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ANALYTICAL METHODS FOR THE DETERMINATION OF GLICLAZIDE IN PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL FLUIDS

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ABSTRACT

Diabetes/ Diabetes mellitus, a group of metabolic diseases is identified in patients with high blood sugar, either because the pancreatic cells does not produce enough insulin, or the body cells do not respond to the insulin that is produced. It is categorized on the response of body cells towards insulin and known as insulin dependent diabetes mellitus (IDDM, Type I) and non insulin dependent diabetes mellitus (NIDDM Type II). Numbers of drugs are used for the management of both types of diabetes and amongst the types of drugs, sulphonyl urea have their own significant. These agents stimulate the pancreatic cells to increase the insulin secretion. Gliclazide, a category II sulphonyl urea is used in the management of NIDDM. In contrast with other sulphonyl urea, gliclazide is a highly efficient drug and a specific dose is required in the management of high blood glucose level in diabetes. The variability in absorption pattern in inter- and intra-patient creates the bioavailability problems during oral administration of gliclazide. Hitherto, various analytical techniques have been developed for the quantification of gliclazide in pharmaceutical dosage forms as well as in biological fluids. This study is based on the overview of different instrumental techniques with their characteristics for quantification of gliclazide.

KEYWORDS: bioavailability, diabetes mellitus, sulphonyl urea, high performance liquid chromatography.

INTRODUCTION

The drugs used for the management of diabetes treat diabetes mellitus by lowering glucose levels in the blood. With the exceptions of insulin, exenatide, liraglutide and pramlintide; all are administered through oral route and are also called oral hypoglycemic agents/ oral anti-hyperglycemic agents. There are different classes of anti-diabetic drugs, and their selection depends on the nature of the diabetes, age and situation of the person, as well as other factors. Diabetes mellitus type 1 (IDDM, Type I) is a disease caused by the lack of insulin. Insulin must be used in Type I, which must be injected. Diabetes mellitus type 2 (NIDDM, Type II) is a disease of insulin resistance by cells.

Treatments for diabetes mellitus include (1) agents that increase the amount of insulin secreted by the pancreas e.g. sulfonylurea, (2) agents that increase the sensitivity of target organs to insulin e.g. biguanides, and (3) agents that decrease the rate at which glucose is absorbed from the gastrointestinal tract e.g. alpha–glucosidase inhibitor.

Sulfonylurea/sulphonylurea) derivatives are a class of anti-diabetic drugs that are applied for the management of diabetes mellitus type 2 (NIDDM, Type II). There are two classes of sulphonylurea and in Category I, Carbutamide, Acetohexamide, Chlorpropamide, Tolbutamide, Tolazamide are used for NIDDM. Glipizide, Gliclazide, Glibenclamide (glyburide), Glibornuride, Gliquidone, Glisoxepide, Glyclopyramide, and Glimepiride are of category II sulphonylurea. Gliclazide, (1-(3-azabicyclo (3,3,0) oct-3yl) 3-p-tolylsulphonyl urea, a second-generation sulfonylurea is an oral hypoglycemic agent used in the treatment of non-insulin dependent diabetes mellitus.[1] It is a second generation sulphonylureas with common proprietary names as diabrezide, diamicron, diaprel, diabeziol etc. The dosage for the 80 mg formulation is 40 to 320 mg daily in two divided doses, while the 30 mg and 60 mg modified release formulation may be given at a dose of 30 to 120 mg once daily at breakfast. According to official compendia, it not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 1-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-3-[(4-methyl phenyl) sulphonyl] urea, calculated with reference to the dried substance. It is a white or almost white powder, practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone, slightly soluble in alcohol.[2] The dosage for the 80 mg formulation is 40 to 320 mg daily in two divided doses, while the 30 mg and 60 mg modified release formulation may be given at a dose of 30 to 120 mg once daily at breakfast.[3] In pure gliclazide prepared by synthetic methods, impurities may be present and these are not more

than 1 percent of gliclazide. These impurities have also been synthesized to be applied as lead marker for identification of metabolites and degradable products during biological elimination in body and stability study analysis, respectively.

Gliclazide is rapidly and well absorbed but may have wide inter- and intra-individual variability. Peak plasma concentrations occur within 4-6 h of oral administration. It is intensively metabolized in the liver by hydroxylation, n-oxidation and oxidation to a number of inactive metabolites like other sulphonylureas.[4,5] CYP2C9 is involved in the formation of hydroxygliclazide in human liver microsomes and in a panel of recombinant human CYP450.[6,7] But the pharmacokinetics of gliclazide MR formulations is affected mainly by CYP2C19 genetic polymorphism instead of CYP2C9 genetic polymorphism. [8,9,10,11] Cytochrome P450 2C9 is responsible for conversion of gliclazide into 7-Hydroxygliclazide and 6-Hydroxygliclazide during metabolism while Cytochrome P4502C9 along with Cytochrome P4502C19 converts the drug into methylhydroxy- gliclazide. Metabolites and conjugates are eliminated primarily by the kidneys (60-70%) and also in the feces (10-20%). Gliclazide is extensively protein bind (87% to 94%) in the circulation which may account for the minimal hepatic first pass effect.[4,5,12,13] In the pancreas, gliclazide binds β-cells sulphonylureas receptors (with its sulphonyl urea moiety) with high affinity which is reflected in the effective daily dosing of gliclazide, in the milligram range.[14] Gliclazide selectively binds to sulfonylurea receptors (SUR-1) on the surface of the pancreatic β-cells. It has been shown to provide cardiovascular protection as it does not bind to sulfonylurea receptors (SUR-2A) in the heart.[15] This binding effectively closes the K^+ ion channels and decreases the efflux of potassium from the cell which leads to the depolarization of the cell. It causes voltage dependent Ca^{++} ion channels to open increasing the Ca^{++} influx. The calcium can then bind to and activates calmodulin which in turn leads to exocystosis of insulin vesicles leading to insulin release. It has been suggested that due to its short term acting, gliclazide may be suitable for use in diabetes patients with renal impairment and also in elderly patients whose reduced renal function may increase the risk of hypoglycemia following sulphonylureas. Gliclazide has been shown to decrease fasting plasma glucose, postprandial blood glucose and glycosolated hemoglobin (HbA1c) levels (reflective of the last 8-10 weeks of glucose control). As gliclazide is extensively metabolized in liver but, in the plasma, unchanged gliclazide represents over 90% of the administered dose. Besides reducing blood sugar, gliclazide can improve the liver function and further delay the occurring of blood vessel neopathy. It is also suggested that due to its short term acting,

gliclazide may be suitable for use in diabetic patients with renal impairment and elderly patients reduced renal function may increase the risk of hypoglycemia following some sulphonylureas.[16] It has good efficacy and appears to particular benefit in patients previously untreated with oral anti-diabetic drugs and is generally well tolerated. [17] The drug is currently prescribed in dose range 40-320 mg per day as tablets once to three times daily. Individual dose requirements vary between patients and partly reflect the variability in pharmacokinetic characteristics in addition to differences in severity of diabetes.[18] The high inter-subject variation in oral absorption of conventional immediate release tablets has been observed but it has no effect on efficacy and safety of the drug. The safety and efficacy parameters of gliclazide are well established. The variation in absorption parameter of gliclazide can be attributed to both the physicochemical properties of gliclazide as well as of formulations (e.g. tablets). Gliclazide is a week acid with a good lipophilicity and a pH dependent solubility. It is practically insoluble in acidic media and its solubility increases as the pH becomes more alkaline. It is a drug of class II of biopharmaceutical classification in which the dissolution rate is the controlling step for drug absorption. But in case of gliclazide, the dissolution rate depends upon the gastric emptying time and upon dissolution rate in small intestine (e.g. alkaline media) where the drug is soluble. The variability in absorption of gliclazide is also related to an early dissolution in acidic environment of stomach leading to more variability in the absorption in the intestine.[18] It is well recognized that the presence of presence of food in gastrointestinal track can affect bioavailability and disposition of orally administered drugs through physicochemical and physiochemical integrations. Delay in gastric emptying and change in pH accompanying food intake also affect the dissolution of drug in gastrointestinal track. The alteration in pharmacokinetics parameters of gliclazide conventional immediate release tablets have also been created by intake of food in healthy volunteers. In this case, two mechanisms responsible for creating alteration were thought to be involved as delay in the absorption of gliclazide when given 30 mins after breakfast and decrease in the extent of absorption of gliclazide when given 30 mins before a meal. Due to the problem of Inter- and intra subject variability in gliclazide absorption leading to variation in bioavailability, modified release tablets of gliclazide are available in the market and these allow a once-daily dosing regimen at breakfast due to their steady state dissolution pattern with subsequent complete absorption. It has also been reported that a consistent and optimal release of gliclazide from modified release tablets of gliclazide leads do a low to moderate variability in its pharmacokinetics parameters and these tablets can be given to diabetic patients without regards to meals i.e. before, during or after meal.[18]

Gliclazide and its metabolites may accumulate in those with severe hepatic and/or renal dysfunction. Symptoms of hypoglycemia include: dizziness, lack of energy, drowsiness, headache and sweating. Hyperglycemic action may be caused by danazol, chlorpromazine, glucocorticoids, progestogens, or β-2 agonists. Its hypoglycemic action may be potentiated by phenylbutazone, alcohol, fluconazole, β-blockers, and possibly ACE inhibitors. It has been found that rifampin increases gliclazide metabolism in human's *in vivo* study.[19] Some of the adverse reactions observed in diabetic patients taking gliclazide are hypoglycemia, gastrointestinal disturbances, skin reactions, hematological disorders, and elevation in hepatic enzyme levels. Gliclazide has been proven to have the same efficacy as glimepiride, one of the newer sulfonylureas, the European GUIDE study has shown that it has approximately 50% fewer confirmed hypoglycaemic episodes in comparison with glimepiride.[19] Gliclazide is contraindicated in Type 1 diabetes, hypersensitivity to sulfonylureas, severe renal or hepatic failure, pregnancy, lactation and miconazole co-prescription.

It is orally administered and has duration of action of 12 hrs or more. As its effects are less prolonged than those of chlorpropamide or glibenclamide, it may be more suitable for elderly patients who are prone to hypoglycemia with long acting sulphonylureas. Several methods have been developed for the determination of gliclazide, either per se or in pharmaceutical preparations and biological fluids. These methods include non-aqueous titration[2], spectrophotometry[20], radioimmunoassay[20], GLC[21,22], TLC[23], HPLC [24,25,26,27,28,29,30] colorimetry[28] etc. Some of the reported analytical methods involve time-consuming and laborious extraction steps [30, 32], complex derivatization techniques [30], lengthy retention time or large volumes of biological samples [30,32,33], solid phase extraction [29], or use of mass spectroscopy for detection and identification of drug. There have been several studies of the pharmacokinetics and pharmacodynamics in diabetic patients [34,35,36,37]. Glowka et al. [38] reported the pharmacokinetic and pharmacodynamic properties of gliclazide in healthy European volunteers. In healthy Korean volunteers, the pharmacokinetic and pharmacodynamic characterization of gliclazide was performed by applying glibenclamide as internal standard in HPLC method. The study reported the maximum hypoglycemic effect approximately after 1.5 hrs of formulation administration. [39] In another study, the bioequivalent study of two formulations of gliclazide in healthy volunteers has been performed by applying glyburide as internal standard (I.S) and the retention time of gliclazide and glyburide (I.S) was reported 4.1 and 6.21 mins, respectively. [40]

Ternary Complex with Eosin and Palladium (Ii)

This method is based on the reaction of gliclazide with palladium and eosin. Alternatively, 4-chloro 7-nitro benzo-2-oxo-1,3-diazide can be used as derivatizing agent for GLZ and measurement of absorbance spectrophotometrically at 400 nm or spectrofluorometrically at 470 nm after oxidation at 400 nm. [41] This concentration range is 0.5-4µg/mL with a detection limit of 0.05 µg/mL compared with 2-20 µg/mL with a detection limit of 0.2 µg/mL for NBD-Cl method. Also, this method can be applied to spiked biological samples. Moreover, the regents used in the proposed method are stable at least 2 weeks while NBD-Cl must be freshly prepared. The method is considered as stability indicating method since the side of complex formation is expected to be the side of degradation (hydrolysis). Ternary complex formation has been used for the determination of palladium (II) via 1,10 phenoanthroline as a cationic component and eosin as anionic counter ion.[42] This method can be applied for the determination of gliclazide in pure form, pharmaceutical preparations and biological fluids. The proposed method is based on ternary complex formation with eosin and Pd (II). The ternary complex form between the metal ion, electronegative ligand and organic base often have higher values of molar extinction co-efficient than binary complexes of the same components. The factors affecting the process are pH, type of buffer, type of metal cation, temperature, time of heating, effect of different sensitizers, different surfactants, concentrations of surfactants and concentration of eosin and Pd (II). The drug reacts via the amide group with the Pd (II) on in the presence of eosin.

High Performance Liquid Chromatography

According to the British Pharmacopoeia, liquid chromatography is recommended for assessment of gliclazide purity. [43] Various high performance liquid chromatography methods have been developed for the determination of gliclazide determination in biological fluids and pharmaceutical formulations. Most of the methods have limitations, including time consuming sample clean-up, laborious extraction steps, low sensitivity (30 ng/mL or more), complex dramatization techniques, use of large sample volumes (1 mL or more) or use of expensive solid extraction cartridges. Park et al.[40] have reported a semi-micro HPLC method for quantification of gliclazide in human plasma. The limit of quantification (LOQ) was reported as 100 ng/mL in this study and total analysis time was 8 mins. In HPLC methods, some of the factors affecting the performance also depend upon the use of columns for separation. The monolithic silica columns used in HPLC exhibit a tailor made bimodal pore structure with both narrow pores or through pores and mesopores. [44] The most unique

features of these columns are their high permeability which is nearly twice or high as that of packed columns. Therefore, monolithic silica can be operated at high flow rate, thus allowing fast separation of various mixtures. By this means much faster separations are possible and the productivity of chromatographic process can be increased by at least one order of magnitude as compared to traditional chromatographic columns packed with porous particles. This enhances the speed of the separation process and reduces back process and unspecific binding without sacrificing resolution.[45, 46] The use of smaller sample volume and shorter analysis time provide advantages as compared with some previous methods that requires large sample volumes (1-2 mL) for and longer run time for analysis of gliclazide.[30,33] The isocratic reversed phase HPLC with monolithic columns with UV-detection for gliclazide has been reported.[44] The mobile phase used in the method was a mixture of 0.01M disodium hydrogen phosphate buffer-acetonitrile (52:48 v/v) adjusted to pH 4.0 at flow rate of 1.2 mL/min. Stock solutions (2 mg/mL) of gliclazide were prepared in methanol and the working standards were prepared in plasma from stock solution and stored at 4^0C. To 450 µL of plasma taken in a glass-stoppered 15 mL centrifuge tube, 50 µL of glibenclamide as internal standard (3 µL/mL) and 500 µL of acetonitrile were added. After mixing (30s), the mixture centrifuged for 10 mins at 4000 rpm. Blank plasma was prepared for heparinized whole-blood sample collected from healthy volunteers and stored at -20^0C. After thawing, stock solution of gliclazide was added to yield final concentrations ranging from 10 to 5000 ng/mL. Internal standard solution was added to each of these samples to yield a concentration of 300 ng/mL. The samples then were prepared for analysis. The average recovery was 94.86 ± 1.96% (n=6) and the limit of quantification was 10 ng/mL for gliclazide.

Another HPLC method has been applied for the quantitative measurement of gliclazide concentrations as low as 0.05 µg/mL in plasma.[44,47] The stock solutions of gliclazide (3.20 mg/L) and nadoxolol (100 µg/mL) were prepared in methanol and were stable for at least one month when stored at 4^0C. Plasma standards were prepared in the concentration range 0.1-1.28 µg/mL. The UV-detector was set at 229 nm for analyzing gliclazide. The mobile phase used was methanol, 2-propanaol, water, 1.16 M perchloric acid (70:29.5:0.5:0.3 v/v) and operated at ambient temperature with a flow rate of 2.0 mL/min. The limit of determination was estimated to be 0.1µg/mL and the detection limit, based on a signal-to-noise ratio 4:1, was 0.05 g/mL. In addition; phenobarbital, primidone, phenacetin, carbamazepin, clobazam, diazepam, caffeine, theophylline, theobromine, salicylic acid, tolbutamide, acebutalol, propranolol, sotalol, propafenone, lidocaine, aminodarone, verapamil, clomipramine, and

imipramine do not co-chromatograph with either gliclazide or internal standard (nadoxolol). Rouini et al. [16] also developed a simple, rapid and applicable HPLC method requiring small sample volume and minimal sample work-up. In the method phenytoin worked as internal standard. The pH of the acetonitrile-water mobile phase (45:55, v/v) was adjusted to 3 with phosphoric acid. Mobile phase was passed through column by a low rate of 0.9 mL/min and elute was monitored at 230 nm. The stock solution of gliclazide (100 µg/mL) and the internal standard (160 µg/mL) were prepared in acetonitrile and were stable or at least 1 month at 4^0C. The working internal standard (12 µg/mL) was prepared in water. A 50 µl of the internal standard working solution was added in 100 µL of serum and afterwards, 100 µL of 0.07M phosphate buffer (pH 4.5) was mixed. After vortex mixing for 10 sec., 1 mL of toluene was added and the mixture was shaken vigorously for 1 min. The mixture was then centrifuge for 5 mins at 10000 rpm. An 800-µL aliquot of the upper organic layer containing gliclazide and internal standard was transferred to a clean glass tube and evaporated under air stream to dryness at 50^0C. The residue was re-dissolved in 10 µL of mobile phase and a 50-µL aliquot was injected onto HPLC column. The limit of quantification of gliclazide was 75 ng/mL (signal-to-noise ratio of 5) and the minimum detectable level was 30 ng/mL (signal-to-noise ratio of 3). Mean recovery for gliclazide and internal standard were 84.5 and 87%, respectively. The methods published by Kimura et al[32] and Senser et al. [33] needed 0.5 and 0.25 mL of serum samples, respectively, compared to 0.1 mL in the reported method. [16] Nadoxolol has been used as I.S. which is not easily available. [47]The perchloric acid used in the mobile phase reduces the column life remarkably.

Determination of Gliclazide by Hplc Using an Anion-Exchange Resin

Kimura et al. [22] developed the method for gliclazide analysis in human serum by HPLC on a column packed with a macroporous anion-exchange resin, Diaion CDR-10. The elution was performed with acetonitrile-methyl alcohol-1.2 M ammonium perchlorate (4:3:7, v/v/v) at a flow rate of 0.4 mL/min. The retention time of gliclazide was 15 mins. The detection limit of gliclazide was 0.2 µg/mL in plasma. For the extraction of gliclazide from plasma, a mixture of 250 µL of human serum and 1 mL of 0.5 M phosphate buffer (pH 7.0) was shaken vigorously for 15 mins with 4 mL of chloroform. The mixture was centrifuged for 15 mins at 1400 g, then 3 mL of chloroform layer withdrawn, mixed with 2 mL of 1N sodium hydroxide, and shaken for 10 min. After centrifugation of the mixture for 5 mins; 1.5 mL of the aqueous layer was added to a mixture of 0.5 mL of 3N hydrochloric acid, 2.5 mL each of 0.5M phosphate buffer (pH 7.0) and chloroform, followed by shaking for 10 mins. After

centrifugation for 5 mins, 2 mL of the chloroform layer was evaporated to dryness under a stream of nitrogen at 50^0C. The residue was redissolved in 50 µL of methyl alcohol containing the internal standard (IS), methyl benzoate in 0.4 µg/50µL amount, and 10 µL aliquot was injected onto the HPLC column. The methyl alcohol containing the extracted drug was stored at 2-4^0C. Gliclazide possesses an oxidative group, and can be detected by electrochemical detection (Ed). The electrochemical detection is a very powerful detection system, which can detect weak currents of less than generated from oxidative or reductive reactions. The optimum conditions for the analysis of gliclazide have been investigated and successfully one such HPLC-Ed method with simple sample preparation and high sensitivity for the determination of gliclazide in plasma has been developed. [48]

High Performance Liquid Chromatography Using Solid-Phase Extraction

A solid phase extraction (SPE) utilizing OASIS® HLB cartridge has also been used for the determination of gliclazide. [49] Five washing steps during the extraction process were applied in this method which is not practical and is time consuming. [49] The SPE method developed by Strausbauch et al. [50] was only validated for urine samples. Martha et al. [51] used two separate method one for measuring the concentration of metformin and the other for glibenclamide. AbuRuz et al. [52] optimized ion pair solid phase extraction technique followed by HPLC determination of gliclazide. The internal standard used in the method was tolbutamide. The stock solution was prepared by dissolving 0.04 gm of glipizide and 0.08 gm of each of metformin and gliclazide in 100 mL methanol. From the stock solution, 0.02 mL was taken and further diluted with water to 10 mL. It was further diluted to produce the remaining working standards. A 50 µL of the aqueous solution of internal standard (tolbutamide) and 0.125 mL of standards containing metformin, gliclazide and glipizide were added to 1 mL plasma resulting serial dilutions of metformin and gliclazide. The plasma samples were prepared by collecting blood samples into glass tubes containing EDTA and centrifuging at 3000 rpm (1610xg) for 15 mins. The separated plasma was kept frozen at -70^0C until analysis. To 1 mL of spiked plasma standards, 50 µL of internal standard solution and 0.125 mL of water were added. A 0.1 mL of 0.4 M HCl was added to each of the standards and the plasma samples then vortex mixed for 30 sec. and centrifuged at 14000 rpm (17500xg) for 5 mins before extraction to prevent blockage of cartridges. The prepared samples and standards were extracted using the optimized ion pair solid phase extraction technique utilizing OASIS® HLB cartridges (1cc, 30 mg) as following-

1. Condition 1:1 mL methanol followed by 1 mL of water
2. Condition 2: 1 mL aqueous solution of 2 mM sodium dodecyl sulphate (ion pair reagent)
3. Load: 1.275 mL spiked plasma
4. Wash: 1 mL 30 % methanol
5. Elute: 1 mL methanol
6. Evaporate with nitrogen stream and constitute in 350 µL of mobile phase and inject 150 µL onto HPLC. The mobile phase was pumped at a flow rate of 1 mL/min and consisted of 2mM sodium dodecyl sulphate, acetonitrile (37.5%) and potassium dihydrogen phosphate (62.5%) (From 0.02 M buffer to produce a final buffer concentration of 0.0125 M). The pH adjusted to 5.3 using HCl for optimal separation. The prepared mobile phase was filtered through 0.45 µm Millipore filters and degassed ultrasonically before use. Based on the UV spectrum of the analytes, the detector wavelength was set at 225 µm. The results of the study conferred the specificity as there were no endogenous substances in the plasma that could interfered with the peaks of interest. The blank analysis did not give responses at the retention times of the peaks of interest. The recovery of sulfonylureas (SU) ranged between 79.7% and 101.9% as a high acidity used in the loading step increased the recovery of SU.

Capillary Electrophoresis with Amperometric Determination

In the formulation development, the potency of the drug is required that is basically related to the purity. In gliclazide, 3-amino-3-azabicyclo[3,3,0] octane (aminoheterocycle) and 3-azabicyclo[3,3,0] octane (azabicycle) are two kinds of impurities in bulk and strictly restricted by the safety requirement of authorities. Amongst the various methods of impurities detection in gliclazide, capillary electrophoresis has been extensively studied and applied as a highly effective analytical method in modern pharmaceutical analysis. This technique has many advantages, such as lower sampling volume and higher separation efficiency. Because amperometric detector is more sensitive than UV-VIS detector and much cheaper than LIF detector, it can be coupled with capillary electrophoresis to provide high sensitivity and selectivity for the determination of electroactive substances in many medical and pharmaceutical analysis area.[53,54,55] Lv et al.[56] developed a method for simultaneous determination of 3-amino-3-azabicyclo[3,3,0] octane (aminoheterocycle) and 3-azabicyclo[3,3,0]octane (azabicycle) by capillary elctrophoresis with amperometric detection in gliclazide bulk drug. The optimal conditions of capillary electrophoresis-amperometric detection were 50 mM borate solution (pH 9.0) as running buffer, 14 kV as separation voltage and 0.95 volt as detection potential. In these conditions, the two analytes were

perfectly separated within 9 min. The linearity range of aminoheterocycle was from $1.0X10^{-6}$ and $1.0X10^{-3}$ M and that of azabicycle was from $2.0X10^{-6}$ to $1.0X10^{-3}$M, respectively. Their detection limits were $5.0X10^{-7}$ and $1.0X10^{-6}$M, respectively, (S/N=3). A carbon disc used as working electrode in the method was prepared by taking a 300 µm diameter pencil lead. Prior to use, the carbon disc electrodes were firstly polished with emery paper and 0.05µm alumina powders, the ultrasonicated in deionised water, and finally carefully positioned opposing the capillary outlet with the aid of a micromanipulator.

Capillary Gas Chromatography with Flame Ionization

A gas chromatographic method was developed that permitted the accurate and specific determination of gliclazide in plasma.[21] In this method, gliclazide was extracted with chloroform and, after clean-up, derivatized with diazomethane followed by heptafluorobutyric anhydride to form N-methyl-N'-heptafluorobutyrylgliclazide that was assayed on a gas chromatograph equipped with a flame ionization detector, an electron-capture detector or a nitrogen-phosphorus sensitive detector. Accurate determinations were possible with flame ionization detection over a concentration range of 1-15 µg/mL of gliclazide in plasma with a relative standard deviation of 5.2%. The LOD with electron-capture detection was found to be 0.02 microgram per sample. [21] Gas chromatography (GC) with flame ionization detection (FID) and packed columns (in particular OV-1, OV-17, and XE-60) has also proven to be useful for the detection of gliclazide in pharmaceutical formulations as well as in blood plasma at 227 nm. [57,58] Gas chromatograph (CE Instruments Thermo Quest, Rodano, Italy), equipped with a flame ionization detector has also been applied for gliclazide detection. In this method, flame ionization detector was used at base body temperature, 275°C in which air (350 mL/min); hydrogen (35 mL/min); and nitrogen (make-up gas, 30 mL/min) were utilized as mobile phase. Helium of purity class 5.0, additionally passed through an OT3-2 oxygen/moisture trap filter was used as carrier gas. The chromatograms were recorded at a constant pressure of carrier gas, 100 kPa, as a mode of the mobile phase flow during the entire GC oven program. Gliclazide concentrations of 1.0–10.0 mg/mL were taken for calibration and phenazone was used as internal standard solution. In this method the retention time for gliclazide and phenazone (I.S) were found 35.82 ± 0.0276 and 29.39 ± 0.0222 min, respectively. The accuracy of the method was defined in terms of gliclazide (analyte) recovery. The mean recovery (n = 6) was 96.5%, and recoveries ranged from 91.10 to 104.03%. The limits of detection and quantification were found to be 30 and 60 ng/mL, respectively. The relationship between peak area and concentration indicated a wide

linearity range from 0.1 to 10 mg/mL. Much better results were obtained when the calculations for the GC with internal standard method were based on peak area rather than on peak height. The most accurate and repeatable results obtained by the GC with internal standard method with calculations based on peak area, regardless of the origin of the drugs indicated the high selectivity and precision of the proposed method. Any influence of matrix constituents was eliminated under the specified conditions. [59]

Spectrofluorimetry and Spectrophotometry

Accurate, sensitive, and simple spectrophotometric and spectrofluorimetric methods were developed for the determination of gliclazide in pharmaceutical formulations and biological fluids.[41] These methods were based on a coupling reaction between gliclazide and 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in borate buffer, pH 7.8, in which a yellow reaction product that could be measured spectrophotometrically at 400 nm was developed. The same product exhibited a yellow fluorescence at 470 nm upon excitation at 400 nm. The absorbance-concentration plot was rectilinear over the range of 2-20 µg/mL with minimum detectability [signal-to-noise (S/N) ratio= 2] of 0.2 µg/mL (6.18 x 10^{-7} M); the fluorescence-concentration plot was rectilinear over the range of 0.2-2.5 µg /mL with minimum detectability (S/N = 2) of 0.02 µg/mL (6.18 x 10^{-8} M).

Voltammetric Determination

In this method, electrochemical oxidation of gliclazide has investigated at glassy carbon electrode in phosphate buffer solutions over the pH range 2.7–11.8 using cyclic and differential pulse voltammetry (DPV).[60,61] In this method, gliclazide exhibited one anodic peak in the pH range of 2.7–6.3 and a second peak was produced above pH 6.3. The oxidation processes was shown to be irreversible and diffusion controlled. The formation of an inclusion complex of gliclazide with *β*-cyclodextrin (*β*-CD) was investigated by cyclic and differential pulse voltammetry. A phase solubility study with spectrophotometric detection has been also applied. The differential pulse voltammetric method and the phase solubility method indicated stability constant of the complex to be 839 and 360 M^{-1}, respectively. In another reported method, the electrochemical oxidation of gliclazide was studied in Britton-Robinson buffers in the pH range of 2.0-12.0 by cyclic voltammetry and differential pulse polarography at carbon paste electrode. Gliclazide gave rise to two voltammetric peaks, corresponding to the oxidation of hydrazide–CO-NH-NR_2 moiety (where R_2 are cycloalkylamino ring). The oxidation process was found to be irreversible and diffusion-

controlled with adsorption characteristics over the entire pH range. [61]

Potentiometric Determination

It is an official method for quantification of gliclazide in pure form.[62] In this method, about 0.250 g is dissolved in 50 mL of anhydrous acetic acid and titrated with 0.1M perchloric acid. The end point is determined potentiometrically. A 1 mL of 0.1M perchloric acid is equivalent to 32.34 mg of $C_{15}H_{21}N_3O_3S$ (gliclazide).

Radioimmunoassay

Radio-immunoassays have been developed for active pharmaceutical ingredient (s) and these methods enable accurate and sensitive determination of drugs in human serum. [13] Suzuki et al. [63] obtained Antisera A and B against gliclazide from guinea pigs immunized with conjugates A and B prepared by coupling gliclazide homologues, 1-(p-toluenesulfonyl)-3-(4'-carboxypiperidino)urea and 1-(4-methyl-3-carboxy benzene sulfonyl)-3-(3-azabicyclo[3,3,0]oct-3-yl)urea, to bovine serum albumin. 3H-Gliclazide was used as a tracer. Dextran-coated charcoal was used to separate bound and free 3H-gliclazide in the reaction mixture. The assays of gliclazide in serum were possible over a concentration range from 0.25 to 20 µg/mL with the antiserum A and from 0.1 to 10 µg /mL with the antiserum B, respectively, using 0.01 mL of human serum without the need for an extraction procedure. The anti-sera used in this method for the assays were specific for gliclazide. Data obtained by the radioimmunoassay with the antiserum A are in good agreement with those by the radioimmunoassay with the antiserum B and gas-liquid chromatography.

Simultaneous Determination Gliclazide in Blood Plasma with other Anti-Diabetic Drugs

For many patients with type 2 diabetes, monotherapy with an oral anti-diabetic agent is not sufficient to reach target glycemic goals and multiple drugs may be necessary to achieve adequate control. [51] In such cases, a combination of metformin and one of the sulfonylureas (SU) is used. [64] This combination can be achieved by taking each of drugs separately or alternatively fixed formulations have been developed. A combination tablet formulation is beneficial in term of its convenience and patient compliance. The measurement of the plasma concentrations of antidiabetic medications is important for studying the pharmacokinetics of these drugs, for adherence and drug monitoring in diabetic hypoglycemia. A supercritical fluid chromatography (SFC)/tandem mass spectrometry method has also been developed for the simultaneous quantification of metformin and gliclazide in human plasma.[65] A SFC method has several advantages like rapid separation without using hazardous organic

solvents, comparing with conventional chromatographic techniques like GC, HPLC, and LC. The diffusion rate of solutes is very high (ten times greater than simple organic solvents) in supercritical fluid (liquid CO_2). As a results decrease in resistance to mass transfer in the column and allow to very fast separation. In this method, acetonitrile : water (80:20 v/v) mixture was used as a mobile phase along with liquid CO_2 in supercritical fluid chromatography and phenformin as an internal standard. The modified plasma samples were analyzed by electro-spray ionization method in selective reaction monitoring mode in tandem mass spectrometry. Supercritical fluid chromatographic separation was applied using nucleosil C_{18} containing column as a stationary phase. The separated products were identified by characteristic peaks and specific fragments peaks in tandem mass spectrometry as m/z 130 to 86 for metformin, m/z 324 to 110 for gliclazide and m/z 206 to 105 for phenformin were obtained. The present method was found linear in the concentration ranges of 6.0-3550 ng/mL and 7.5-7500 ng/mL for metformin and gliclazide, respectively. [65]

CONCLUSION

The therapeutic oral dose of gliclazide, which is a more potent hypoglycemic than other sulfonylureas is not more than 1/5 of that of tolbutamide. However, the detection limits of conventional methods of assay of serum sufonylureas by coloimetry and by pyrolysis-GLC are 10 and 5µg/mL, respectively. These methods are not satisfactory for the determination of serum gliclazide. On the other hand, the detection limit of gas liquid chromatography with electron-capture detection is 0.1µg/mL of gliclazide which would seem to be useful for clinical examination of serum gliclazide, but this method usually requires tedious procedures, such as acylation of the extracted sample, and gives lower reproducibility. Also, neither co-administered drugs (e.g. vitamins or antibiotics) nor possible metabolites can be detected in the present analytical system. Hence, the another conventional analytical methods such as HPLC, colorimetry etc. may be applied for the detection of gliclazide in pure, dosage forms and as in body fluids and the selection of the analytical techniques will depend upon various factors associated with type of samples, requirements of results sensitivity etc.

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