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A NOVEL ROUTE FOR THE SYNTHESIS OF IMPURITIES G OF ANTIDIABETIC DRUG GLICLAZIDE

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ABSTRACT

The study reports the synthesis and characterization of Gliclazide Impurity G, developed for the first time with high yield and excellent purity. The synthetic strategy was optimized to ensure efficiency and reproducibility, providing a reliable pathway for laboratory and potential industrial applications. Purity assessment of the synthesized impurity was carried out using High-Performance Liquid Chromatography (HPLC), which confirmed its high chemical integrity and suitability for analytical studies. Structural elucidation was further performed by employing proton nuclear magnetic resonance (^{1}H NMR) spectroscopy and mass spectrometry, both of which provided detailed confirmation of the molecular framework and functional groups. These combined techniques validated the identity of the impurity with high precision. The successful preparation and complete characterization of Gliclazide Impurity G hold significant value for pharmaceutical quality control, regulatory submissions, and future research aimed at understanding drug metabolism, degradation pathways, and ensuring safety in therapeutic formulations.

KEYWORDS: Gliclazide Impurity G; Synthesis; Characterization; HPLC; ^{1}H NMR; Mass spectrometry.

INTRODUCTION

Gliclazide is an established oral antihyperglycemic medication widely prescribed for the management of non-insulin-dependent diabetes mellitus (NIDDM), also referred to as type 2 diabetes mellitus.[1] Belonging to the second-generation class of oral hypoglycemic sulfonylureas, gliclazide has proven to be more potent than glibenclamide, while still maintaining a pharmacological profile broadly comparable to tolbutamide and other agents in the sulfonylurea family.[2] Its therapeutic effectiveness is particularly noteworthy due to its prolonged duration of action, which extends up to twenty-four hours, allowing for convenient once-daily dosing in many patients.[3] This advantage not only enhances patient compliance but also ensures more stable glycemic control throughout the day.[4]

Beyond its primary role in glycemic regulation, gliclazide has attracted research interest for additional pharmacological properties. Independent of blood sugar management, gliclazide demonstrates antioxidant and antiplatelet activity, features that distinguish it from other sulfonylureas.[5] These secondary effects are largely attributed to the unique azabicyclo ring structure present exclusively in gliclazide.[6] While these findings are promising, the long-term clinical significance remains uncertain. Large-scale, randomized, controlled trials are still required to establish whether these properties translate into meaningful benefits such as reduced diabetic complications, improved cardiovascular outcomes, or decreased overall mortality in patients with type 2 diabetes.[7] Such investigations are vital because diabetic patients are predisposed to oxidative stress and vascular complications, and a drug that combines hypoglycemic action with antioxidant and antiplatelet activity could significantly alter treatment strategies.[8]

Impurities in Active Pharmaceutical Ingredients (APIs).

Alongside the therapeutic benefits of gliclazide, another critical aspect in pharmaceutical development is the presence of impurities. Impurities are chemical substances that coexist with the active pharmaceutical ingredient (API) either as by-products of synthesis, degradation products, or contaminants introduced during formulation and manufacturing.[9] Even at very low concentrations, impurities have the potential to alter the efficacy and safety of a drug. Consequently, impurity profiling has become an essential component of modern drug development, quality control, and regulatory evaluation. Drug safety is no longer considered solely a function of the pharmacological and toxicological properties of the API itself, but also of the impurities that may accompany it.[10]

The classification of impurities can generally be divided into two major categories.

1. Impurities related to the active medicinal ingredient – these may arise from incomplete synthesis, side reactions, or structural variations in the final compound.
2. Impurities generated during formulation, manufacturing, or storage – these may occur as degradation products or contaminants introduced during handling and packaging.[11]

The importance of monitoring and controlling impurities is underscored by the stringent standards imposed by leading pharmacopeias such as the United States Pharmacopeia (USP), Indian Pharmacopeia (IP), and British Pharmacopeia (BP).[12] These authorities have progressively tightened the permissible limits for impurities in APIs to safeguard patient health and ensure drug consistency.

Impurity Profiling in Gliclazide

In the case of gliclazide, the European and British Pharmacopoeia have documented a number of recognized impurities, including Impurity A, B, C, D, E, F, and G.[13] These structural variants are either intermediates formed during the synthetic process or degradation products arising under certain storage or formulation conditions. Identifying and quantifying these impurities is vital to ensure that gliclazide preparations meet both safety and therapeutic standards.[14]

The conventional approach of isolating impurities directly from the final API often presents challenges, as this method may not provide the required purity levels or sufficient yields for detailed analysis.[15] To overcome this limitation, synthetic methods are developed to produce pure forms of the impurities. Synthesizing these impurities in the laboratory not only allows their structural confirmation and characterization but also facilitates the study of their biological impact.[16] Once pure reference standards are available, they can serve as benchmarks in analytical methods such as chromatography and spectroscopy, thereby enabling precise identification of similar impurities in commercial batches of gliclazide of particular interest is **Impurity G**, for which no published synthesis methods currently exist in the scientific literature.[17,18] This absence has created a gap in the impurity profiling of gliclazide, since the inability to synthesize and study Impurity G limits both structural understanding and toxicological assessment.[19] Recent research has focused on addressing this gap by presenting synthetic approaches for Impurity G along with comprehensive structural analysis. The development of such methodologies is a critical advancement in ensuring complete impurity profiling of gliclazide.[20]

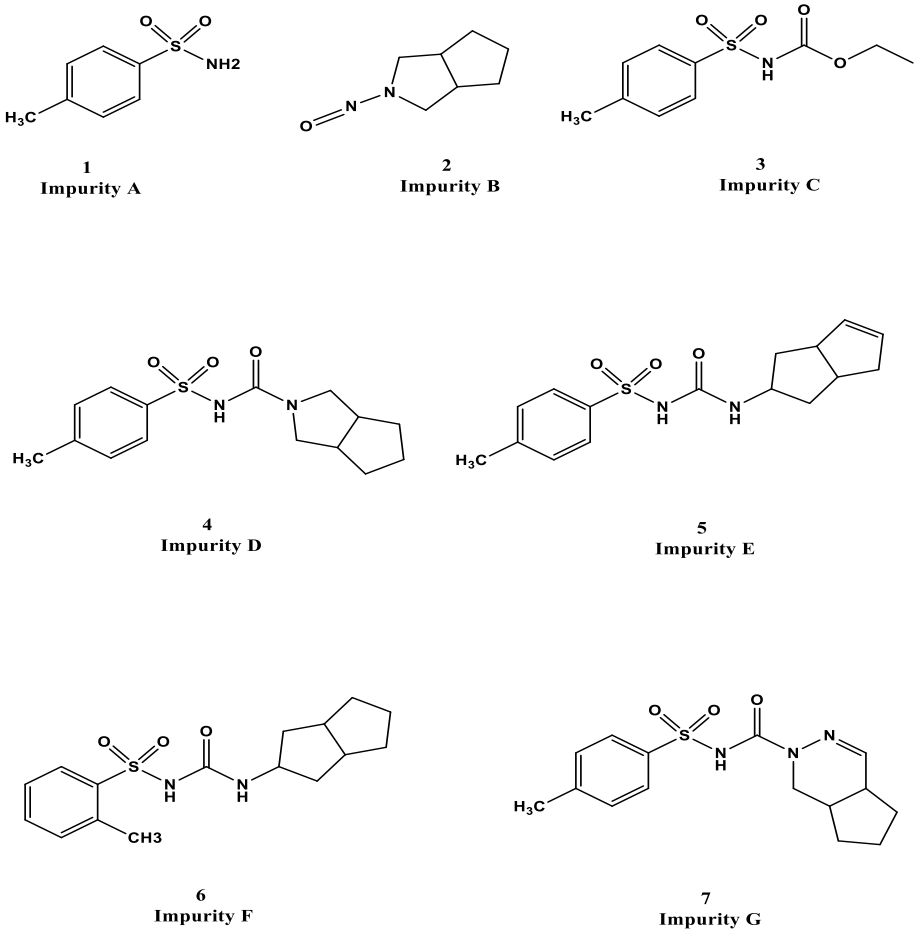

Fig. 1: structure of gliclazide impurities.

Review of literature

Table no 1: Review of literature.

S.No.	Title	Definition of Research Work	Reference
1	Gliclazide impurity F: N-[(perhydrocyclopenta[c]pyrrol-2-yl)aminocarbonyl]-o-toluenesulfonamide	This paper reports the isolation and structural characterization of impurity F of gliclazide. The study focuses on the crystallographic analysis, providing detailed molecular geometry, bond lengths, and torsional parameters, which help in understanding its formation and stability during gliclazide synthesis.	[21]
2	Gliclazide	This work provides an overview of gliclazide as an oral antidiabetic agent. It discusses the mechanism of action (sulfonylurea stimulating insulin secretion), pharmacokinetics, therapeutic use in Type 2 diabetes mellitus, and clinical benefits compared to other sulfonylureas.	[23]
3	Improved synthesis of 1-[hexahydrocyclopenta[c]pyrrol-2(1H)-yl]-3-(4-methylbenzenesulfonyl)urea	This paper describes a novel and efficient synthetic route for gliclazide (and its intermediates). The improved methodology enhances yield, purity, and scalability compared to earlier methods. It reduces reaction steps and employs optimized conditions suitable for pharmaceutical manufacturing.	[24]
4	Gliclazide: A Preliminary Review of its Pharmacodynamic Properties and Therapeutic Efficacy in Diabetes Mellitus	This review article evaluates the pharmacological profile and clinical performance of gliclazide. It highlights its glucose-lowering effect, low incidence of hypoglycemia, potential antioxidant benefits, and therapeutic role in managing Type 2 diabetes mellitus patients.	[25]

MATERIALS AND METHODOLOGY

The Bruker 300, variant 500 spectrometer was used to record 1H-NMR spectra at 300 and 500 MHz Chemical shifts were reported in parts per million relative internal standard. A Perkin-Elmer spectrometer was used to record infrared (IR) spectra of KBr dispersions in the solid stage. Perkin-Elmer PESCIEX mass spectrometer API2000 was used to record the mass spectra. Scheme 1 describes the experimental process as follows, and Scheme 4 indicates that the essential raw material for the synthesis of Compound.[26] Solvents and reagents have been purchased from commercial vendors.

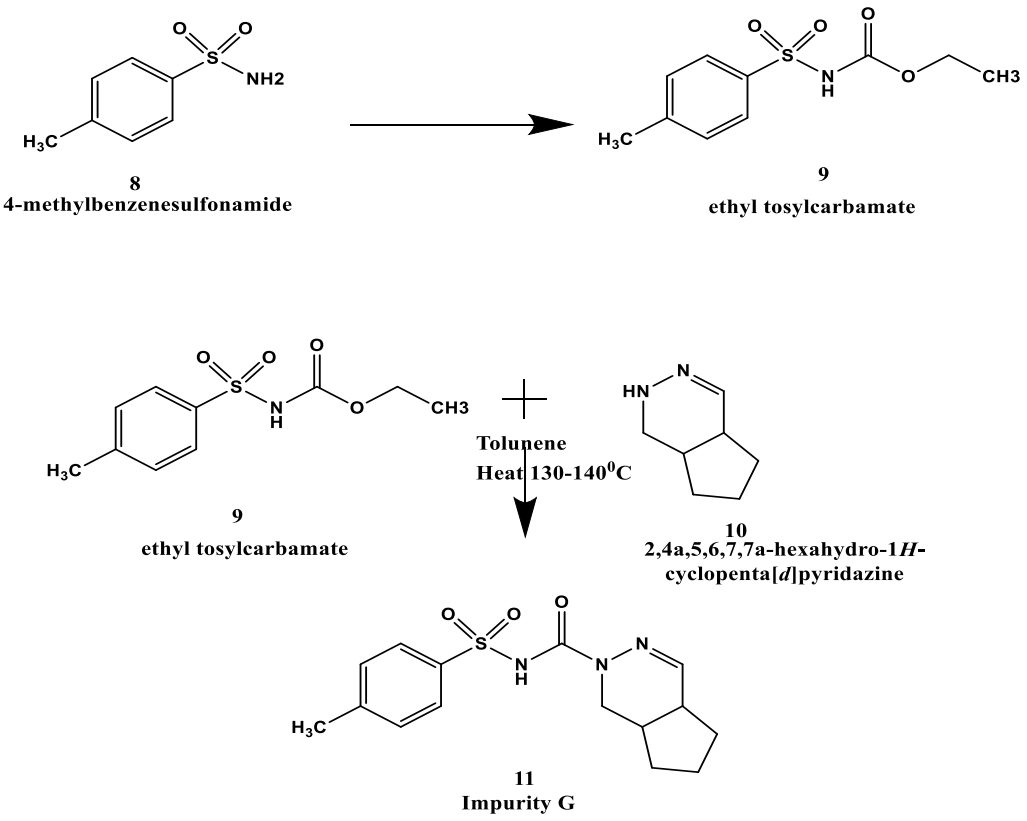

Fig. 2: Scheme for the Synthesis of Gliclazide EP impurity G.

Synthesis of Ethyl Tosylcarbamate

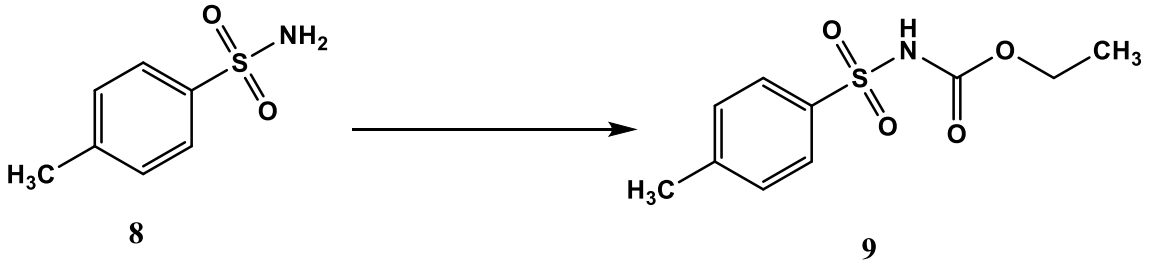

Fig. 3: Scheme for the Synthesis of Ethyl Tosylcarbamate.

In 250 ml round bottom flask, to a suspension of **p-Toluene sulfonamide** (1 eq) in 20 ml of methanol, 1N solution of potassium hydroxide (30 ml) in methanol was added and the mixture were stirred get clear solution. Then methanol was removed from the reaction mixture by rotary evaporator at 60^0C. The residue was suspended in 150 ml of ethyl methyl ketone and potassium carbonate (9.5 eq) was added to it. **Ethylchloroformate** (8.5 eq) was dropwise added to the reaction mixture and refluxed at 90^0C for 13 hrs. The reaction progress was monitored by TLC (2: 8 Ethyl acetate: Hexane). The reaction mixture was cooled to RT and filtered, washed with ethyl methyl ketone. The filtrate was concentrated and water was added to the obtained residue and the product was extracted with ethyl acetate. The Ethyl acetate layer was dried over sodium sulphate, filtered and distilled at 65^0C to obtain an **Ethyl Tosylcarbamate** as white solid.

Synthesis of N-tosyl-1, 4a, 5, 6, 7, 7a-hexahydro-2H-cyclopenta[d]pyridazine-2-carboxamide

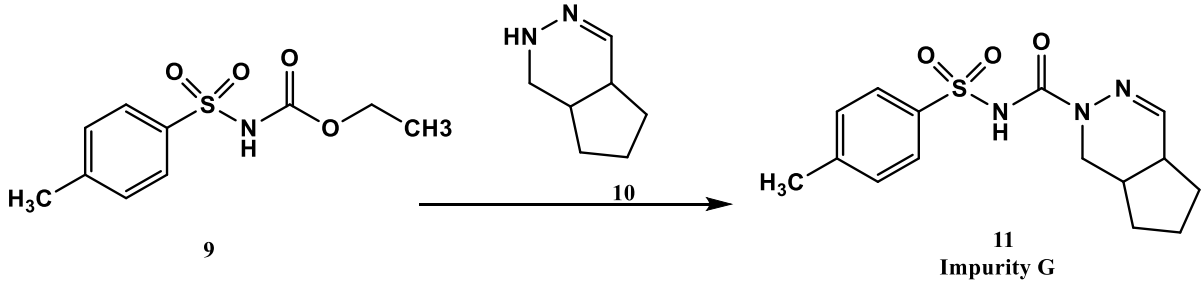

Fig. 4: Scheme for the Synthesis of N-tosyl-1, 4a, 5, 6, 7, 7a-hexahydro-2H-cyclopenta[d]pyridazine-2-carboxamide.

A mixture of ethyl tosylcarbamate and 2,4a,5,6,7,7a-hexahydro-1H-cyclopenta[d]pyridazine in toluene were heated to refluxed for 2 hours at 130-140^0C. The reaction process was monitored by TLC. After the conversion, the reaction mixture was cooled to RT. the toluene was distilled off and the crude product was purified by column chromatography by using silica gel, methanol: MDC (0.5: 9.5) as an eluting solvent. MS; m/z Calcd for C15H19N3O3S (M+H); Found 322.13, IR (KBr, V_{Max} CM^{-1})1435.73 C-N, 1702.12 C C=O, 2952.44 H-C-H. Hnmr solvent used: DMSO (d6) δ 1.157 ppm (1H,m,-CH), δ 1.404 ppm (1H,m,-CH),δ 1.510 ppm (1H,m,-CH), δ 1.737 ppm (1H,m,-CH), δ 1.885 ppm (1H,m,-CH), δ 2.166 ppm (1H,m,-CH),δ 2.332 ppm (3H,d,-ArCH3), δ 2.501 ppm (1H,m,-NCH),δ 3.000 ppm (1H,q,-CH), δ 3.551-3.595 ppm (1H,dd,-NCH),δ 7.000 ppm (1H,d,-N=CH), δ 7.27 ppm (1H,s,-NH),δ 7.356 ppm (2H,dd,-2ArCH), δ 7.825 ppm (2H,dd,-2ArCH). HPLC purity 99.70%.

RESULTS AND DISCUSSION

The chemical synthesis of impurity F was previously reported in *Acta Cryst.* (2012). E68, o44, and its synthetic pathway has been well established in the literature. Building upon this background, the present work focused on the stepwise preparation of impurity G (compound 11), employing a multistep synthetic route, as illustrated in the scheme.

In the first step, ethylchloroformate was reacted with p-toluenesulfonamide (compound 8) in the presence of bases such as KOH and K_2CO_3. The reaction was carried out in ethyl methyl ketone under controlled heating at 130–140 °C, leading to the successful formation of ethyl tosylcarbamate (compound 9). The use of dual bases (KOH and K_2CO_3) ensured efficient deprotonation of the sulfonamide group, thereby facilitating nucleophilic substitution with ethylchloroformate. This intermediate served as a crucial precursor for subsequent coupling reactions.

Finally, the obtained ethyl tosylcarbamate (compound 9) was combined with 2,4a,5,6,7,7a-hexahydro-1H-cyclopenta[d]pyridazine (compound 10) in toluene. The mixture was heated at 130–140 °C for 14 hours, allowing the condensation reaction to proceed smoothly. This step yielded the target product, N-tosyl-1,4a,5,6,7,7a-hexahydro-2H-cyclopenta[d]pyridazine-2-carboxamide (compound 11). The prolonged heating under reflux conditions in an aromatic solvent facilitated complete

conversion and high product yield, confirming the robustness of the synthetic strategy.

IR INTERPRETATION OF GLICLAZIDE EP IMPURITY G

Mol.Wt : 321.39
Mol. For : C15H19N3O3S

Gliclazide EP Impurity G; 1H-Cyclopenta[d]pyridazine Gliclazide; N-Tosyl-5,6,7,7a-tetrahydro-1H-cyclopenta[d]pyridazine-2(4aH)-carboxamide; N-[(4-Methylphenyl)sulfonyl]-1,4a,5,6,7,7a-hexahydro-2H-cyclopenta[d] pyridazine-2-carboxamide.

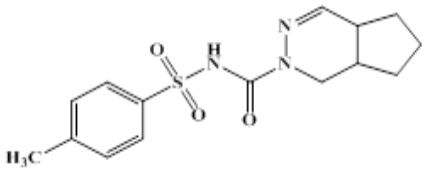

Fig. 5: structure of gliclazide impurity G.

Table no. 2: IR Interpretation of gliclazide ep impurity G.

S.No	Wave number (cm–1)	Group	Stretch
1	1435.73	Carbon and Nitrogen of Imino Group	C-N
2	1702.12	Carbonyl group of Keto group	C=O
3	2952.44	Alkyl Group	H-C-H

The signals of the IR spectrum and their interpretation are consistent with the structural formula.

MASS INTERPRETATION OF GLICLAZIDE EP IMPURITY G

Mol.Wt : 321.39
Mol. For : C15H19N3O3S

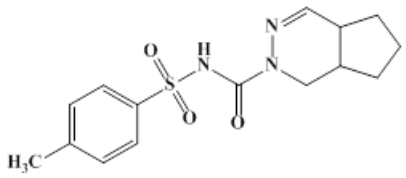

Fig. 6: structure of gliclazide impurity G.

Gliclazide EP Impurity G; 1H-Cyclopenta[d]pyridazine Gliclazide; N-Tosyl-5,6,7,7a-tetrahydro-1H-cyclopenta[d]pyridazine-2(4aH)-carboxamide: N-[(4-Methylphenyl) sulfonyl]-1,4a,5,6,7,7a-hexahydro-2H-cyclopenta[d] pyridazine-2-carboxamide

Table no. 3: MASS Interpretation of gliclazide ep impurity G.

m/z	fragments
322.13 (+ve mode)	[M+1]
320.07 (-ve mode)	[M-1]

The molecular mass of GLICLAZIDE EP IMPURITY G (321.39) has been confirmed with fragments appears [M+1] 322.13 in positive mode and [M-1] 320.07 in negative mode. The signals of the mass spectrum and their interpretation are consistent with the structural formula. **HNMR INTERPRETATION OF GLICLAZIDE EP IMPURITY G**

Mol.Wt : 321.39
Mol. For : C15H19N3O3S

Gliclazide EP Impurity G; 1H-Cyclopenta[d]pyridazine Gliclazide; N-Tosyl-5,6,7,7a-tetrahydro-1H-cyclopenta[d]pyridazine-2(4aH)-carboxamide; N-[(4-Methylphenyl)sulfonyl]-1,4a,5,6,7,7a-hexahydro-2H-cyclopenta[d] pyridazine-2-carboxamide

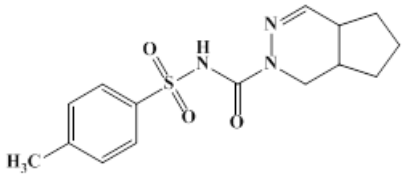

Fig. 7: structure of gliclazide impurity G.

SOLVENT USED: DMSO (d6)

δ 1.157-1.235 ppm (1H,m,-CH), δ 1.404-1.492 ppm (1H,m,-CH), δ 1.510-1.582 ppm (1H,m,-CH), δ 1.737-1811 ppm (1H,m,-CH), δ 1.885-1.990 ppm (1H,m,-CH), δ 2.166-2.212 ppm (1H,m,-CH) δ 2.332-2.388 ppm (3H,d,-ArCH3), δ 2.501-2.675 ppm (1H,m,-NCH), δ 3.000-3.052 ppm (1H,q,-CH), δ 3.551-3.595 ppm (1H,dd,-NCH),δ 7.000-7.006 ppm (1H,d,-N=CH), δ 7.27 ppm (1H,s,-NH), δ 7.356-7.408 ppm (2H,dd,-2ArCH), δ 7.825-7.846 ppm (2H,dd,-2ArCH).

Note: one proton from cyclopenta pyridazine merge with DMSO residual solvent, one proton from sulfonamide NH merge with DMSO Water residual and it is under standard able.

HPLC INTERPRETATION OF GLICLAZIDE EP IMPURITY G

Mol.Wt : 321.39
Mol. For : C15H19N3O3S

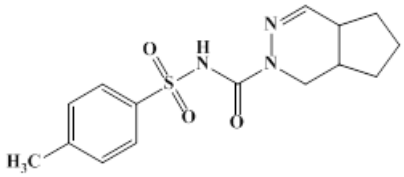

Fig. 8: structure of gliclazide impurity G.

Gliclazide EP Impurity G; 1H-Cyclopenta[d]pyridazine Gliclazide; N-Tosyl-5,6,7,7a-tetrahydro-1H-cyclopenta[d]pyridazine-2(4aH)-carboxamide; N-[(4-Methylphenyl)sulfonyl]-

1,4a,5,6,7,7a-hexahydro-2H-cyclopenta[d] pyridazine-2-carboxamide.

CONCLUSION

In this work, we present a reliable and efficient synthetic method for the preparation of **gliclazide impurity G**, obtained in good yield and with a high degree of purity. The developed procedure not only provides a reproducible route for synthesizing this impurity but also offers valuable insights into the reaction conditions required to optimize the process. To confirm the identity and structural integrity of the synthesized compound, a comprehensive spectral characterization was carried out. The data include Infrared (IR) spectroscopy**,** which highlights the characteristic functional group vibrations; ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy**,** which provides detailed information on the proton environment and confirms the molecular framework; and Mass Spectrometry (MS)**,** which validates the molecular weight and fragmentation pattern. Together, these analytical results establish the successful synthesis and structural confirmation of gliclazide impurity G, making this study a useful contribution for both pharmaceutical research and quality control in drug development. Acknowledgements; Authors are grateful to Dr. Mohamed Rafi, Globe Synth Pharma Tech Pvt. Ltd, Salem, Tamilnadu for providing necessary support for the laboratory work and practical analysis.

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