. INTRODUCTION

Drugs used in diabetes treat diabetes mellitus condition by lowering the glucose levels in human beings. Diabetes mellitus type-1 is recognized as condition with deficiency of insulin in body. Type-2 diabetes is disease of resistance to insulin by cells. According to a recent survey published in daily newspaper Times of India 16 June 2014 issue (on increasing risk of higher population being effected by diabetes conducted by Metroplis Healthcare), it was discovered that almost 57% of sample population (38,966) indicated high diabetes levels. An alarming concern was attributed towards a fact that study included over 41% young population (age group of 20-40 years). Outcomes of study strongly recommended a routine screen for disease conditions and proper treatment schedule for effected population.

Secretagogues class of anti-diabetic drugs are drugs that increase insulin output from pancreas. Drugs in secretagogues category can be divided into:

- Sulfonylureas
- Biguanides
- Thiazolidinediones
- Alpha-glucosidase inhibitors
- Meglitinides
- Combination of Sulfonylureas and metformin (biguanide)

Sulfonylureas exerts is pharmacological activity via higher release of insulin from β cells in pancreas. Structurally sulfonylureas consist of S-phenylsulfonylurea structure with different substitutions on urea N end and phenyl ring. {Martindale, (2005)}.

First generation agents:
Tolbutamide, Acetohexamide, Tolazamide and Chlorpropamide.

Second generation agents:
Glipizide, Glibenclamide, Glimipride, Gliclazide, Glycopyramide and Gliquidone.

Sulfonylureas may induce hypoglycemia due to excess insulin production and release. These drugs also induce weight gain and show teratogenicity. According to study result for clinical trail
by UK Clinical practice research datalink on more than 10 million patients, 15,687 receiving sulfonylurea drugs as monotherapy vs 76,811 diabetic patients treated with metformin, difference in mortality rates was 4.46 vs 1.36 for sulfonylurea drugs vs metformin treatment. Drug-drug interaction studies become very important for this class of drugs as inducer/inhibitor drugs may potentiate or prolong the effects of sulfonylureas, thus leading to risk of hypoglycemia and other related side effects. {Bertram G. Katzung (1996)}.

**Tolbutamide:**

Tolbutamide is first generation potassium channel blocker, sulfonylurea oral hypoglycemic drug sold under brand name ORINASE. Orinase was developed by Upjohn Co. as an oral antihyperglycemic agents used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). {Martindale, (2005)}.

Pharmacological action of this category (i.e. Sulfonylureas) was first witnessed during a European pharmaceutical research trial for antibiotics. One of the contenders for new sulfa antibiotic witnessed serious side effects (Blackouts, convulsions and coma) during clinical trial at University of Montpellier. An insulin researcher in same university heard of these adverse events and recognized them as common indications for hypoglycemia. Drug was first brought into market under brand name Rastinon by West German Pharmaceutical Company (Hoechst). Upjohn entered in to cross licensing agreements with Hoechst to market tolbutamide under brand name ‘Orinase’. {Martindale, (2005)}.

Based on large scale clinical trials on over 5000 patients during 1955-1957, Upjohn filled an approval for Orinase in 1956. Drug was aimed for treatment option to undiagnosed and asymptomatic diabetic population base. Drug saw a drop in sales post reports of serious side effects including death from cardiovascular problems (beginning with the Washington Post). Many patients learned of this before their physicians and FDA recommendations, and lead to a public firestorm over proposed treatment regimen. The question of whether the reported cardiovascular adverse events were due to Orinase or not, has not been conclusively settled. {Martindale, (2005)}. 
Major side effects observed with tolbutamide are Hypoglycemia, Weight gain, Hypersensitivity (cross allergicity with sulfonamides) and drug interactions (Increased hypoglycemia with cimetidine, Insulin, Salicylates and Sulfonamides). Salicylates displace tolbutamide from its binding site on plasma binding proteins which lead to increase in free tolbutamide concentrations and thus hypoglycemic shock.

Tolbutamide is predominantly metabolized via liver enzymes through oxidation to form 1-buty1-3-p-carboxyphenylsulfourea. Major metabolizing enzymes are P450 2C9 and 2C19. {Martindale, (2005)}.

Accurate quantification of drug is of utmost importance for determination and correlation of pharmacokinetic (PK) and pharmacodynamic (PD) evaluation. Several reports have been published with emphasis on exact correlation of these factors. Several regulatory agencies have issued guidance to industry/researchers on deciding the critical parameters to prove the suitability of quantification method for intended application. Selection of appropriate extraction technique and detection method is very important to achieve accurate and reproducible method for quantification of xenobiotics. Drug analysis plays important role in the development, manufacture and therapeutic use of drugs. Drug analysis means identification, characterization, and quantification of drugs. It is also useful in assuring quality during the manufacture of drug formulations. Bioanalytical methods play essential roles in in-vitro and pharmacokinetic studies i.e., studies of the absorption, distribution, metabolism and elimination of drugs on animals and humans.

Analysis of drug in biological fluids generally involve two steps viz., extraction from complex biomatrices (blood, plasma, serum, liver microsomes, hepatocytes, Caco-2 cells, bile, urine, feces, tissues etc.) and measurement of compound of interest in extracted fluids by chromatographic method coupled to detection module. There is a growing need for development of bioanalytical method/s in drug discovery research and drug therapy (post market surveillance and clinical).
Development and validation of simple, selective, accurate and reproducible bioanalytical method/s is difficult as quantification of drugs has to be suitable for determination of drug at very low concentration levels (e.g. micro or ng/mL levels). Assessment of aqueous solubility, metabolic stability, CYP inhibition, CaCO-2 permeability and various pharmacokinetic parameters (such as AUC, tmax, Cmax, Kel, Vd , t1/2) in discovery and/or clinical studies is important for decision making on treatment regime.

Considering the ambiguity on adverse events for long-term clinical trials, an appropriate screen for drug-drug interaction possibilities for tolbutamide is inevitable. According to a recent research publication by Xiangjun Qiu and his colleagues (Xiangjun Qiu et.al. (2012)) studies with and without bupropion treatment to in-vivo model didn’t showed any significant change in CYP-2C9 mediated tolbutamide hydroxylation. On a separate note there are only few analytical methods available for support to quantification of tolbutamide in biological matrix.