CHAPTER 1

Abstract
Diabetes is a metabolic disorder condition in human beings resulting due to elevated glucose levels in human body for prolong period. Indication's of high glucose comprise recurrent urination, amplified thirst, and improved hunger. If gone untreated, diabetes can lead to several impediments.

Glucose is generated from our dite, we eat. Insulin represents hormone that facilitates glucose enter into living cell to furnish or release energy. For person with type-1 diabetes, body doesn't produce insulin. For type-2 diabetes, more widespread category, human body doesn't construct or utilize insulin well. In abscence of sufficient insulin, the glucose remains in blood. According to a survey report published by International Diabetes Federation (Retrived on 29 November 2014), around three hundred eighty seven million peoples worldwide are effected from diabities. Out of these, around ninety percent case were diagnosed with type-2 diabetes. Another medical condition asscociated with Diabities is prediabetes. This reflects a condition of blood sugar elevated than usual but not high enough to considered as diseased condition i.e. diabetes. Prediabetes condition reflectecs a higher probability of attaining type 2 diabetes.

Tolbutamide is first age group potassium direct blocker, sulfonylurea oral hypoglycemic drug. Major side effects observed with tolbutamide are Hypoglycemia, Weight pick up, Hypersensitivity (cross allergicity with sulfonamides) and medication communications (Increased hypoglycemia condition associated Insulin, Cimetidine, Sulfonamides and Salicylates). Salicylates dislodge tolbutamide from its coupling site on plasma tying proteins which prompt increment in free tolbutamide focuses and therefore hypoglycemic astound. Drug saw a drop in sales post reports of severe side effects including demise from cardiovascular problems (beginning with the Washington Post). Many patients got aware about these facts/news even before their doctors and regulatory 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, have not been conclusively settled. Considering the ambiguity on adverse events for long-term clinical trials, an appropriate screen for drug-drug interaction possibilities for tolbutamide was inevitable.

A drug-2 interaction may be elaborated as situation in which a substance (mostly another
drug) affects the action of medication. At times both are present together at site of action. This effect can be synergistic (medication activity amplified) or antagonistic (medication activity reduced) or novel activity is monitored, that none of the products exerts individually. Usually, drug interactions among different medications are observed (i.e. drug-drug interaction). Nevertheless, interactions can also develop between medication and food substances (drug-food interactions), and also between medications and medicinal plants and herbs (drug-plant interactions). Person under exposure of antidepressant medication e.g. monoamineoxidase inhibitors is advised not consume food containing tyramine as hypertensive condition may develop (drug-food interaction). These interactions can develop due to inadvertent misuse or lack of information on pharmacoactive ingredients contained in pertinent substances.

Drug discovery and clinical evaluation process has undergone a chronological changes over last few decades. Earlier discovery strategy included; assortment of primary pharmaco-active compounds from sequence of novel synthesized compounds using specific pharmacological assays. Safety characteristics were evaluated by screening the selected compounds at high dose in assays aimed at indication apart from desired activity of new chemical entity (NCE). These assessments were followed with pharmacokinetic study, that were primarily aimed to determine residential half-life time and exposure. Safety assessment depend on acute and sub-acute toxicity studies, that result information expressing on organ construct rather than on organ functionality. Pharmacokinetic and toxicological studies were tailored in the direction of development studies during clinical pharmacology and clinical trial phases. This approach has been challenged during last 20 years for several reasons:

Few negative observations on organ function, e.g., ventricular tachy-arrhythmia are detectable only at later stages. Conversely, negative observations during repeated dose toxicity studies in animals may be inappropriate for human population. Due to species differences and other factors.

Innovative scientific advancements, e.g. high-throughput screening, combinatorial chemistry, insilico replicas, pharmaco-genomics and pharmaco-proteomics extended newer potentials.

The rate at which the new medications are introduced in market has gone drastically down as
compared to rate at which the new chemical entities are being synthesized in laboratory. The
time required for getting a compound from bench-side (Synthesis Laboratory) to bed-side
(Clinic) has increase dramatically as compared to earlier days.

These factors led to change in drug discovery strategy:

• Parallel in place of sequential participation of the different scientific disciplines.

• The phrase “Safety Pharmacology” was introduced.

• International Conference on Harmonization (ICH) Safety Pharmacology working group
  was established.

• Easily available and mainly useful assays must be chosen.

Precise quantification of medication has prime importance for determination and correlation
of pharmacokinetic (PK) and pharmacodynamic (PD) assessment. Analytical method
optimisation and validation of straightforward, specific, accurate and reproducible bioanalytical
method’s is difficult as quantification of drugs has to be suitable for quantification of drugs at
very low concentration levels (e.g. micro or ng/mL levels) and performance of method has to be
evaluated as per regulatory guidelines before analysis of any study samples. Assessment of
aqueous solubility, metabolic stability, plasma protein binding, Cytochrome P450 inhibition,
CaCO-2 permeability and various pharmacokinetic parameters (such as AUC, t_{max}, C_{max}, K_{el}, V_d,
t_{1/2}) in discovery and/or clinical studies is important for decision making on treatment regime.

During conduct of proposed research, analytical (HPLC-UV) and bio-analytical (LC-MS/MS)
methods were developed and validated as per ordained guidance requirements.

A novel, accurate, specific, simple, sensitive and reproducible high-performance liquid
chromatography (HPLC) assay method has been developed and validated for the estimation of
tolbutamide. The analytical method involves dilution of samples with Internalstandard
(Diphenhydramine) solution in 80% Methanol from formulation media (0.5% Methyl cellulose
and Tween-80). The chromatographic analysis was performed on a Waters Alliance system with
an Symmetry shield C_{18} column maintain on 40°C temperature and gradient mobile phase
[acetonitrile:- 0.01 M Ammonium acetate in Milli-Q water, pH adjusted to 5.0 with acetic acid]
at a stream rate of 1.00 mL/min with analytical run time of 10 min. The eluate was monitored for absorbance using an UV detector set at 258 nm wavelength. Method validation was performed in accordance to requirements established in Organization for Economic Co-operation and Development (OECD); Principles of Good Laboratory Practices (ENV/MC/CHEM (98)17 - 1997); APVMA guidelines (Validation of analytical methods for active constituents, agricultural and veterinary chemical products, October 2004) and ICH Guidelines (Validation of analytical procedures:- Text and methodology Q2 (R1)) and results met acceptance criterion. The response curve was linear over concentration range of 250 to 25000 µg/mL ($r^2 = 0.997$). The intra- and inter-day precision values were within the range of 0.99-6.35 and 4.32-5.51, correspondingly. The validated HPLC method was successfully applied to solubility and formulation assays, stability and homogenity studies.

A very delicate, fast examine strategy has been created and accepted for the quantitation of tolbutamide in rodent plasma with fluid chromatography coupled to pair mass spectrometry with electro shower ionization in the positive-particle mode. The examine methodology includes extraction of tolbutamide and ISTD from rodent plasma with protein precipitation. Chromatographic partition was accomplished utilizing an isocratic mobile phase (5 mM ammonium formate with 0.05% formic acid:-acetonitrile: - 30:-70, v/v) at a flow gradient of 0.4 to 0.7 mL/min on an Atlantis dC18 column (maintained at 40 ± 1°C) with a total run time of 3.0 min. The MS/MS ion transitions monitored were 271.0 and155.0 for tolbutamide and 256.1→152.2 for internal standard. Method validation was performed as per USFDA bioanalytical method validation guidelines and the results met the acceptance criteria. The linearity range extended from 5 to 600 µg/mL. The intra- and inter-day precisions were in the range of 0.41 to 6.59 and 4.28 to 5.70, respectively. The validated LC-MS/MS method was successfully applied to metabolic stability, CYP inhibition, plasma protein binding, CaCO$_2$ permeability and pharmacokinetic studies.

Analytical methods sensitivity (on column load) for Analytical (HPLC-UV) and Bioanalytical (LC-MS/MS) methods were 100 nano-grams and 29 pico-grams, respectively Lowest acheived till date by any researcher. Further these methods were applied to determine solubility, microsomal stability, plasma protein binding, Cytochrome P450 inhibition, CaCO2 permeability
and pharmacokinetic parameters. Results indicate good solubility and low metabolic clearance for tolbutamide. In terms of inhibition of specific CYP isoenzymes, no significant inhibition was seen for 3A4, 2C9 and 2C19, while inhibition was seen for 2D6. This fact has never been reported by any researcher till date and would certainly be helpful in further studies. No significant change in pharmacokinetics was seen upon repeated dose (400, 800 and 1600 mg/Kg) till 28 days to rats.

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Research suggests a sensitive and robust analytical strategy for tolbutamide estimation and gives insight on various pre-clinical assessment of tolbutamide via \textit{in-vitro} and in-vivo experiments.