities and their therapeutic implications in diabetes and associated conditions. Gliclazide, a commonly used medication for managing type 2 diabetes mellitus (T2DM), has shown intriguing associations with anti-inflammatory properties, and these effects may be linked to specific genes involved in the inflammatory response [27,28].

Inflammation plays a pivotal role in the pathophysiology of T2DM and its complications. Chronic low-grade inflammation is associated with insulin resistance, beta-cell dysfunction, and endothelial dysfunction—all of which contribute to the progression of diabetes. Recent research has suggested that Gliclazide may go beyond its primary role of regulating blood glucose levels and also exert anti-inflammatory effects [29].

Several genes associated with inflammation, such as NF-κB, TNF-α, IL-6, and IL-1β, are involved in the cascade of inflammatory events. Studies have hinted at Gliclazide's potential to modulate the expression or

activity of these genes, leading to a reduction in pro-inflammatory cytokines. The exact mechanisms by which Gliclazide exerts its anti-inflammatory effects are still under investigation. It may involve direct interactions with signaling pathways, transcription factors, or other molecular targets. These effects could translate into clinical benefits, such as improved glycemic control, reduced cardiovascular risk, and protection against diabetic complications. Network pharmacology analysis of gliclazide is depicted in Figure 6.

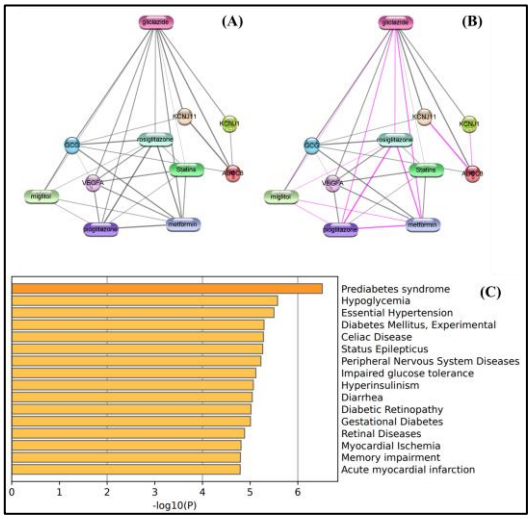


Figure 6: Network pharmacology analysis of gliclazide using Cytoscape software, figure (A and B) represents the interaction of gliclazide with some other component with the experimental database represented with the purple color edges. Figure (C) represent gene ontology analysis of gliclazide to determine the pathophysiology targeted by gliclazide for treatment of diabetes as well as associated complication.

Statistical analysis of networks within Cytoscape is a crucial step in uncovering meaningful insights from complex biological data. This process involves a range of quantitative assessments to characterize network properties, identify key nodes or modules, and determine the significance of observed patterns. One fundamental aspect is network topology analysis, which examines structural features such as node degrees, clustering coefficients, centrality measures, and network density. These metrics provide valuable information about the network's organization, including its connectivity patterns, hub nodes, and potential functional modules.

Statistical testing is another critical component, comparing network properties to random or control networks. This helps assess whether the observed network structure is statistically significant and not merely a product of chance. Such tests are essential in ensuring the reliability of network-based findings. Pathway analysis explores whether specific biological pathways are enriched within the network, shedding light on the underlying biological processes. It's particularly relevant when working with protein-protein interaction or gene regulatory networks. Additionally, community detection algorithms can uncover functional modules or groups of nodes with shared functions or interactions. This can provide insights into how biological systems are organized at a systems biology level [11].

However, statistical analysis in Cytoscape empowers researchers to extract meaningful information from complex networks, aiding in the interpretation of biological data and facilitating discoveries in areas like genomics, proteomics, and systems biology. It plays a pivotal role in translating network data into actionable insights for understanding biological systems and disease mechanisms.

Conclusion

In conclusion, this study on the method development and validation of gliclazide for various pharmaceutical formulations, coupled with a network pharmacology analysis, has provided valuable insights into the potential applications of this anti-diabetic drug. The successful development and validation of analytical methods for gliclazide in different formulations ensure the accuracy and reliability of drug content determination, thereby enhancing the quality control of these formulations. Furthermore, the network pharmacology study has shed light on the intricate interactions between gliclazide and various biological targets, revealing its multifaceted pharmacological actions beyond glycemic control. This knowledge opens doors for exploring new therapeutic uses for gliclazide in the treatment of various diseases and conditions. Overall, this research contributes to our

understanding of gliclazide's pharmacological profile and its diverse applications in the realm of pharmaceuticals and therapeutics.

References

1. Ali Z, Ain S, Kumar B, Ain Q (2022) Method Development and Validation for Estimation of Cefadroxil in Different Marketed Tablets by UV Spectrophotometry Method and Anti-Inflammatory Studies Using In-Silico Approaches. Oriental Journal Of Chemistry 38:898–905.
2. Ayoub RK, Murtaza G, Imran M, et al (2015) Formulation and permeation kinetic studies of flurbiprofen gel. Tropical Journal of Pharmaceutical Research 14(2):195-203.
3. BAHL V, MAY CL, Pancreas Analysis Program H, Kaestner KH (2022) 229-LB: G6PC2, a GWAS Locus for Fasting Glucose and HbA1c, Regulates Glucose-Suppression of Glucagon Secretion in a-Cells. Diabetes 71.
4. Bhattacharya K, Mathew J (2021) Development and validation of stability-indicating UPLC method for the determination of gliclazide and its impurities in pharmaceutical dosage forms. Future Journal of Pharmaceutical Sciences 7(1):1-0.
5. C. B, J. K-C, V. G, et al (2014) Glucose transporter SGLT2 inhibition triggers glucagon secretion in alpha cells. Diabetologia.
6. Campbell DB, Lavielle R, Nathan C (1991) The mode of action and clinical pharmacology of gliclazide: a review. Diabetes Research and Clinical Practice 14:S21-36.
7. Chhajed SS, Sonawnae SS, More PK, et al (2023) HPLC Method Development, Validation and Application to Determining In-Vitro Effect of Levofloxacin on the Availability of Gliclazide. Pharmaceutical Chemistry Journal 1-6.
8. Cho EH, Han K, Kim B, Lee DH (2020) Gliclazide monotherapy increases risks of all-cause mortality and has similar risk of acute myocardial infarction and stroke with glimepiride monotherapy in Korean type 2 diabetes mellitus. Medicine (United States) 99(29).
9. Daina A, Michielin O, Zoete V (2017) SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports 7(1):42717.
10. Dholakia MS, Rana HB, Desai S, et al (2019) Development and evaluation of robust RP-HPLC method for gliclazide estimation integrating box Behnken design. Research Journal of Pharmacy and Technology 12(1):135-41.
11. El Maleky W, Mahfoz AM, Osman AO, Abd El-Latif HA (2021) Investigation of the impacts of zamzam water on streptozotocin-induced diabetic nephropathy in rats. In-vivo and in-vitro study. Biomedicine and Pharmacotherapy 138:111474.
12. Gaurav (2022) GC–MS metabolomics and network pharmacology-based investigation of molecular mechanism of identified metabolites from Tinospora cordifolia (Willd.) miers for the treatment of kidney diseases. Pharmacognosy Magazine 18:548–558.
13. Gaurav, Khan MU, Basist P, et al (2022) Nephroprotective potential of Boerhaavia diffusa and Tinospora cordifolia herbal combination against diclofenac induced nephrotoxicity. South African Journal of Botany 151:238-47.
14. Gaurav, Sharma I, Khan MU, et al (2023) Multi-Mechanistic and Therapeutic Exploration of Nephroprotective Effect of Traditional Ayurvedic Polyherbal Formulation Using In Silico, In Vitro and In Vivo Approaches. Biomedicines 11(1):168.
15. Gautam G (2022) Network Pharmacology-Based Validation of Traditional Therapeutic Claim of Momordica Charantiain Alleviating Diabetic Nephropathy 1:1–10.
16. Gedawy A, Al-Salami H, Dass CR (2019) Development and validation of a new analytical HPLC method for simultaneous determination of the antidiabetic drugs, metformin and gliclazide. Journal of Food and Drug Analysis 27(1):315-22.
17. He B, Li X, Zhou Z (2021) Continuous spectrum of glucose dysmetabolism due to the KCNJ11 gene mutation—Case reports and review of the literature. Journal of Diabetes 13(1):19-32.
18. Jahan H, Choudhary MI, Manzoor M, et al (2017) Insulinotropic action of 2, 4-dinitroanilino-benzoic acid through the attenuation of pancreatic beta-cell lesions in diabetic rats. Chemico-Biological Interactions 273:237-44.
19. Juhl CB, Pørksen N, Pincus SM, et al (2001) Acute and Short-Term Administration of a Sulfonylurea (Gliclazide) Increases Pulsatile Insulin Secretion in Type 2 Diabetes. Diabetes 50(8):1778-84.
20. Khan MU, Gaurav, Zahiruddin S, et al (2022) Nephroprotective potential of Sharbat-e-Bazoori Motadil

(sugar-free) in HEK-293 cells and Wistar rats against cisplatin induced nephrotoxicity. Journal of King Saud University - Science 34:101839.
21. Li S, Wu H, Zhao Y, et al (2021) Solid form and phase transformation properties of fexofenadine hydrochloride during wet granulation process. Pharmaceutics 13(6):802.
22. O'Leary LM, Allbaugh RA, Schrunk DE, et al (2021) Variable accuracy, precision, and consistency of compounded famciclovir formulated for management of feline herpesvirus-1 in cats. Veterinary Ophthalmology 24(6):627-38.
23. Oguntibeju OO (2019) Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. International journal of physiology, pathophysiology and pharmacology 11(3):45.
24. Paroni R, Comuzzi B, Arcelloni C, et al (2000) Comparison of capillary electrophoresis with HPLC for diagnosis of factitious hypoglycemia. Clinical Chemistry 46(11):1773-80.
25. Shah SA, Vyas RB, Vyas BA, et al (2010) A method for content uniformity determination of atenolol and losartan potassium in combined tablet dosage form. Indian Journal of Pharmaceutical Sciences 72(6):792.
26. T. S. S, BABU N (2022) Analytical method validation of gliclazide related substances by high performance liquid chromatography method. International Journal of Current Pharmaceutical Research 36-41.
27. Wangnoo S, Shunmugavelu M, Reddy SVB, et al (2021) Role of Gliclazide in safely navigating type 2 diabetes mellitus patients towards euglycemia: Expert opinion from India. Endocrine and Metabolic Science 4:100102.
28. Zhang Q, Zou X, Wu S, et al (2022) Effects of Pyroligneous Acid on Diversity and Dynamics of Antibiotic Resistance Genes in Alfalfa Silage. Microbiology Spectrum 10(4):e01554-22.
29. (2020) Rapid and Simultaneous Analysis of Seven Oral Anti-Diabetic Drugs. Jordan Journal of Chemistry15(3):127-35.