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Bioavailability studies of selected oral anti-diabetic drugs in human volunteers - A review

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ABSTRACT

The intestinal absorption of oral-anti diabetic drugs in type-II Diabetes mellitus is altered when concomitantly administered with other drugs like, antacids, antinuclear drugs, antibiotics and others. Drug therapy becomes more complex as many individuals are on multiple drug therapy and administer many drugs during same period of time. An increasing number of drug related problems caused by drug interactions extended to include drug- drug interactions, drug – food interactions, drug-diseases interactions. A closer monitoring and supervision of drug therapy is required so that drug related problems can be prevented or detected at an early stage. To find out the pharmacokinetic changes of oral anti- diabetic drugs when administered with other drugs meant for co-existing diseases. The following parameters should be standardized. They are HPLC analytical methods, Study design, Software to calculate pharmacokinetic parameters. In this article several HPLC methods of the analysis for anti-diabetic drugs, reports of in-vitro and in-vivo studies of anti-diabetic drugs were discussed. According to the results the changes in pharmacokinetic parameters of anti-diabetic drugs were observed and discussed. It is concluded that bioavailability studies of anti-diabetic drugs to be conducted in human volunteers with updated protocols and HPLC methods of analysis. To rectify the problems associated with concomitant administration and for better management of Type –II diabetes therapy.

Keywords: Bioavailability, Antidiabetic drugs, Pharmacokinetics, Drug interactions, Concomitant administration, HPLC.

INTRODUCTION

Bioavailability and bioequivalence studies provide important information in the overall set of data that ensure the availability of safe and effective medicines to patients and practitioners.

Bioavailability and Bioequivalence measures are frequently expressed in systemic exposure measures, such as area under the plasma concentrations-time curve (AUC) and maximum concentration (C_{max}). These measures of systemic exposure are assumed to relate in some way to safety and efficacy outcomes that may be expressed in biomarkers, surrogate endpoints, or clinical benefits endpoints. Based on this assumption, Bioavailability and bioequivalence information has been determined to have practical and public health value for pharmaceutical sponsors, for regulatory agencies, and for patients and practitioners. The term diabetes mellitus describes a metabolic disorder of multiple an etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Currently diabetic mellitus is a great threat to the world community with more than 100 million persons suffering from diabetes. The prevalence and incidence of diabetes is increasing in most populations, being more prominent in developing countries. India leads the world largest number diabetic subjects and is being termed the "diabetes capital of the world". With 40.9 million people currently suffering from diabetes and expected to rise 69.9 million by 2025.Chronic elevation of blood glucose levels leads to many co-existing complications like diabetic retinopathy, diabetic neuropathy, peptic ulcer, and diabetic foot ulcer.

Drug therapy in Type II diabetes becomes more complex as many individuals are on multiple drug therapy and administer many drugs during the same period of time to treat secondary diabetic complications. A closer monitoring and supervision of drug therapy is required so that drug related problems can be prevented or detected at an early stage. An increasing number of drug related problems are caused by drug interactions. Currently clinicians come across the problem of erratic absorption of oral anti diabetic drugs when administered with other drugs prescribed for co- existing diseases. Due to this bioavailability of oral anti diabetic drugs is altered. Identification of drugs in biological fluids like serum, plasma, saliva and urine is playing very important role in clinical research.

HPLC methods of analysis are the tool for identification of drugs in biological fluids. Various methods are available for identification drugs by HPLC with (or) without mass spectroscopy. Sample preparation for HPLC analysis is a main process for quantifying drugs. Errors associated with sample preparation may produce fault results in quantitative estimation of drugs. Due to that there is a chance of alteration in calculation of pharmacokinetic parameters. It may result in wrong conclusion in clinical research. In this article various methods of HPLC analysis for anti-diabetic drugs published in reputed journals were discussed. In-vitro and in-vivo study reports of anti- diabetic drugs were discussed. To avoid the above mentioned problems in bioavailability studies of anti- diabetic drugs. Suitable method for HPLC analysis of each drug to be selected and the bioavailability studies to conducted in human volunteers. The findings will act as boon for the management of diabetic therapy.

Bioavailability: (Mei-Ling Chen *et al.*, 2000)

Bioavailability studies may provide additional useful information's about metabolism, transport, distribution, and elimination of the drug, dose proportionality, nutrients effects on drug absorption, etc. From a drug product performance of the formulation(s) used in the clinical trials that provide evidence of safety and efficacy. Based on different study goals and designs, a distinction may be made between BA studies that are intended to provide pharmacokinetics

information and those that are intended to focus on product quality. The former studies may be termed "pharmacokinetics bioavailability" studies whereas the latter studies have been termed "product quality BA" studies.

Pharmacokinetics Studies:
The statutory definition of Bioavailability and Bioequivalence, expressed in rate and extent absorption of the active moiety or ingredient to the site the action, emphasizes the use of pharmacokinetics measures to indicate release of the drug substances from the drug product with absorption into the systemic circulation. This approach rests on an understanding that measurement of the active moiety or ingredient at the site(s) of action is generally not possible and that some predetermined relationship exists between the drug concentrations at the site of action relative to that in the systemic circulation.
A goal in Bioavailability and Bioequivalence studies is to assess rate extent of drug absorption. Extend of absorption is readily measured by AUC either to the last sampled time point (AUC_{0-t}) or following extrapolation to time infinity ($AUC_{0-\infty}$).

These measures include
(i) Total exposure (AUC_{0-t} or $AUC_{0-\infty}$ for single-dose studies and AUC_{0-t} for steady-state studies).
(ii) Peak exposure (C_{max}), and
(iii)Early exposure (partial AUC to peak time of the reference product for an immediate-release drug product).

Reliance on systemic exposure measures will reflect comparable rate and extent of absorption, which in turn, will achieve the underlying goal of assuring comparable therapeutic effects.

Moieties To Be Measured
Parent Drug Vs Metabolites
Moieties to be measured in Bioavailability and Bioequivalence studies are the active drug ingredient or active moiety in the administered dosage form (parent drug) and, when approach both active ingredient or active moiety and active metabolites. According to this approach, both active ingredient or active moiety and active metabolites should be measured in bioavailability studies, if analytically feasible. For Bioequivalence studies, only the parent drug should be measured, although there are situations in which active metabolites are to be measured.

2. Pharmacokinetic changes of antidiabetic drugs:
Pharmacokinetics of antidiabetic drugs alters by drug-drug interactions, Drug-disease interactions, Drug-food interaction and Pharmacogenetic variabilities. This type of interactions makes drug therapy in Type II diabetes becomes more complex. As many individuals are on multiple drug therapy and administer many drugs during the same period of time to treat secondary diabetic complications. A closer monitoring and supervision of drug therapy is required so that drug related problems can be prevented or detected at an early stage. Some selected interactions mentioned above are discussed below:

2.1. Anti - Diabetic Drugs with Antacids and Adsorbents: (Naggar VF *et al.*, 1980).

The in- vitro study of Metformin hydrochloride, glibenclamide, acetohexamide,carbutamide and glymidine on various antacids or adsorbents was studied at 37□ C like magnesium trisilicate, aluminium hydroxide, calcium carbonate, magnesium oxide resulted in probable variation in dissolution rates . Concluded in-vivo studies to conduct to assess the drug – antacid interaction.

2.2. Rifampicine with Natiglinide in healthy subjects: (Mikko Niemi *et al.*, 2003).
A randomized crossover study of rifampicine with natiglinide resulted as rifampicine modestly decreases the plasma concentration of natiglinide probably by inducing its oxidative biotransformation.

2.3. Sex Differnces in the Pharmacokinetics of Pioglitazone in Rats: (Yukiyoshi Fujita et al., 2003)-Clinical studies have suggested that pioglitazone, an insulin sensitizer, has strong effect in women than men. To determine the sex difference in the pharmacokinetics of pioglitazone the plasma and white adipose tissue levels resulted as. No significant different changes in found in the tissue- to- plasma concentration and elimination rate of pioglitazone were faster in male rates than in female rats.

2.4. Glibenclamide with Doxycyclin in albino rats: (Thokchom IS et al., 1993)
The drug interaction of glibenclamide with doxycycline was done in 40 male albino rats which were divided into 4 groups of 10 each. Both glibenclamide and doxicycline are bound to plasma protein more than 90% resulted as doxicyclin enhances the hypoglycemic effect of glibenclamide.

2.5. Effect of Genetic Polymorphisms in Cytochrome P450 (CYP) 2C9 AND CYP2C8 on the pharmacokinetics of oral antidiabetic drugs: (Kirchneiner J et al., 2005)
Type 2 diabetes mellitus affects up to 8% of adult population in western countries. Treatment of these diseases with oral anti diabetic drugs is characterized by considerable inter individual variability in pharmacokinetics, clinical efficacy and adverse effects .Genetic factors are known to contribute to individual difference in bioavailability, drug transport, metabolism and drug action. Only scarce data exist on clinical implications of this genetic variability on adverse drug effects or clinical outcomes in patients taking oral antidiabetics. In this study the effect of genetic polymorphism in cytochrome P450 (CYP2C9 & CYP2C8) on the pharmacokinetics of antidiabetic drugs studied. It is concluded pharmacogenetic variability plays an important role in the pharmacokinetics oral antidiabetic drugs; however, to date, the impact of this variability on clinical outcomes in patients is mostly unknown and prospective studies on the benefit of CYP genotyping are required

2.6. Enhanced Bioavailability of Pioglitazone by Quercetin in Rats: (Sudhir N. Umathe et al., 2008)
Concomitant administration of pioglitazone with quercetin (a naturally occurring dietary flavonoid, ubiquitously present in many antidiabetic herbal preparations) has been carried out. Pioglitazone is metabolized by multiple cytochrome P450 (CYP) isoenzymes, mainly by CYP2C8, CYP3A4 and CYP2C9 to several active and inactive metabolites. Quercetin is an inhibitor of CY3A4 and reduces the active metabolite of pioglitazone and increases its plasma concentration it may increase the toxicity of pioglitazone.

2.7. Pharmacokinetics and Bioavailability of a Metformin/Glyburide Tablet Administered alone and with food: (Marathe PH et al., 2000)

Two randomized crossover studies were conducted to evaluate the pharmacokinetic (including food effect (including food effect) of fixed-combination metformin/glyburide tablets. The effect of high-fat meal on the bioavailability of a metformin/glyburide (500 mg/5 mg) tablet was assessed relative to the fasted condition in study 2. The fixed combination metformin/glyburide tablets showed bioequivalence for the metformin component with the reference metformin tablet and comparable bioavailability for the glyburide component with the reference glyburide tablet. Food does not appear to affect the bioavailability of either component to an appreciable extent.

2.8. Enhanced Bioavailability of Pioglitazone by Clindamycin - A In vivo Evaluation in Human Volunteers: (our unpublished data).

A two phases cross over study with a wash out period of 4 weeks conducted for concomitant administration of pioglitazone 20 mg as a tablet (PIOGLIT) and clindamycin (CLINCIN) 150 mg as a tablet studied in 10 diabetic patients. Pioglitazone is metabolized by multiple cytochrome P450 (CYP) isoenzymes, mainly by CYP2C8, CYP3A4 and CYP2C9 to several active and inactive metabolites. From the results it is observed that clindamycin affects the pharmacokinetics of pioglitazone by moderate inhibition of CYP3A4. Hence the metabolism of pioglitazone purely depends on activity of CYP2C8 enzyme only and extends the time period of metabolism of pioglitazone and remains in the systemic circulation for longer period as a result the pioglitazone showed longer hypoglycemic action. It may lead to the increasement of other complications like adverse reaction and toxicity of pioglitazone.

3. HPLC methods for analysis of anti – diabetic drugs.

Determination of drug in biological fluids by using HPLC plays very important role in pharmacokinetic and pharmacodynamic evaluation. HPLC variables as such mobile phase, column, sample preparation, flow rate and others are very important to determine the concentration of drug in systemic circulation. Identification method of some selected antidiabetic drugs with the HPLC variables are discussed below.

3.1. Glipizide:

Sample preparation:

Glipizide with 5 ml of chloroform. Tolbutamide was added an internal standard to all samples. Samples were vortexes and subsequently centrifuged and the organic phase was evaporated to dryness under a stream of nitrogen. The samples were reconstituted in 200µ1 of freshly prepared NBD-C1 solution (1mg/ml NBD-C1-octanol/amyl acetate [98:2 vol/vol]) and heated at 120 °C for 80 min. Samples were then filtered using Milipore Ultrafree MC filters (amicon; Millipore, Billerica, MA).

Mobile Phase : The mobile phase consisted of 35% acetonitrile in water.

Flow rate : The flow rate runs at 0.5 ml/min.

Retention time : The retention time is 2 minutes and 25 seconds.

Wavelength : The fluorescence detection (excitation = 470 nm and emission = 530 nm).

Column : The column used is Luna C-8 column (3- µm particles, 150 × 4.6 mm; Phenomenex, Torrance, CA) equipped with a Security Guard cartridge (Phenomenex).

3.2. Gliclazide: (Mohammad-Reza Rouini et al., 2003)
Sample preparation:
To 100µl of serum were added 50µl of the Internal Standard (160 µl/ml of gliclazide in Acetonitrile) working solution and 100 µl of 0.07 M phosphate buffer (pH 4.5). After vortex mixing for 10 s, 1 ml of toluene was added and the mixture was shaken vigorously for 1 min. The mixture was then centrifuged for 5 min at 10,000 rpm (8500 g) (Eppendorf 5415C, Germany). A 800 µl aliquot of the upper organic layer containing gliclazide and I.S. was transferred to a clean glass tube and evaporated under air stream to dryness at 50°C. The residue was redissolved in 100 µl of mobile phase and a 50 µl aliquot was injected onto the HPLC column.

Mobile phase : Mobile phase consisted of acetonitrile and water 45:55, v/v and pH was adjusted to 3 with phosphoric acid.
Flow rate : At a flow-rate of 0.9 ml/min.
Retention time : The retention time is 3 minutes.
Wavelength : The Ultraviolet absorbance was monitored at 230 nm wavelength.
Column : A Techsphere C_8 column (3 µm, 150 mm×3.9 mm I.D.; HPLC, UK.

3.3. Chlorpropamide:
Sample preparation:
Samples were extracted with dichloromethane and the organic layer evaporated to dryness. The residue was dissolved in methanol, and 10 lal aliquot injected onto the column. Tolbutamide was used as the internal Standard for chlorpropamide. The UV detector response Vas linear over the range 0-200 g ml-l with a correlation coefficient of 0.999; detection limit: 0.002 g n~l-l. Within-day and between-day assay variation was generally <7 %. No interference from endogenous instatements was observed. The utility of the method Vas demonstrated by determining chlorpropamide in samples from six healthy volunteers following a single oral dose of 250 rag. The procedure is simple and requires small volumes of plasma.

Mobile phase: The mobile phase was methanol: 0.2 % acetic acid (3:2) adjusted to pH 6.7 with 1M NaOH.
Flow rate: Flow-rate was 0.8 ml min -1 with an operating pressure of 2000 p.s.i.
Retention time: The retention time is 2 minutes and 30 seconds.
Wavelength: The UV detector was set at 254 nm and the sensitivity kept at 0.10 a, u.f.s.
Column: Radial pack C18 column (1000.8 mm I.D., 10 ~ tm particles) were obtained prepacked from Waters Associate, Goteborg, Sweden.

3.4. Pioglitazone: (Ketan K. Nerukar et al., 2002)
Sample preparation: To 1.0 ml pooled human blood plasma in 15 mk centrifuge tunbes appropriate aliquots of stock solutions of the srug were added and tubes were vortexed for one minute. To the tubes 100µl of rosiglitazone solution as internal standard (200 ng/ml) were added and the tubes vortexed again for one minute. Into these tubes 5 ml of dichloromethane : hexane (1:1) mixture was added and vortexed for 2 minutes and centrifuged at 4000 rpm for 10 minutes. Then the tubes were kept in ice acetone bath maintaining at -80°C for 10 minutes. The upper 4 ml organic layer was collected and dried at 40°C under stream of nitrogen. The residue was reconstituted in 200 µl of mobile phase and 20µl of the aliquot was introduced into the HPLC.

Mobile phase: The mobile phase comprised of Acetonitrile + 50 mM KH_2PO_4 (50:50) with pH adjusted to 5.35 with 20% phosphoric acid
Column **:** Hypersil BDS (150 × 4.6mm) 5µm.
Flow rate : At a flow-rate of 1.0 ml/min.
Retention time : The retention time is 6 minutes and 18 seconds.
Wavelength : The Ultraviolet absorbance was monitored at 230 nm wavelength.

3.5. Rosiglitazone: (Matthew W. Hruska et al., 2004)
Sample preparation: 10 µl of Internal standard (betaxolol dissolved in methanol) was added to plasma samples (200 µl) in microcentrifuge tubes and vortexed briefly. Acetonitrile (600 µl) was then added to each sample vortexed for 2 min and centrifuged at 3000 × g for 10 min. Supernatant was evaporated using nitrogen gas in a heating block set at 45° C. Dried samples were reconstituted with 200 µl of mobile phase and a 75 µl aliquot was injected onto the column.
Mobile phase: An isocratic mobile phase of 10mM sodium acetate (pH 5) - Acetonitrile (60:40) with pH adjusted to 5.35 with 20% phosphoric acid
Column **:** 250 mm × 4.6 mm, 5 µl alltima phenyl column.
Flow rate : At a flow-rate of 1.0 ml/min.
Retention time : The retention time is 13 minutes and 2 seconds.
Wavelength : The Ultraviolet absorbance was monitored at 247 nm wavelength.

3.6. Glibenclamide: (Ioannis Niopas *et al.*, 2002).
Sample preparation: To plasma sample (0.5 ml) calibration standard (25 µl) and internal standard (25 µl) were added. To control blanks and to quality control standards 50 and 25 µl of acetonitrile/water 1:1 were added, respectively. The contents were shaken with 2 ml of dichloromethane/hexane 1:1 for 10 min. They were then centrifuged at 2000 × g for 10 min and the organic phase was transferred to a lean test tube and evaporated to dryness at 30°C with the aid of a gentle stream of air. One milliliter of mobile phase and 2 ml of isooctane were added to the residue and after shaking for 10 min and centrifugation for 5 min, isooctane was aspirated off. The residue was transferred to an auto sampler vial and a 100 µl volume was injected into the HPLC system for quantitation.
Mobile phase: The mobile phase comprised of acetonitrile/water/acetic acid (500:500:0.3, by volume).
Column **:** A Hypersil MOS C8 analytical column (3 µm particle size 100 × 30.2 mm i.d.) purchased from Aldrich (St. Louis, MO)
Flow rate : At a flow-rate of 0.5 ml/min.
Retention time : The retention time is 4 minutes and 3 seconds.
Wavelength : The Ultraviolet absorbance was monitored at 325 nm wavelength.

3.7. Metformin: (Kah Hay Yuen *et al.*, 1998)
Sample preparation: A 250 µl aliquot of plasma sample was measured into an eppendorf microcnetrifuge tube and deprotenized by adding 10 µl of 60% perchloric acid. The mixture was vortex-mixed for 1 min using a vortex mixer and then centrifuged at 12,800 g for 3 min. The supernatant was transferred to a new eppendorf microcentrifuge tube and 50 µl were injected onto the column.
Mobile phase: The mobile phase comprised of 0.01 M potassium dihydrogen orthophosphate (Adjusted to pH 3.5 with glaial acetic acid) and acetonitrile (60:40, v/v).

Column **:** A Supelcosil Lc-CN column from supelco (Bellefonte, PA, USA; 5 µm, 250×4.6 mm I.D.) fitted with a refillable guard column (Upchurch Scientific, Oak Harbour, WA, USA), packed with Spherisorb CN-5 µm (Keystone Scientific, Bellefonte, PA, USA).
Flow rate : At a flow-rate of 1.0 ml/min.
Retention time : The retention time is 5 minutes and 9 seconds.
Wavelength : The Ultraviolet absorbance was monitored at 234 nm wavelength.

3.8. Nateglinide:
Chromatography:
NA was quantified using Shimadzu HPLC system (Duisburg, Germany) consisting of pump LC 9A, an automatic sampler SIL 6A, a column heater CTO 6A and an ultraviolet detector SPD 6A. The class LC 10 software Version 1.6 (Shimadzu) was used for data analysis and processing. The compounds were separated at 50 C on a ProntoSIL 120-5-C18 AQ (5 µm, 250×3 mm I.D.) (BISCHOFF Chromatography, Leonberg, Germany) with guard column and quantified by UV detection at 210 nm. For preparation of the mobile phase, a 0.1 M potassium hydrogen phosphate solution was adjusted to a pH of 4.0 with potassium hydroxide (30% in water). Seven hundred ml of this aqueous solution was mixed with 80 ml of methanol and 300 ml of acetonitrile to constitute the mobile phase. The mobile phase was prepared weekly and was delivered at a flow rate of 1.0 ml/min. The substance was quantified using its peak height ratio to an internal standard (Carbamazepine).

Sample preparation:
In a 1.5 ml plastic tube, 50µl of plasma were mixed with 10µl of internal standard working solution and 100µl of acetonitrile for 10 min in an Eppendorf thermomixer 5437 (Hamburg, Germany). After centrifugation for 10 min at 3000 × g, the supernatants were transferred into an injection vial. Samples were evaporated to dryness at 50 C under stream of nitrogen. The residues were reconstituted with 30 µl of the mobile phase and 20 µl were injected into the HPLC.

3.9 Simulataneous determination of Metformin and Glipizide, Gliclazide, Glibenclamide or Glimperide in Plasma: (AbuRuz S et al., 2005)
Sample preparation:
Patients' blood samples were collected into glass tubes containing EDTA and entrifuged at 3000 rpm (1610×g) for 15 min. The separated plasma was kept frozen at -70°C until analysis. To 1 ml of patients' plasma samples or spiked plasma standards 50µl of the internal standard solution and 0.125 ml of water were added. 0.1 ml of 0.75 M HCl for Method 1 and 0.1 ml of 0.4 M HCl for Method 2 was added to each of the standards and patients samples then vortex mixed for 30 s and centrifuged at 14,000 rpm (17,500 × g) for 5 min before extraction to prevent blockage of the cartridges.
The extraction procedure was an optimization of one developed previously for SPE of M [ion pair solid phase extraction (IPSPE)] the main difference was the addition of acid during the loading step.
The samples and standards were prepared as mentioned above and then extracted using the optimized ion pair solid phase extraction technique utilizing Oasis® HLB cartridges (1 cc, 30 mg) (which was connected to Waters extraction vacuum Manifold) as following:
1. Condition 1: 1 ml methanol followed by 1 ml of water.

2. Condition 2: 1 ml aqueous solution of 2mM sodium dodecyl sulphate (ion pair reagent);
3. Load: 1.275 ml spiked or patient plasma (as prepared above);
4. Wash: 1 ml 30% methanol
5. Elute: 1 ml methanol
6. Evaporate with nitrogen stream and reconstitute in 350µl of the mobile phase and inject 150 µl onto the HPLC.

Mobile phase: Mobile phase consisted of 2 mM sodium dodecyl sulphate, acetonitrile (37.5%) and potassium dihydrogenphosphate (62.5%) (from 0.02M buffer to produce a final buffer concentration of 0.0125).

1. Method 1 the pH was adjusted to 7.3 using NaOH ,
2. Method 2 it was adjusted to 5.3 using HCl for optimal separation.

Flow rate : At a flow-rate of 1 ml/min.
Retention time : The retention time were
Method 1: M - 4.7 min, P – 12.5 min, Gm – 8.4 min and Gb – 6.2 min
Method 2: M - 5 min, Gp – 8.1 min, Gc – 15.6 min and T – 10.1 min
Wavelength : The Ultraviolet absorbance was monitored at 225 nm wavelength.
Column : C_{18} Supelco analytical column (250 mm × 4.6 mm, 5 µ; Sigma, Poole, England). The guard column was a Supelco Discovery (20 mm × 4mm, 5µ; Sigma, Poole, England).

4. Softwares to calculate pharmacokinetic parameters:

The concentration of drugs are determined with the comparison of standard chromatograms in pharmacokinetic evaluations. All the pharmacokinetic and statistical analysis may be carried out by using the following softwares, *Kinetica* (Version 4.4.1, Innaphase, USA) (Bhavesh *et al.*, 2007), *Win online, Edfast, Intellipharm Pk, ModKine* such as Peak plasma concentration (C_{max}), Time to C_{max} (T_{max}), AUC from 0 to 12h (AUC_{0-12}), t½ were calculated.

DISCUSSION

Identification of drugs in biological fluids by HPLC is vital tool for clinical research .Various methods available for HPLC analysis of anti-diabetic drugs as single and simultaneous estimation with other drugs. Sample preparation and utilization of instruments varies drug to drug. Practical errors during sample preparation may alter results of analysis. The following parameters are to be properly maintained during HPLC analysis such as Mobile phase blend, Retention time, Column selection, Soft ware's to calculate pharmacokinetic parameters. The methods mentioned in this article are practically performed and published in reputed journals. They were produced accurate results and also economic. The results of various pharmacokinetic studies mentioned in this article were proved the changes in pharmacokinetic parameters of anti-diabetic drugs. Alteration of pharmacokinetic parameters resulted due to the Concomitant administration of drugs, metabolic disorders, drug – drug interaction. Before conducting bioavailability studies it important to select a proper HPLC method for analysis, Pharmacology of drugs, Genetic polymorphism of individuals should be discussed to prepare standard protocols.

CONCLUSION

Bioavailability and Bioequivalence studies provides drug information's to prevent Drug interactions, adverse reactions and to individualize drug dosage regimen. Recently, clinicians and clinical pharmacologist focusing their attention to address these complications for the better management of type II diabetes mellitus. It is very essential to conduct pharmacokinetic studies of antidiabetic drugs with the drugs meant for secondary diabetic complications like Peptic ulcer, Diabetic retinopathy, renal failure and Hypertension. Several methods available for the analysis of drugs by HPLC. The methods mentioned in this article can be utilized for Bioavailability and Bioequivalence studies of anti-diabetic drugs. Several studies were conducted by *In vitro* and *In vivo* (animals) methods and the results were proved the changes in bioavailability of drugs. In future bioavailability studies of oral anti-diabetic drugs to be conducted in human volunteers to improve the management of Type - II diabetes therapy.

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