CHAPTER 3

Literature Review
Review of literature was very critical during conduct of proposed research due to limited number of literature references for *in-vitro* and *in-vivo* drug detection examine fallout of Tolbutamide on pre-clinical species. As pharmacological activity of Tolbutamide was observed during early 1940s during European pharmaceutical research trial for antibiotics at University of Montpellier there were very limited Investigational Drug discovery Data compilation available. Drug was first brought in market as 'Rastinon' by West German Pharmaceutical Company (Hoechst). Upjohn later considering their business interests entered in cross licensing agreements with Hoechst to market tolbutamide under brand name ‘Orinase’. Clinical efficacy was major contributor for drug of choice for asymptomatic diabetic during 1960s.

Tolbutamide is oral anti-hyperglycemic medication utilized for cure of non-insulin-dependent diabetes mellitus. It comes under sulfonylurea class of insulin secretagogues medications, that demonstrate by activating β cells of the pancreas to discharge insulin. As a symptom, these medications may bring about hypoglycemia and are encouraged to be dose with predictable sustenance admission (Fed condition) to diminishing this danger.

Sub-atomic Weight:- 270.348 (Average Mass) ; 270.103813142 (Monoisotopic Mass)

Synthetic Formula:- C12H18N2O3S

IUPAC Name:- 3-butyl-1-(4-methylbenzenesulfonyl) urea

Synthetic Structure
Physiochemical Properties:-
Melting Point:-128.5 ºC

pKa:-5.16

Log-P:-2.34

Water Solubility:- 109 mg/L (at 37 ºC)

Pharmacodynamics:-

Tolbutamide is original sulfonylurea antidiabetic medication and is prescribed to be utilized as a part of conjunction with eating routine. Tolbutamide lessens glucose by triggering pancreas to release insulin. For activity, this medication needs the pancreas to have the capacity to deliver insulin.

Action:-

Tolbutamide brings down blood glucose in patients experiencing non-insulin-dependent diabetes mellitus, by specifically fortifying sharp arrival of insulin from dynamic beta cells of pancreatic islet tissue by an obscure procedure that incorporates a sulfonylurea (receptor-1) on the beta cell. Medication prevents the ATP-potassium channels exhibit on the beta cell layer and potassium efflux, these outcomes in depolarization and calcium convergence, calcium-calmodulin tying, kinase enactment, and release of insulin-containing granules by exocytosis.

Ingestion:-

Medication is promptly assimilated after oral dose. In people, it is noticeable in plasma post 30-60 minutes of oral dosing with a maximum plasma concentration attained at 3-5 hours post dose. Its bioavailability rate is unaltered with food, but is increased at high pH.
Plasma protein binding:-

Tolbutamide is highly bound to plasma proteins (i.e. ≥ 95%). According to a study by Thiesen JJ et.al. Published in Journal of Clinical Pharmacology (1976); shared significant information on plasma protein binding property of tolbutamide. Drug concentration range of 50 to 300 µg/mL were quantified in 21 typical and 14 alcoholic subjects. At 100 µg/mL level, the plasma protein adherence values were 97.8 ± 0.3 % in normals and 95.1 ± 4.2 % in alcoholics. Lower plasma protein binding values in alcoholic human plasma (p less than 0.02) indicated that extent of tolbutamide plasma binding is reliant on albumin content of plasma.

Metabolism:-

Tolbutamide is metabolised primarily via oxidation of p-methyl moiety to result in hydroxytolbutamide and along these lines metabolized to carboxytolbutamide (Rechard Thomas et.al. and S.J.Gee et.al). The first reaction is CYP450 dependent ( R.G.Knodeli et.al. and M.E. Veronse et.al.) While later oxidation reaction occurs in the cystol (Rechard Thomas et.al.). Metabolism of tolbutamide has been studied extensively in humans to account for adverse drug reactions resulting from inhibition of metabolism pathways (S.M. Pond et.al. and D.J. Beck et.al.) And as a sculpt for genetic polymorphisms (J. Scot et.al and G.F. Peart et.al.).

Few critical references in this regards were:-


→ R.Knodile & S.M. Heel.et.al;(1987) magazine of pharmacology & experimental therapeutics; Vol-141 Page-1012 to 1089.

→ M.I. Veronase, And J.I. Minrs;(1990) Drug Metabolism and Disposition; Vol-28 Page-357 to 363.
Elimination:-

Tolbutamide and its metabolites are primarily eliminated from body via urine and feces. Major route of elimination for oral dosed drug is in form of 1-butyl-3-p-carboxyphenyl sulfonylurea in pee inside of 24 hours that accounts for 75 - 85% of total dose. Elimination half life is approximately 7 hours, but due to high inter-individual variations the half life ranges between 4 to 25 hours. Among all drugs of same class (i.e. sulfonylureas) tolbutamide has shortest duration of pharmacological action.

Toxicity:-

For oral route of administration, reported LD$_{50}$ values for mouse is 2600 mg/Kg. Reported value for rat acute toxicity is 2.0629 mol/Kg.

Conversion of mol/Kg to mg/Kg dose strength:-

Mol Wt.:-270

Dose Volume:-1 mL/Kg

Dose (mg/Kg) will be **557** mg/Kg.

Considering this high dose value, I have kept 3 dose levels (i.e. 400, 800 and 1600 mg/Kg) for estimation of pharmacokinetic profile of this drug.
Drug Interactions:-

As discussed earlier, Tolbutamide has a very specific path of metabolism involved and hence it accounts for several drug-drug interaction possibilities if not dosed and monitored properly. Following are some of marketed drugs that have shown evidence of drug-drug interaction possibilities with tolbutamide:-


Table-1: Drug interactions of tolbutamide with Acebutolol, Acenocoumarol, Acetylsalicylic acid, Atenolol, Bisoprolol, Bosentan Capecitabine, Carvedilol, Celecoxib, and Chloramphenicol

(Reference:- Drug Bank; Accession Number:- DB01124 (APRD00267))
**Table-2:-** Drug interactions of tolbutamide with Dapsone, Delavirdine, Digoxin, Esmolol, Floxuridine, Fluconazole, Fluorouracil and Fluoxetine {Reference:- Drug Bank; Accession Number:- DB01124 (APRD00267)}

<table>
<thead>
<tr>
<th>Drug</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dapsone</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Dapsone. Consider alternate therapy or monitor for changes in Dapsone therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Delavirdine</td>
<td>Delavirdine, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Tolbutamide therapeutic and adverse effects if Delavirdine is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Tolbutamide increases the effect of digoxin</td>
</tr>
<tr>
<td>Esmolol</td>
<td>The beta-blocker, esmolol, may decrease symptoms of hypoglycemia.</td>
</tr>
<tr>
<td>Floxuridine</td>
<td>Floxuridine, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Tolbutamide therapeutic and adverse effects if Floxuridine is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Fluconazole, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Fluconazole therapeutic and adverse effects if Delavirdine is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Fluorouracil</td>
<td>Fluorouracil, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Tolbutamide therapeutic and adverse effects if Fluorouracil is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Fluoxetine. Consider alternate therapy or monitor for changes in Fluoxetine therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
</tbody>
</table>

**Table-3:-** Drug interactions of tolbutamide with Flurbiprofen, Fosphenytoin, Gemfibrozil, Glimepiride, Glipizide, Ibuprofen and Indomethacin {Reference:- Drug Bank; Accession Number:- DB01124 (APRD00267)}

<table>
<thead>
<tr>
<th>Drug</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flurbiprofen</td>
<td>Flurbiprofen, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Tolbutamide therapeutic and adverse effects if Flurbiprofen is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Fosphenytoin</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Fosphenytoin. Consider alternate therapy or monitor for changes in Fosphenytoin therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>Gemfibrozil, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Gemfibrozil therapeutic and adverse effects if Gemfibrozil is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Glimepiride. Consider alternate therapy or monitor for changes in Glimepiride therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Glipizide</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Glipizide. Consider alternate therapy or monitor for changes in Glipizide therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Ibuprofen, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Ibuprofen therapeutic and adverse effects if Ibuprofen is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Indomethacin, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Tolbutamide therapeutic and adverse effects if Indomethacin is initiated, discontinued or dose changed.</td>
</tr>
</tbody>
</table>
Table-4:- Drug interactions of tolbutamide with Ketamine, Ketoconazole, Labetalol, Losartan, Lumiracoxib, Mefenamic acid, Mestranol and Metoprolol {Reference:- Drug Bank; Accession Number:- DB01124 (APRD00267)}

<table>
<thead>
<tr>
<th>Drug</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Ketamine. Consider alternate therapy or monitor for changes in tolbutamide therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>Ketoconazole, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Tolbutamide therapeutic and adverse effects if Ketoconazole is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Labetalol</td>
<td>The beta-blocker, labetalol, may decrease symptoms of hypoglycemia.</td>
</tr>
<tr>
<td>Losartan</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Losartan. Consider alternate therapy or monitor for changes in Losartan therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Lumiracoxib</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Lumiracoxib. Consider alternate therapy or monitor for changes in Lumiracoxib therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>Mefenamic acid, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Tolbutamide therapeutic and adverse effects if Mefenamic acid is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Mestranol</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Mestranol. Consider alternate therapy or monitor for changes in Mestranol therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>The beta-blocker, metoprolol, may decrease symptoms of hypoglycemia.</td>
</tr>
</tbody>
</table>

Table-5:- Drug interactions of tolbutamide with Miconazole, Montelukast, Nadolol, Nateglinide, Nicardpine, Oxpresolol, Paclitaxel and Phenylbutazone {Reference:- Drug Bank; Accession Number:- DB01124 (APRD00267)}

<table>
<thead>
<tr>
<th>Drug</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miconazole</td>
<td>Miconazole, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Tolbutamide therapeutic and adverse effects if Miconazole is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Montelukast</td>
<td>Montelukast, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Montelukast. Consider alternate therapy or monitor for changes in Montelukast therapeutic and adverse effects if Montelukast is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Nadolol</td>
<td>The beta-blocker, nadolol, may decrease symptoms of hypoglycemia.</td>
</tr>
<tr>
<td>Nateglinide</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Nateglinide. Consider alternate therapy or monitor for changes in Nateglinide therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Nicardpine</td>
<td>Nicardpine, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Tolbutamide therapeutic and adverse effects if Nicardpine is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Oxpresolol</td>
<td>The beta-blocker, oxpresolol, may decrease symptoms of hypoglycemia.</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Paclitaxel. Consider alternate therapy or monitor for changes in Paclitaxel therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>Phenylbutazone increases the effect of the hypoglycemic agent</td>
</tr>
</tbody>
</table>
**Table-6:** Drug interactions of tolbutamide with Phenytoin, Pindolol, Piroxicam, Propranolol, Rifampicin, Sitaxentan, Somatropin and Sulfadiazine {Reference:- Drug Bank; Accession Number:- DB01124 (APRD00267)}

<table>
<thead>
<tr>
<th>Drug</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenytoin</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Phenytoin. Consider alternate therapy or monitor for changes in Phenytoin therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Pindolol</td>
<td>The beta-blocker, pindolol, may decrease symptoms of hypoglycemia.</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>Piroxicam, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Tolbutamide therapeutic and adverse effects if Piroxicam is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Propranolol</td>
<td>The beta-blocker, propranolol, may decrease symptoms of hypoglycemia.</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Rifampin may decrease the effect of sulfonylureas, tolbutamide.</td>
</tr>
<tr>
<td>Sitaxentan</td>
<td>Sitaxentan, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tolbutamide, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in Tolbutamide therapeutic and adverse effects if Sitaxentan is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Somatropin recombinant</td>
<td>Somatropin may antagonize the hypoglycemic effect of Tolbutamide. Dose adjustments of Tolbutamide may be required.</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>Tolbutamide and Sulfadiazine are strong CYP2C9 inhibitors and substrates. Decreased metabolism and clearance of both agents may occur during concomitant therapy. Consider alternate therapy or monitor for changes in the therapeutic and adverse effects of both agents if concomitant therapy is initiated, discontinued or dose(s) changed.</td>
</tr>
</tbody>
</table>

**Table-7:** Drug interactions of tolbutamide with Sulfamethoxazole, Sulfinpyrazone, Sulfisoxazole, Tamoxifen, Timolol, Torasemide and Trimethoprim {Reference:- Drug Bank; Accession Number:- DB01124 (APRD00267)}

<table>
<thead>
<tr>
<th>Drug</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfamethoxazole</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Sulfamethoxazole. Consider alternate therapy or monitor for changes in Sulfamethoxazole therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Sulfinpyrazone</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Sulfinpyrazone. Consider alternate therapy or monitor for changes in Sulfinpyrazone therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Sulfisoxazole</td>
<td>Tolbutamide and Sulfisoxazole are strong CYP2C9 inhibitors and substrates. Decreased metabolism and clearance of both agents may occur during concomitant therapy. Consider alternate therapy or monitor for changes in the therapeutic and adverse effects of both agents if concomitant therapy is initiated, discontinued or dose(s) changed.</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may decrease the metabolism and clearance of Tamoxifen. Consider alternate therapy or monitor for changes in Tamoxifen therapeutic and adverse effects if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Timolol</td>
<td>The beta-blocker, timolol, may decrease symptoms of hypoglycemia.</td>
</tr>
<tr>
<td>Torasemide</td>
<td>Tolbutamide, a strong CYP2C9 inhibitor, may increase the serum concentration of Torasemide, a CYP2C9 substrate, by decreasing its metabolism and clearance. Consider alternate therapy or monitor for changes in the therapeutic and adverse effects of Torasemide if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>The strong CYP2C9 inhibitor, Tolbutamide, may decrease the metabolism and clearance of Trimethoprim, a CYP2C9 substrate. Consider alternate therapy or monitor for changes in therapeutic and adverse effects of Trimethoprim if Tolbutamide is initiated, discontinued or dose changed.</td>
</tr>
</tbody>
</table>
Drug investigation implies distinguishing proof, portrayal, and evaluation of medications. It is additionally valuable in guaranteeing quality amid the production of medication plans. Bioanalytical techniques assume key parts in-vitro and pharmacokinetic studies i.e., investigations of the ingestion, dispersion, digestion system and end of medications on creatures and people.

Investigation of drug in biological fluids generally involves 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). There is a requirement in the pharmaceutical laboratories for the development of analytical methods, to quantify drug levels in animal plasma and tissues. The objective is to attain more selective, sensitive, reproducible and high through-put assays and analytical methods than currently existing methods. The established analytical method’s can then be subjected to assessment of pharmacokinetic parameters from different animal species to ascertain the bioavailability and exposure profiles for clinical and pre-clinical studies. Advancement and acceptance of straightforward, particular, exact and reproducible bioanalytical technique /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.

Few HPLC-UV, LC-MS/MS and GC-MS system’s assay reports utilized for specified unit (tolbutamide) have been shared and displayed till date. Few of these technique/s utilize complex extraction systems and scientific strategies, amplified and dreary specimen extraction method including critical volume of dissolvable/s and/or natural frameworks for the extraction.

In terms of understanding on metabolism path for Tolbutamide and investigation of clinical ambiguities, following research articles/publications were of critical importance:-

- **Rechard C. Thomas and George J. Ikeda** (1966) have published their research work on metabolism of Tolbutamide in rats and humans using Tritium labeled tolbutamide. In human; 85% of drug was eliminated via metabolic pathway through urine. This accounted for 67% of p-hydroxymethyl and 33% of p-carboxy via radioactivity assessments for 2 compounds on Paper and Thin layer chromatography. In rats; 80% of drug was excreted via urine in form of p-hydroxy methyl predominantly.

- **D.R.Redman et.al.** (1973) have published results indicating lowering of induction capacity of liver microsomal proteins for tolbutamide in development onset diabetic patients. Tolbutamide half-life was monitored in mature onset diabetic patients with treatment for 2 weeks with 1.0 and 1.5 gm dosages. Slight (not significant) shortening of half life was seen for study. Further treatment with phenobarbital (60 to 90 mg) was also not able to show any induction of microsomes involved in metabolism for tolbutamide.

- **C.J.Doacke et.al.;** (1991) conducted studies to establish which isoenzyme (cytochrome P450 monooxygenase system) is responsible for metabolism of phenytoin and tolbutamide. Research showed competitive inhibition by
phenytoin on tolbutamide methyl hydroxylation (mean $K_m=85.6; \ n=3$). Tolbutamide was additionally discovered to be hindering the Phenytoin 4-hydroxylation. Sulphaphenazole was intense inhibitor for both medications (i.e. Phenytoin and Tolbutamide) with IC50 estimations of 0.4 and 0.6 µM, individually. Mephenytoin was discovered to be poor inhibitor for both medications (i.e. Phenytoin and Tolbutamide) with IC50 esteem more prominent than 400 µM. Analysts likewise watched that Anti-rabbit P450IIC3 IgG repressed both (i.e. Phenytoin and Tolbutamid by 62 and 68%, respectively.

- **Laskar JM et al.** (1998) have published results indicating metabolic efficiency of CYP-2C19 as being equally efficient catalyst in oxidative metabolism of Tolbutamide, as CYP-2C9. They shared reaction kinetic parameters for 2 isoenzymes as showing comparable metabolic capability (i.e. 2C19: $K_m=650$ µM, $V_{\text{max}}=3.71 \text{ min}^{-1}$ and 2C9: $K_m=178-407$ µM, $V_{\text{max}}=2.95-7.08 \text{min}^{-1}$). They also observed CYP-2C19 antibodies potentially inhibiting the reaction to over 90% as compared to over 85% inhibition by CYP-2C9 antibodies. Lack of relation between microsomal tolbutamide hydroxylation and CYP-2C19 content was also observed. Group mentioned low levels of CYP-2C19 appearance in liver as reson to preclude importance of specific isoenzyme (i.e.CYP2C19) role in hepatic metabolism of tolbutamide.

- **R. Scot Obachi** (1999) conducted study on 29 different drugs to examine the in-vitro half life prediction approach from microsomal intrinsic clearance and microsomal binding data. Tolbutamide showed a low binding with microsomes and disregarding binding considerations lead to poor prediction/ calculation of human clearance values. Human liver microsomal incubation experiments were conducted in triplicate and reaction were terminated by addition of acid to incubation mixture (i.e. Hydrochloric acid). Binding was estimated via equilibrium dialysis experiment. Fraction unbound in plasma was 0.04 and a
equal distribution of drug was seen in blood to plasma ratio experiment (i.e. 0.55). Values indicate low binding and absence of erythrocyte uptake of tolbutamide in blood. Protein concentration was kept high (i.e. 10 mg/mL) for microsomal incubation experiments. A relatively high in-vitro $T_{1/2}$ value (71 ± 12 minutes) with a low intrinsic clearance value (0.90 ± 0.15) was observed.

- **Michel R. Wastret et al.** (1999) published their research on part of CYP-2C19 in tolbutamide hydroxylation by human liver microsomes. They utilized immunochemical way to deal with survey the part of individual CYPs. Polyclonal antibodies were developed to CYP-2C9 filtered by human liver and were on the other hand assimilated against recombinant CYP-2C19 coupled to strong stage support. Monospecific against 2C9; was found to hinder speed of tolbutamide hydroxylation at 93 ± 4 % and 78 ± 6 % in CYP-2C19 deficient and CYP-2C19-containing human hepatic microsomal concentrates, separately. Study presumed that CYP-2C19 metabolizes no less than 14 to 22% of tolbutamid.

- **Dermet F. Mcginity et al.** (2000) directed an examination on diverse test substrates for building up a computerized measure to focus enzymology of compound oxidation by distinctive cytochrome P450s (CYP 1A2, 2C9, 2C19, 2D6 and 3A4). These were coexpressed practically in Escherichia coli. Tolbutamide was discovered to be metabolized by CYP-2C19 (~30%) and CYP-2C9 (~70%). HPLC test for metabolic concentrate was performed at 230 nm wavelength. CLint was dictated by $V_{max}/K_{m}$ and was discovered to be 0.014 and 0.03 ± 0.01 μL*min$^{-1}$ pmol$^{-1}$ for CYP-2C9 and 2C19, separately. While no distinguishable results were accounted for other three isoenzymes (i.e. CYP1A2, CYP2D6 and CYP3

- **Rai Yuan et al.** (2002) common their perception on assessment of cytochrome P450 test substrates regularly utilized by the pharmaceutical business as a part of
examining medication interactions. Tolbutamide was utilized as test substrate for CYP-2C9 isoenzyme. Analysts have eluded tolbutamide 4'-hydroxylation as prefered test response for around 80% entries. Studies with cDNA communicated chemicals demonstrate that at test focuses ≤ 500 µM, around 90% of Tolbutamide is metabolized by CYP-2C9 and about 10% by CYP-2C8. Tolbutamide hydroxylation takes after Michaelis-Menten energy with Km values between 60 to 400 µM. They likewise alluded study perceptions of Laskar; about association of 2C19 in digestion system of tolbutamide. They have additionally demonstrated importance of natural dissolvable impact i.e. at focus levels of 1% v/v; isopropanol, DMSO and methanol; each repress tolbutamide hydroxylation by ≥ 40%. Be that as it may, Acetonitrile does not influence and hence should be the solution of choice for conduct of any microsomal incubation experiment for tolbutamide.

- **Dan Lee et.al.** (2010) common their perceptions on impact of diverse organic solvents on role of cytochrome P450 substrates in rodent liver microsomes. Tolbutamide methyl hyroxylation was utilized as chemical movement checking response for surveying effect of distinctive dissolvable qualities on response rate. At 0.25% level methanol, DMSO, acetonitrile and CH3) 2CO didnt influenced the reaction rate but ethanol at 0.25% levels was able to inhibit the reaction upto 27%. No inhibition was seen for acetonitrile level of 1% in incubation mixture. All solvents were inhibiting the reaction 60% at 5% composition levels. Hence during the conduct of any incubation experiment; care must be take to control the organic solvents to (or below) these specified levels to assess actual inhibition and avoid any false interpretation.

- **Faihad I. Al-jenobi** (2010) has published observations on effect of some common Saudi people home grown meds on metabolic productivity of CYP-2C9 in human liver microsomes. Marker reaction used for monitoring the inhibitory
effects of methanolic extracts of eleven different Saudi folk herbal medications was CYP-2C9 mediated metabolism of tolbutamide to 4’-hydroxytolbutamide. Among the drugs tested only two drugs (i.e. aniseed and curcuma) showed statistically significant inhibitory effects on CYP-2C9 mediated tolbutamide metabolism. Curcuma showed around 45% inhibition at highest substrate concentration (i.e. 10 and 25 µg/mL) and aniseed showed around 55% inhibition at 2.5 µg/mL substrate concentration.

- **Haw-Wen Chain *et al.*; (2013) has published their research work on study of Andrographolide and andrographis paniculata extract on CYP-2C enzymes modulation in rats and their impact on tolbutamide pharmacokinetics. Rats were intragastrically dosed with 2 and 50 g/Kg of andrographis paniculata separate and andrographolide every day for 5 days. On 5th day tolbutamide was dosed at 20 mg/Kg measurement level to same rats, a control gathering was likewise kept to study the impact of multiple/repeated dosed concentrate and andrographolide on tolbutamide. Study demonstrated andrographis paniculata concentrate and andrographolide decreased the AUC \(_{0-12h}\) of tolbutamide by 37 and 18% separately. Biochemistry study showed increased levels of protein, mRNA and few CYP isoenzymes (CYP-2C6/II, CYP-1A1/2 and CYP-3A1/2). However in spite of reduced AUC and increased expression of drug metabolizing enzymes, hypoglycemic effect of tolbutamide was not effected in glucose tolerance test.

For understanding the analytical and bioanalytical efforts on tolbutamide; several research articles were studied. Mentioned literature (not limited to below) were reviewed and referred to develop better and simplified analytical approach for Tolbutamide, considering anticipated preclinical assay matrices. Till date no research work has been conducted on this drug, to establish a comprehensive analytical and pre-clinical strategy.
Review on available bio-analytical research publications for Tolbutamide quantization:-

**Title:** Determination of tolbutamide hydroxylation in rodent liver microsomes by HPLC:- impact of psychoactive medications on in vitro action

**Creator/s:** John W. Ho. and David E. Moody

**Diary:** Journal of Life Sciences, Vol. 52, Page- 21 to 28

**Survey:** Authors have shown HPLC investigation approach for estimation of restraint of microsomal tolbutamide hydroxylation by sixty psychoactive medications for appraisal of medication interaction possibilities by these. In human and rat species, tolbutamide is primarily metabolised via oxidation of para-methyl group to result hydroxytolbutamide and further result in formation of carboxytolbutamide.

Sprague Dawley (male) rats were excised to remove liver organ and same was perfused to remove traces of blood or other body fluids. Microsomes were ready using differential centrifugation of perfused liver. Tolbutamide was added at substrate @ levels of 150 µM at a microsomal protein fixation level of 0.4 mg.

Responses were ended by expansion of acidic (3N HCl) arrangement. Tests were subjected to fluid extraction (utilizing Diethyl ether). Natural layer was isolated, vanished and dried buildup was reconstituted in watery media (like HPLC portable stage).

**Analytical conditions:-**

**Framework:** Varian - 5060 Chromatography instrument with UV-VIS identifier

**Detector:** UV-VIS with altered wavelength channel and Anspec - 1200 strip chart recorder.

**Column:** Analytichem International C18 RP (4.6 mm, 15 cm, 5µm)

**Mobile Phase:** 0.01 M monobasic Ammonium phaosphate in methanol (45:-55, v/v) pH 5.

**Flow Rate:** 0.7 mL/min

**Injection:** 10 µL
In terms of detection wavelength, the $\lambda_{\text{max}}$ for tolbutamide, Internalstandard (chlorpropamide), Hydroxy-tolbutamide and Carboxy-tolbutamide were 227, 230, 232 and 234nm. These maximas are very close and result in overlap of absorption bands.

At lower wavelengths the baseline noise and interference levels will also be high at lower wavelengths. In text authors have mentioned use of 240 nm as detection wavelength, but contradictorily in fig-1 the absorbance has been mentioned to be monitored at 340 nm.

Title: Development and Validation of Reverse Phase-HPLC technique for quantitative examination of tolbutamide in pharmaceuticals

Creator’s: K. Ammani, D. Madhu Latha and P. Jitendra Kumar

Diary: Int. J. of Chemical Sci., Vol. 11 (4) Page- 1607 to 1614; 2013

Review: Authors have demonstrated HPLC analysis approach for quantitation of tolbutamide in pure and pharmaceutical formulations. Validation was performed for tolbutamide (pure) with conduct of following tests:

Linearity:-

- Calibration curve was plotted for 20, 40, 60, 80, 100 and 120 µg/mL.

Precision:-

- Intraday and interday precision was assessed by analyzing 6 replicate runs at single concentration level (i.e. 60 ppm).

Specificity:-

- Effect of several different excipients and additives was evaluated.

Ruggedness:-

- 3 different make HPLC systems (Shimadzu, Agilent and Waters) were used for analysis of tolbutamide. For column chemistry also 3 different makes (Hypersil, Phenomenex and Hichron) were evaluated.
Limit of detection (LOD) and limit of quantification (LOQ):

- numerical calculation of LOD and LOQ was performed using standard deviation of Y-intercepts obtained for calibration curve regression line.

* Accuracy:-

Accuracy was assessed by spiking 50, 100 and 150% attention in 40 µg/mL samples.

* Robustness:-

3 analysis parameters (i.e. Mobile phase, pH and wavelength) were varied somewhat to review impact on chromatography.

No details on excipients and additives used for assessment of specificity was shared in article. Also in ruggedness; different make instruments and analytical columns were mentioned to be evaluated, but no supportive data is shared. Usually different make instruments have different system volumes and hence will result in different retention times. In such case relative retention time is better comparison. Also different C-18 columns will lead to different chromatographic profile due to different extent of ligand coverage and silica sources.

As per published information, Limit of finding obtained was 5 µg/mL and Limit of quantification was 1.54 µg/mL, here LOD value obtained is higher then LOQ. While as per definition LOD is concentration for signal to noise value of 3, while LOQ is concentration for signal to noise value of 10. The LOD value cannot be less than LOQ value. Due to non-availability of complete data used for calculation, conclusion cannot be drawn on this point.

**Analytical conditions:-**

System:- Shimadzu Chromatograph with LC 20AT pump

Detector:-LC 7000 UV detector with fixed wavelength of 231 nm.

Column:-Zodiac C18 R P (4.6 mm, 250 mm, 5µm)

Mobile Phase:-0.1 % ortho phosphoric acid:- Methanol:- Acetonitrile :-:- 3:-1:-6; v/v, pH 5.9 with ortho phosphoric acid.
Floow Rate:-1.0 mL/min
Injection:-20 µL
Mentioned conditions were used to determine the Tolbutamide strength in commercial formulation sample (Rastinone Tablet- 500 mg). % assay obtained was 98.05% of actual label claim values.

Title:- A simplified method to determine 5 cytochrome P450 probe drugs by HPLC in a single run.
Authorrs:- Ying Liu, Jenjie Jiao, Cali Zhueng and Janshi Louie
Review:- Authors shared HPLC analysis approach for determination of 5 specific probe substrate drugs (i.e. Caffeine, Chlorzoxazone, tolbutamide, metoprolol and midazolam) plasma concentration levels. Method includes liquid-liquid extraction of drugs from plasma with internal standard (Diazepam). Accuracy, precision and recovery experiments were conducted to assess the validation. Also 5 drugs cocktail formulation was dosed to six rats for pharmacokinetic profile determination. Linearity range was as follows:-

Caffeine:- 0.2 to 50 µg/mL
Chlorzoxazone:- 0.2 to 100 µg/mL
Tolbutamide:- 0.5 to 50 µg/mL
Metoprolol :- 0.5 to 50 µg/mL
Midazolam:- 0.5 to 50

Sample preparation included; addition of 2 mL of chloroform to 0.2 mL plasma sample containing 20µL of internal standard. Samples were vortexed, centrifuged and supernatant layer was dissipated at 40°C under nitrogen stream. Dried deposit was reconstituted in 180 µL of MP and subjected for analysis.
Acceptance was executed according to SFDA rules by setting up specificity, linearity, extraction recuperation, affectability, precision and exactness. Exactness and precision was set up by 5X at three diverse focus levels i.e. Low (1 µg/mL) medium (10 µg/mL) and high (50 µg/mL) quality control tests for three different analys

Systematic conditions:-

Framework:- Agilent 1100
Detector:-UV-230 nm.
Column:-C18 (4.6 mm, 250 mm, 5µm)
Mobile Phase:- 50 mM Phosphate salt (pH 3.4) :- Methanol :-:- 35:-65; v/v
Flow Rate:-1 mL/mi
Injection:-40 µL

Combined formulation of Caffeine (2.5 mg) Chlorzoxazone (5 mg) tolbimate (2.5 mg) metoprolol (10 mg) and midazolam (5 mg) per K bodyweight was administered to six rats via caudal vein injection. Venous blood samples were collected till 22 hours postdose and plasma harvested was stored at -20ºC until analysis.

Proposed approach has limitation of not being sensitive enough to capture elimination profile for low bioavailable drugs e.g. Chlorzoxazone, metoprolol and midazolam. Also wavelength used 230 nm is not best suitable for all these drugs.

Method provides a important information on initial approaches for pre-clinical drug-drug interaction assessment for 2C9, CYP 1A2, 2D6, 2E1 and 3A4. Instead of combined analysis, assessment of metabolising enzymes induction and/ or inhibition process should be on single substrate and drug, as presence of other non-relevant compounds (not specific to one isoenzyme) may influence the absorption and/or clearance.

**Title:** - Validated technique for rapid inhibition screening of six cytochrome P-450 enzymes by liquid chromatography–tandem mass spectrometry.

**Author/s:**- Xieoyan Li, Qang Li, Dafeng Zhong, Xieoyan Chain and Lenling Wang
Review:- Authors shared LC-MS/MS analysis approach for determination of six specific probe substrate drugs (i.e. Phenacetin, Tolbutamide, S-mephenytoin, Dextromethorphan and midazolam) CYP inhibition in vitro media concentration levels. Method includes addition of 200 µL of ice cold organic solution (acetonitrile) to reaction mixture to arrest the metabolic reaction and further liquid-liquid extraction of supernatant layer for estimation of primary metabolite (i.e. 4-Hydroxy tolbutamide). Accuracy, precision, stability and recovery experiments were conducted to assess the validation. Also 1 anticancer drug (XC-302) was assessed for its CYP inhibition potential. Method includes two drugs taken for 3A4 isoenzyme and major metabolites were monitored for estimation of rate of metabolism.

Tolbutamide and its primary metabolite were procured from Shenyang Pharmaceutical University of China. Tolbutamide substrate concentration was 100 µM in reaction mixture.

Two different method of extraction approaches were followed, Approach-1 for Hydroxytolbutamide and Hydroxychlorzoxazone and Approach-2 for rest five substrates (2 for 3A4).

Approach-1; is primarily of our concern and has been discussed further in details:-

100 µL aliquot was fortified with 100 µL of interior standard (100 ng/mL of Osalmide) and further subjected to pH change utilizing 600 µL of 30mM pH 7.0 Phosphate salt. Fluid extraction was performed utilizing 3 mL of Di-ethyl ether. Tests were vortexed, blended and centrifuged for partition of Di-ethyl ether layer. Concentrate was dissipated under nitrogen stream @ 40 ºC and were reconstituted with 150 µL of MP.

Acceptance was executed according to USFDA rules by building up linearity, extraction recuperation, affectability, steadiness, exactness and accuracy. Exactness and precision was set up by assesing six imitates at four diverse fixation levels i.e. LLOQ, Low, medium and High control tests for three different days. Cutoff of location for assay (LOD) was evaluated by deciding focus levels for response to noise ratio estimation of 3. Grid impact was resolved at middle quality control fixation level. Strength of investigation was evaluated by breaking down triplicate quality control tests at 3.0 and 1800 ng/mL.
After investigations were led for validation assessment:-

- 2 hours steadiness of tests at room temperature.
- *Three store defrost (FT) cycles at -80 °C
- *20 days LT at -80 °C
- *Re-infusion reproducibility of reconstituted examples following 4 hour.

**Expository conditions:**

Framework: - Agilent 1100

Detector:-Thermo Finnigan TSQ Quantum Ultra triple Quad.

Column:-Zorbax SB C18 (4.6 mm, 150 mm, 5µm)

MP:- Methanol:- Water:- 1% (v/v) Ammonia water:-:- 80:-20:-0.5; v/v

Stream Rate:-0.5 mL/min

Injection:-20 µL

Analysis was performed in negative mode utilizing electro splash ionization source. Source temperature was adjusted to 320 °C and MRM move utilized was 285 m/z (Precursor/essential particle) and 186 m/z (Product particle). MRM move utilized for inside standard was 228 m/z (Precursor/essential particle) and 210 m/z (Product particle).

Creator has said that pH of extraction media was acclimated to 7.0 to enhance the extraction proficiency (Page - 134) The recuperation acquired was low and had varieties clear (i.e. Recuperation at Low, Middle and High Quality control focu levels was 15.8, 14.0 and 17.5 respectively). Although these level of recoveries were sufficient for attainment of required sensitivity levels, but these low recovery levels may not be suitable for use of this extraction
approach for other *in-vitro* or *in-vivo* tests for tolbutamide (may miss slight varieties in levels).

**Title:**- Liquid chromatography/tandem mass spectrometry method for simultaneous evaluation of activities of five cytochrome P450s utilizing a five-drug combined formulation and application to cytochrome P450 phenotyping studies in rats.

**Creator’s:**- Quansheng Li, Naning Song, Chngxiao Liu and Hurong Fan


**Review:**- Five specific probe substrate drugs (i.e. Phenacetin, Tolbutamide, Mephenytoin, Dextromethorphan and midazolam) quantization strategy with SPE extraction and LC-MS/MS recognition was shared by creators.

Acceptance was performed in understanding to administrative rules by building up linearity, extraction recuperation, affectability, exactness and accuracy. Accuracy and precision was built up by examining five reproduces at three distinctive focus levels for three different days.

After analyses were directed for assessment:-

- 24 hours tests at room temperature.
- Three FT cycles
- 3 months LT at - 20 °C
- Re-infusion reproducibility of reconstituted examples following 24 hour

Test handling:- Internal standard was added to tests and subjected to SPE extraction by means of Oasis HLB cartridges. Oasis HLB cartridges conditioned using 1 mL methanol and after that equilibrated with 1 mL of Milli-Q water. Tests were stacked on to cartridges and were eluted in drop-wise way. Washing of cartridges was performed with 1 mL of water, took after by 1 mL of
5% methanol in water arrangement. Analytes were eluted in 2X 1 mL of methanol and concentrate was dried to dryness under delicate nitrogen stream.

Deposits were reconstituted in 0.15 mL of MP composition solution.

Stability of urine samples was assessed in presence and absence (both) of β-glucrodinase enzyme. 0.5 mL aliquot of urine sample were incubated with 0.25 mL of 20 mM acetic acid (pH 4.75) solution and further with 0.25 mL aliquot of 5000-units/ mL of β-glucrodinase solution (in 20 mM ammonium formate; pH 4.75). Samples were incubate in water tub (37 °C) for 12 hours for deconjugation. Samples for validation (i.e. CC/ QCs) were not subjected to β-glucrodinase treatment. Post treatment samples were extracted from Oasis HLB cartridges as mentioned in above section.

Internal standard used for tolbutamide and its metabolites was meloxicam (1 µg/mL).

**Analytical conditions:-**

**System:** Surveyor quaternary narrowbore LC Pump, Autosampler & Oven

Detector:-TSQ Quantum triple Quad.

(Xcalibur 1.1. Software)

**Column:** Shiseido C18 (4.6 mm, 150 mm, 5µm) @ 35ºC

**MP:** Methanol:- 20 mM Ammonium Formate (0.1% Formic Acid) :-:- 75:-25; v/v

**Stream Rate:**-0.4 mL/min

**Injection:**-20

Detection was performed in negative mode using electro spray ionization source set a potential of -4000V. Source temperature was set to 280 ºC and MRM transition used was 269 m/z (Precursor/ essential particle) and 170 m/z (Product particle) for tolbutamide. MRM move utilized for two noteworthy metabolites were:-
4-Hydroxytolbutamide- 285 m/z (Precursor/essential particle) and 186 m/z (Product particle).

Carboxytolbutamide- 299 m/z (Precursor/essential particle) and 92 m/z (Product particle).

To comprehend the potential for in-vivo collaboration between individual test substrates exhibit in mixed dose, potential metabolic impedance of individual CYP isoenzyme/s was assessed by looking at the phenotypic lists of each pharmacokinetic profile compared with pooled dosage pharmacokinetic results. Tolbutamide was administered at 2 mg/Kg in individual and pooled formulations to a group of rats (n=6). Urine samples were for 2 time intervals (i.e. 0-6 and 6-12 Hours). Values for 6-12 hours time interval only were reported as ratio of 4-Hydroxytolbutamide + Carboxytolbutamide to tolbutamide. Mean phenotypic values for individual dosage group were 156 (139-165) while phenotypic values for cocktail formulation group were 190 (146-283) with p-value of 0.705. Statistical analysis results showed no important diversity between entity probe drug profile and cocktail formulation profile. Only urine samples were anlayed for tolbutamide and its metabolites and in study samples the levels were measured after incubation with β-glucrodinase. While in stability results section the values for stability samples incubated for 12 hours at 37ºC with β-glucrodinase were not reported. As the conclusion of in-vivo interaction study (for 2C9 CYP isoenzyme) was solely dependent of tolbutamide and its metabolites concentration profile in urine samples, stability at mentioned condition is very critical parameter.

Title:- Plasma protein binding of diazepam and tolbutamide in constant alcoholics.

Author/s:- Therin Ji, Danbegh P, Selers SM and Dolmon L.


Review:-

Authors shared critical information on plasma protein binding properties for diazepam and
tolbutamide. Cirrhotics is a chronic disease state in which the patients liver cells gets replaced with fibrous tissues (Stiffer) and lose their functionality. Plasma protein retention was assessed by balance dialysis at 37°C. In this experiment known concentration of drug substance were spiked in plasma. Plasma samples are placed in one compartment of equilibrium dialysis device (set-up) that has 2 chambers separated by equilibrium dialysis membrane. The membrane has a specific molecular weight cut-off and molecules higher than that value cannot pass through membrane. Drug molecules bound to plasma proteins are unable to cross the membrane, while free fraction establishes a equilibrium across the membrane.

Plasma composition for alcoholic patients differs in terms of albumin content and same was evident from the study results. Drug concentration of more than a fixation scope of 50 to 300 µg/mL was investigated in 21 typical and 14 alcoholic subjects. At 100 µg/mL fixation level, the plasma protein bound extent was 97.8 ± 0.3 % normals and 95.1 ± 4.2 % in alcoholics. Decreased plasma protein binding in alcoholic subject plasma (p less than 0.02) indicate that extent of tolbutamide plasma binding is dependent on albumin concentration.

Title:- Determination of Tolbutamide and its Metabolite in Human Plasma by High Performance Liquid Chromatography and its Application to Pharmacokinetics

Author/s:- Dan LeeN, Peipai PAN, et.al


Review:-

Tolbutamide and its metabolite (i.e. 4-hydroxytolbutamide) were quantified from plasma samples using HPLC-UV. Strategy was approved and connected to pharmacokinetic investigation of tolbutamide in subjects post single oral measurement dose of 500 mg.

Acceptance was performed in understanding to administrative rules by setting up linearity,
extraction recuperation, affectability, exactness and accuracy. Accuracy and exactness was set up by dissecting six recreates at three diverse fixation levels for three different days. Alignment bend were direct for a focus scope of 0.5 to 100 µg/mL for tolbutamide and 0.01 to 2.0 µg/mL for 4-hydroxytolbutamide. Exactness (Relative standard deviation) for in betweenbatch and with-in group were under 9.3 % for tolbutamide and 7.55% for 4-hydroxytolbutamide.

Test preparing:- Internal standard was added to tests and subjected to fluid extraction utilizing diethyl ether. Supernatant concentrate was dissipated to dryness under tender nitrogen stream.

Deposits were reconstituted in an aliquot of MP.

ISTD utilized for tolbutamide and its metabolite was carbamazepine.

Analytical conditions:-

System:- High performance liquid chromatography system

Detector:-UV at 230 nm

Column:-ZORBAX SB-C18 (4.6 mm, 150 mm, 5µm)

MP:- Acetonitrile:- Water (HPLC Grade) :- 0.1% Trifluoro acidic corrosive :-:- (Gradient structure)

Stream Rate:-1 mL/mi

Detection was performed on ultra-violet detector using 230 nm as detection wavelength. To assess the applicability of developed method, samples from a clinical study conducted on healthy subjects were analysed for plasma profile of tolbutamide and its metabolite (4-hydroxytolbutamide).

__________________________________________________________

Title:- Determination of Tolbutamide and Hydroxytolbutamide by LC-MS/MS in Rat and its request to assessment of CYP2C9 activity
Review: Tolbutamide and its metabolite (i.e. 4-hydroxytolbutamide) were quantified from plasma tests utilizing fluid chromatography coupled mass spectrometry. Strategy was accepted and connected to pharmacokinetic investigation of tolbutamide in sprague-Dawely rats dosed at 3 mg/Kg dosage level, in vicinity and deficiency of bupropion (15 mg/Kg).

Acceptance was performed in agreement to administrative rules by building up linearity, extraction recuperation, affectability, soundness, precision and accuracy. Accuracy and precision was set up by dissecting five duplicates at three diverse focus levels for three different days. Alignment bend (CC) were straight for a focus scope of 5 to 1000 ng/mL for tolbutamide and 10 to 2000 µg/mL for 4-hydroxytolbutamide.

Sample processing: Internal standard was added to samples (10 µL of 1.0 µg/mL of carbamazepine) and subjected to protein precipitation using 2 fold higher volume of organic solvent (i.e. Acetonitrile). Organic extract was separated after centrifugation of samples. Acetonitrile exhibited better efficiency for protein precipitation vs other organic solvents (i.e. methanol).

Analytical conditions:

System: HPLC 1200 Agilent Tech. (Quaternary pump, Degasser, Au to-sampler and Thermo stated column oven.

Detector: Bruker Esquire HCT mass spectrometer

Column: Agilent Zorbax SB-C18 (2.1 mm, 150 mm, 3.5µm) @ 30°C

MP: Acetonitrile: 0.1% Formicacid in Water (HPLC Grade) (Gradient organizatio
<table>
<thead>
<tr>
<th>Time (Interval)</th>
<th>Composition for 0.1% Formic acid in Water (%)</th>
<th>Composition for Acetonitrile (%)</th>
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</thead>
<tbody>
<tr>
<td>0 - 1.5 min</td>
<td>90 - 15 %</td>
<td>10 - 85 %</td>
</tr>
<tr>
<td>1.5 - 6.0 min</td>
<td>15 - 15 %</td>
<td>85 - 85 %</td>
</tr>
<tr>
<td>6.0 - 7.0 min</td>
<td>15 - 90 %</td>
<td>85 - 10 %</td>
</tr>
<tr>
<td>7.0 - 10.0 min</td>
<td>90 - 90 %</td>
<td>10 - 10%</td>
</tr>
</tbody>
</table>

Flow Rate:-0.4 mL/min

Injection:-10 µL

Finding was performed on MS (spectrometer) detector using selected ion monitoring mode in positive ion electro spray interface. Following mass by charge ions were used for SIM transition:-

Tolbutamide:-271.2

Hydroxytolbutamide:-286.9

Internal Standard:-236.8

To assess the applicability of developed method, samples from a pre-clinical study conducted on SD rats were analyzed for plasma and urine concentration profile of tolbutamide and its metabolite (4-hydroxytolbutamide).

To validate method selectivity, blank plasma was analyzed with and without spiking of analytes. Accuracy and reproducibility was assessed by analysing five replicates of control samples (Tolbutamide:- 10, 80 and 800 ng/mL; Hydroxytolbutamide:- 20, 160 and 1600 ng/mL) on three separate days. Stability evaluation was done by analyzing three replicates at all three
concentration levels for following parameters:-

- 2 hours stability of samples at room temperature.
- Three freeze-thaw cycle at -20 ºC
- 14 days LT storage @ -20 ºC
- Re-injection reproducibility of processed samples after 24 hours

Pharmacokinetic study was performed in two phases:-

In phase-1; One group of Sprague-Dawley Rats (n=6) were dosed with tolbutamide 3 mg/Kg dose level, while another group (n=6) were dosed with combined dose of tolbutamide @ 3 mg/Kg and bupropion @ 15 mg/Kg. Post-dosing; urine sample were composed for 0-8, 8-12 and 12-24 hours.

Phase-2; was conducted after 2 weeks recovery on same animals, Freshly dosed and 0.3 mL blood aliquot collected at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 36 and 48 hours.

Urinary metabolic ratio of hydroxytolbutamide/ tolbutamide over study duration (i.e. till 24 hours) for drug in presence and absence of bupropion did not showed any difference in two treatments.

Plasma concentration profiles for tolbutamide and metabolite in presence and absence of bupropion also does not showed any significant changes in two treatments. One major observation in results was that plasma levels for tolbutamide were shown as 20,000 to 25,000 ng/mL; while validation was performed for a range till 1000 ng/mL. This shows a disconnect in validation range and concentration values obtained from study (as dilution of samples has not been indicated in research article anywhere).

Title:- Determination of ionization constant (pk_a) for poorly soluble drugs by using surfactants:-
a novel approach.

**Author/s:** V. Ravichandiren, K. Maslamani and V. Devaranjan

**Journal:** Der Pharmacia Letter, Vol. 3 (4) Page- 183 to 192 (2011)

**Review:**

Ionization constant is very critical information among physiochemical property of compound which determines its absorption, distribution and excretion pathways/ extent. Ionization constant information assists in pre-formulation strategies and analytical method development. Authors share a novel approach for determination of ionization constant for poorly soluble drugs using surfactants.

Drugs selected for experiment included: Carvedilol, Ibuprofen, Glipizide, Tolbutamide and Nimesulide. Potentiometric titration was used for Carvedilol, Ibuprofen, Glipizide and Tolbutamide. Nimesulide was assayed using spectrophotometric method.

**Instruments Used:**

Potentiometric Titrator:- Autotitrator (799 GPT Titrino, Metroham)

Spectrophotometer:- Spectramax plus UV-Visible plate Reader (Molecular Devices)

Potentiometric titration curves were plotted for tolbutamide in 2% Tween-80, 2% Chremophor EL and 2% Labrasol.

Results of potentiometric titration were in close agreement with reported pKa value of tolbutamide (i.e. 5.30).

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**Title:** Andrographis paniculata extract and Andrographolide Modulate the Hepatic Drug
Metabolism System and Plasma Tolbutamide Concentrations in Rats

Creator/s:- Haw-Wen Chen, Pei-Fen Liu, Chien-Chun Li, Chin-Shiu Huang, Chiung-Tong Chen, Jia-Rong Chiang, Hsien-Tsung Yao, Cheng-Tzu Liu and Chong-Kuei Lii


Review:-

Authors have published their research work on andrographolide and andrographis paniculata extract on CYP2C enzymes modulation in rats and their impact on tolbutamide pharmacokinetics. Andrographolide is one of most plenteous terpenoid in A. paniculata, which is utilized for treatment of diabetes.

Rats were intragastrically dosed with 2 and 50 g/Kg of andrographis paniculata extricate and andrographolide day by day for 5 days. Dose was arranged in Cremophor EL/ ethanol/ Water (30/10/60; v/v/v) vehicle to dosage level/s of 20 mg/Kg of tolbutamide on 5th day. Blood tests were gathered from tail vein at 0.25, 0.5, 1, 2, 4, 8 and 12 hours. 50 µL aliquot of plasma isolated from blood was taken for examination. Tests were hastened with 100 µL of acetonitrile, centrifuged and supernatant was investigated on LC-MS instrument. Alignment bend (CC) was plotted for a fixation scope of 1 to 300 µg/mL.

Systematic condition

Framework:- HPLC 1100 Agilent LC framework

Detector:-Agilent MSD mass spectrometer with electrospray ionization source.

Column:-Agilent Zorbax Eclipse XBD-C8 (3 mm, 150 mm, 3µm) @ 25°C

MP:- Acetonitrile containing 10 mM Ammonium acetic acid derivation:- 0.1% Formic acid in Water (HPLC Grad)
<table>
<thead>
<tr>
<th>Time (Interval)</th>
<th>Composition for 0.1% Formic acid in Water (%)</th>
<th>Composition for Acetonitrile containing 10 mM Ammonium acetate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1 min</td>
<td>70 - 50 %</td>
<td>30 - 50 %</td>
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<tr>
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<td>50 - 98 %</td>
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<td>3 - 9 min</td>
<td>2 - 2 %</td>
<td>98 - 98 %</td>
</tr>
<tr>
<td>9 - 10 min</td>
<td>2 - 70 %</td>
<td>98 - 30 %</td>
</tr>
<tr>
<td>10 - 15 min</td>
<td>70 - 70 %</td>
<td>30 - 30 %</td>
</tr>
</tbody>
</table>

Stream Rate:-0.5 mL/min
Injection:-10 µL

Detection was performed on mass spectrometer identifier utilizing chose particle observing (SIM) mode in positive particle electro splash interface.

Study indicated andrographis paniculata concentrate (APE) and andrographolide (AND) diminished the AUC$_{0-12h}$ of tolbutamide by 37 and 18% individually. Mean AUC$_{0-12h}$ for control, andrographis paniculata extract (APE) and andrographolide (AND) studies were 1393, 881 and 1143 µg/mL*h, respectively. Mean Cmax values for control, andrographis paniculata extract (APE) and andrographolide (AND) studies were 151, 133 and 140 µg/mL, respectively.

Biochemistry study showed increased levels of protein, mRNA and few CYP isoenzymes (CYP2C6/II, CYP1A1/2 and CYP3A1/2). In presence of increased rate of metabolism observed for tolbutamide, authors had expected that hypoglycemic effect of drug would be reduced by andrographis paniculata extract (APE) and andrographolide (AND).

However in spite of reduced AUC and increased expression of drug metabolizing enzymes, hypoglycemic effect of tolbutamide was not effected in glucose tolerance test.
Few other research articles include;

Wei Zhiang et.al. (2010) published LC-MS/MS approach for assessment of potential inhibitory impacts of new substance elements on 4 distinctive isoenzymes. Production was entitled as 'Concurrent determination of tolbutamide, omeprazole, midazolam and dextromethorphan in human plasma utilizing Liquid chromatography combined with mass spectrometer—A high throughput way to assess drug–drug interactions'. Tolbutamide was utilized as test substrate for CYP2C9. 50µL plasma aliquot was extracted using protein precipitation. Chromatographic separation was achieved on Hypersil GOLD AQ column. MRM transition of 271 (molecular ion) to 172 (fragment ion) was used for detection. Quantitation range for tolbutamide was 50 to 50000 ng/mL.

Two technical notes utilizing high scan ability of mass spectrometer published by Thermo Fisher Scientific and Waters application labs for tolbutamide assessment were also referred.