



**TREATMENT STRATEGY AND BIO ANALYTICAL METHODS FOR ESTIMATION OF  
ANTI DIABETIC AGENTS IN THEIR BIOLOGICAL SAMPLES –A REVIEW**

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Article Received on 21/12/2018

Article Revised on 11/01/2019

Article Accepted on 31/01/2019

**ABSTRACT**

This paper describes treatment strategy and the various bio-analytical methods reported for quantitative estimation of various drugs in biological fluids, which are used in the treatment of Diabetes mellitus. Quantification of drugs in biological fluids is always typical task due to the matrix effect and real time concentration in the in vivo systems. For the toxicity studies, bio analytical method is pre requisite. Quantification of active drug moiety in the presence of the metabolites and other biological products is typical task for the analyst, but in spite of this for pharmacokinetic application method is mandatory for estimation metabolites and the drug moieties to assess the therapeutic efficacy of drug moieties.

**KEYWORDS:** This paper describes treatment therapeutic efficacy of drug moieties.

**1. INTRODUCTION**

**Definition of Diabetes Mellitus**

A disease in which the body's ability to produce or respond to the hormone insulin is impaired, resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in the blood. Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

**Types of Diabetes Mellitus**

**Type 1 Diabetes (b-Cell Destruction, Usually Leading to Absolute Insulin Deficiency)**

Categorized into following types:

- Immune-Mediated Diabetes
- Genetic defects in insulin action
- Diseases of the exocrine pancreas
- Idiopathic diabetes

**Type 2 Diabetes (Ranging From Predominantly Insulin Resistance With Relative Insulin Deficiency to Predominantly an Insulin Secretary Defect With Insulin Resistance):**

This form of diabetes, which accounts for; 90–95% of those with diabetes, previously referred to as non–insulin dependent diabetes, type 2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. At least

initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of b-cells does not occur, and patients do not have any of the other causes of diabetes listed above. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region.

The various specific types were mentioned in the following

- Genetic defects of the b-cells
- Endocrinopathies
- Drug or chemical-induced Diabetes

**General Principles of Treatment**

Diet and exercise are fundamental in the treatment of diabetes. Dietary recommendations must be customized for each individual to achieve the general objectives of treatment. It should be remembered that obesity is common in type 2 diabetics so one of the main objectives should be weight reduction. The calorie content of the diet should be adjusted in each individual in accordance with the body mass index and regular physical activity. As far as the nutrient proportions of the diet, it is recommended that proteins should constitute 10%-20% of calorie intake and fats less than 30%, with less than

10% saturated fats. With regard to carbohydrates, emphasis should be placed on total intake rather than on their origin, although rapidly absorbed carbohydrates should be avoided.

### Treatment of Type 1 Diabetes Mellitus

Patients with type 1 diabetes mellitus (DM) require lifelong insulin therapy. Most require 2 or more injections of insulin daily, with doses adjusted on the basis of self-monitoring of blood glucose levels. Long-term management requires a multidisciplinary approach that includes physicians, nurses, dietitians, and selected specialists.

In some patients, the onset of type 1 DM is marked by an episode of diabetic ketoacidosis (DKA) but is followed by a symptom-free "honeymoon period" in which the symptoms remit and the patient requires little or no insulin. This remission is caused by a partial return of endogenous insulin secretion, and it may last for several weeks or months (sometimes for as long as 1-2 year).

### Treatment for Type 2 Diabetes Mellitus

#### General Principles and Therapeutic Approach

The diet -which generally must be low-calorie due to the frequency of associated obesity- and a program of regular exercise are the basis of the treatment of type 2 diabetes mellitus. When acceptable metabolic control is not achieved, either because the patient does not adapt to changes in life style or because, in spite of complying with the diet and exercising regularly, therapeutic objectives are not attained, pharmacological treatment must begin.

#### Pharmacological treatment

**Sulfonylureas:** Mechanism of action : The SUs stimulate the second phase of insulin secretion by pancreatic beta cells, that is to say, the release of preformed insulin. Therefore, the SUs require the presence of a critical mass of beta cells with insulin secretory capacity in order to act. Therefore, the SUs will not be effective in patients who are pancreatectomized or have type 1 diabetes mellitus. The SUs act through high-affinity receptors located in the pancreatic beta cells. Binding to these receptors inhibits the opening of ATP-sensitive potassium channels and avoids potassium outflow from the cell, thus triggering cell membrane depolarization. As a result, the calcium channels open, increasing intracellular calcium content and calcium binding to calmodulin, which produces microfilament contraction and the exocytosis of insulin granules.

#### Examples of combination medications for type 2 diabetes

- Metformin and glipizide (Metaglip)
- Rosiglitazone and glimepiride (Avandaryl)
- Pioglitazone and metformin (ACTOplus Met)
- Metformin and glyburide (Glucovance)
- Rosiglitazone and metformin (Avandamet)
- Pioglitazone and glimepiride (duetact)

### Insulin Estimation Procedures

#### Insulin ELISA

For the quantitative determination of insulin in serum and plasma: All reagents and microplate strips should be equilibrated to room temperature (18-25°C) prior to use. Gently mix all reagents before use. A standard curve must be performed with each assay run and with each microplate if more than one is used at a time. All standards, controls, and samples should be run in duplicate. 1. The microplate should be equilibrated to room temperature prior to opening the foil pouch. Designate enough microplate strips for duplicate determinations of the standards, controls, and samples. The remaining microplate strips should be stored at 2-8°C in the tightly sealed foil pouch containing the desiccant. 2. Pipette 25 µl of each standard, control, and sample into their respective wells. See Reagent Preparation for control reconstitution instructions. 3. Pipette 100 µl of Detection Antibody into each well. Insulin ELISA Page 4 of 8 July 31, 2012 4. Cover microplate with a plate sealer and incubate for 1 hour at room temperature, shaking at 700-900 rpm on a microplate shaker. 5. Decant the contents of the wells and wash the microplate 6 times with 350 µl of Working Strength Wash Buffer per well (see Reagent Preparation) using a microplate washer. Alternatively, fill the wells with Working Strength Wash Buffer using a wash bottle (do not use a multichannel pipette). Between washes, invert the microplate to discard the liquid and firmly tap the inverted microplate on absorbent paper towels. After the final wash, (automated or manual), remove any residual Wash Buffer and bubbles from the wells by inverting and firmly tapping the microplate on absorbent paper towels. 6. Pipette 100 µl of TMB Substrate into each well. 7. Cover microplate with a plate sealer and incubate for 15 minutes at room temperature, shaking at 700-900 rpm on a microplate shaker. 8. Pipette 100 µl of Stop Solution into each well and gently shake the microplate to mix the contents. Remove any bubbles before proceeding with the next step. 9. Place the microplate in a microplate reader capable of reading the absorbance at 450 nm. The microplate should be analyzed immediately after the addition of the Stop Solution, and no longer than 30 minutes after.

#### Insulin [125I] Ria Kit

##### Assay procedure

**Assay parameters:** Bo/T 45 ± 5% E-50 70 ± 10µIU/ml.  
**Calibration Standards:** are calibrated against the international reference standard NIBSC 66/304.

Specificity Cross reactivity was defined by weight at the 50% displacement level in per cent.

**Analytical sensitivity:** The analytical sensitivity is 5 µIU/ml, defined as the concentration corresponding of the mean cpm of zero standard minus its double standard deviation.

**Precision and reproducibility:** Patient samples were assayed in one run with 20 replicates, and in 10 runs with duplicates to determine the intra-assay and the interassay precision, respectively.

**Recovery:** Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of insulin.

**Expected values:** It is recommended that each laboratory establish its own reference intervals. As a guide, 5 – 35 $\mu$ IU/ml was obtained from normal patients on an empty stomach. The results obtained should only be interpreted in the context of the overall clinical picture. None of in vitro diagnostic kits can be used as the one and only proof of any disease or disorder.

## 2. Description

**Different drugs and their combinations quantitative and qualitative methods were described as follows**

### 2.1: Repaglinide & Metformin

Sharma, Kuldeep et al. reported LC-MS/MS-ESI method for simultaneous quantification of metformin and Repaglinide with the title “LC-MS/MS-ESI method for simultaneous quantitation of metformin and repaglinide in rat plasma and its application to pharmacokinetic study in rats” is described as follows: A highly sensitive and specific LC-MS/MS-ESI method has been developed for simultaneous quantification of metformin (MFN) and repaglinide (RGN) in rat plasma (50  $\mu$ L) using phenacetin as an internal standard (IS). Simple protein precipitation was used to extract MFN and RGN from rat plasma. The chromatographic resolution of MFN, RGN and IS was achieved with a mobile phase consisting of 0.2% formic acid in water–acetonitrile (1:1, v/v) with a time program flow gradient on a Chromolith RP-18e column. The total chromatographic run time was 3.5 min and the elution of MFN, RGN and IS occurred at 1.64, 2.21 and 2.15 min, respectively. A linear response function was established for the range of concentrations 0.855–394 and 0.021–21.7 ng/mL for MFN and RGN, respectively. The intra- and inter-day precision values for MFN and RGN met the acceptance as per FDA guidelines. MFN and RGN were stable in battery of stability studies viz., bench-top, auto-sampler and freeze–thaw cycles. The developed assay was applied to a pharmacokinetic study in rats.<sup>[1]</sup>

### 2.2. Metformin

ZHANG et al. reported LC-MS/MS determination of metformin in human plasma with the title “LC-MS/MS method for determination of metformin in human plasma” is described as follows : After protein precipitation with acetonitrile, the analyte and internal standard, mildronate, were separated on a Zorbax SB-C<sub>18</sub>(150 mm $\times$ 2.1 mm, 5  $\mu$ m) Narrow-Bore analytical column using the mobile phase of methanol and 10 mmol $\cdot$ L<sup>-1</sup> ammonium acetate (containing 1% formic acid) (5: 95) at a flow rate of 0.3 mL $\cdot$ min<sup>-1</sup>. Detection was carried out by electrospray positive ionization mass

spectrometry in the multiple reaction monitoring (MRM) mode. The MRM transitions of  $m/z$  130.1 $\rightarrow$  $m/z$  71.0 and  $m/z$  147.1 $\rightarrow$  $m/z$  58.2 were used to quantify metformin and internal standard, respectively. Metformin and internal standard were eluted at 1.27 and 1.26 min, respectively. The calibration curve was linear over the concentration range of 0.010-3.000 mg $\cdot$ L<sup>-1</sup> with the lower limit of quantitation (LLOQ) 0.010 mg $\cdot$ L<sup>-1</sup>. Intra and inter day RSD were both less than 6%, and the relative errors(RE) were within 6%. The mean extract recoveries were (86.6 $\pm$ 5.4)%. In the stability studies, metformin in plasma was found to be stable under various storage conditions. It is a rapid, sensitive, selective and reliable method for the determination of metformin in human plasma. The method is successfully applied to a bioequivalence study of metformin hydrochloride enteric-coated tablets in healthy volunteers.<sup>[2]</sup>

### 2.3 Pioglitazone

CHEN et al. reported LC-MS/MS method to determine the concentrations of atorvastatin calcium and pioglitazone hydrochloride in Beagle dog plasma with the title “Concentration determination of atorvastatin calcium and pioglitazone hydrochloride in Beagle dog plasma by LC-MS/MS” is described as follows : To establish an LC-MS/MS method to determine the concentrations of atorvastatin calcium and pioglitazone hydrochloride in Beagle dog plasma, and to verify the method. The drugs were extracted by precipitating protein, with diazepam as an internal standard. The Shim-pack XR-ODS(2.0 mm $\times$ 100 mm, 2.2  $\mu$ m) column at 40°C and the Shim-pack GVP-ODS(2.0 mm $\times$ 5 mm, 2.2  $\mu$ m)pre-column were used. The mobile phase consisted of phase A(5 mmol $\cdot$ L<sup>-1</sup> ammonium acetate, pH 5.55) and phase B(acetonitrile), the phase B accounted for 10% of the total mobile phase rate in the first 0.6 min, then changed to 90% from 0.6 min to 1.2 min, and kept at 90% until 3.5 min, then returned to 10% from 3.5 min to 4.1 min. The analysis was stopped until 5.5 min, the total mobile phase rate was 0.3 mL $\cdot$ min<sup>-1</sup>, injected volume was 20  $\mu$ L. Electrospray ion source was applied in mass spectrometry, and quantitative analysis was undertaken in multiple reaction monitoring. Atorvastatin calcium and pioglitazone hydrochloride had a good linear relation between 0.05-50 ng $\cdot$ mL<sup>-1</sup>, 0.5-1000 ng $\cdot$ mL<sup>-1</sup>, respectively. The intra-and inter-day precision RSD were both less than 15% ( $n=5$ ). Drugs in dog plasma were stable at room temperature for 24 h, and at 4°C for 24 h after being extracted, even after freezing and thawing three times and stored at -70°C for two months. The developed method is simple and accurate, which can be used to determine the concentration and the pharmacokinetics of atorvastatin calcium and pioglitazone hydrochloride in Beagle dogs.<sup>[3]</sup>

### 2.4. Pioglitazone & Candesartan

Vijaya Kumari Karra et al. reported A simple and rapid liquid chromatography-tandem mass spectrometric (LC-MS/MS) assay for simultaneous quantification of

pioglitazone and candesartan in human plasma with the title “Simultaneous determination of pioglitazone and candesartan in human plasma by LC-MS/MS and its application to a human pharmacokinetic study” is described as follows : A simple and rapid liquid chromatography-tandem mass spectrometric (LC-MS/MS) assay method has been developed and fully validated for simultaneous quantification of pioglitazone and candesartan in human plasma. Irbesartan was used as an internal standard. The analytes were extracted from human plasma samples by solid-phase extraction technique using a Strata-X 33Å 1/4m polymeric sorbent. The reconstituted samples were chromatographed on a C18 column by using a 80:20 (v/v) mixture of acetonitrile and 0.1% formic acid as the mobile phase at a flow rate of 0.8 mL/min. The calibration curves obtained were linear ( $r^2 \geq 0.99$ ) over the concentration range of 15–3000 ng/mL for pioglitazone and 5–608 ng/mL for candesartan. The results of the intra- and inter-day precision and accuracy studies were well within the acceptable limits. A run time of 2.7 min for each sample made it possible to analyze more than 300 plasma samples per day. The proposed method was found to be applicable to clinical studies.<sup>[4]</sup>

### 2.5 Pioglitazone & Alogliptin

Abdel Ghany et al. reported LC-MS/MS analysis of alogliptin and pioglitazone in human plasma with the title “Enhanced LC-MS/MS analysis of alogliptin and pioglitazone in human plasma: Applied to a preliminary pharmacokinetic study” A new fast LC-MS/MS method was developed for determination of alogliptin and pioglitazone in human plasma. Linearity ranges of 10–400 ng mL<sup>-1</sup> for alogliptin and 25–2000 ng mL<sup>-1</sup> for pioglitazone, were found to be suitable for their bioanalysis covering the  $C_{min}$  and  $C_{max}$  values of the drugs. Direct precipitation technique was used for simultaneous extraction of the drugs successfully from human plasma samples. Chromatographic separation was achieved on a BEH C<sub>18</sub> column (50 mm × 2.1 mm, 1.7 μm) with 0.1% aqueous formic acid: acetonitrile (40:60, v/v) at a flow rate of 0.3 mL min<sup>-1</sup>. The validated method was applied to a preliminary pharmacokinetic study on human volunteers. Monitoring the transition pairs of m/z 340.18 to 116.08 for alogliptin and m/z 356.99 to 133.92 for pioglitazone, using triple quadrupole mass spectrometer with multiple reaction monitoring, was achieved in the positive mode. The validated method is accurate and suitable for further clinical applications and possible bioequivalence studies.<sup>[5]</sup>

### 2.6 Metformin, Glimperide & Pioglitazone

Sengupta et al. reported Simultaneous Quantitation of Metformin, Glimperide and Pioglitazone in Human Plasma with the title “LC-MS-MS Development and Validation for Simultaneous Quantitation of Metformin, Glimperide and Pioglitazone in Human Plasma and Its Application to a Bioequivalence Study” A simple, precise and reproducible liquid chromatography-tandem mass spectrometry method has been developed

and validated according to the Food and Drug Administration guidelines for the simultaneous quantitation of antidiabetic drugs metformin, glimperide and pioglitazone in human plasma using glipizide as an internal standard. Quantitation was performed on a triple quadrupole mass spectrometer employing electrospray ionization technique, operating in multiple reaction monitoring and positive ion mode. Inter-batch and intra-batch coefficient of variation across four validation runs for the quality control samples was less than 7%. The accuracy determined at quality control levels was within 92.81–105.13%. The method was applied to a bioequivalence study.<sup>[6]</sup>

### 2.7 Pioglitazone & Telmisartan

Sengupta et al. reported simultaneous quantitation of pioglitazone and telmisartan in rat plasma with the title “Development and validation of a high throughput LC-MS/MS method for simultaneous quantitation of pioglitazone and telmisartan in rat plasma and its application to a pharmacokinetic study” Management of cardiovascular risk factors in diabetes demands special attention due to their co-existence. Pioglitazone (PIO) and telmisartan (TLM) combination can be beneficial in effective control of cardiovascular complication in diabetes. In this research, we developed and validated a high throughput LC-MS/MS method for simultaneous quantitation of PIO and TLM in rat plasma. This developed method is more sensitive and can quantitate the analytes in relatively shorter period of time compared to the previously reported methods for their individual quantification. Moreover, till date, there is no bioanalytical method available to simultaneously quantitate PIO and TLM in a single run. The method was validated according to the USFDA guidelines for bioanalytical method validation. A linear response of the analytes was observed over the range of 0.005–10 μg/mL with satisfactory precision and accuracy. Accuracy at four quality control levels was within 94.27%–106.10%. The intra- and inter-day precision ranged from 2.32% to 10.14% and 5.02% to 8.12%, respectively. The method was reproducible and sensitive enough to quantitate PIO and TLM in rat plasma samples of a preclinical pharmacokinetic study. Due to the potential of PIO-TLM combination to be therapeutically explored, this method is expected to have significant usefulness in future.<sup>[7]</sup>

### 2.8 Rosiglitazon

Rashid et al. reported LC-MS/MS method for concurrent determination of sildenafil and rosiglitazone in rat plasma with the title “A highly sensitive LC-MS/MS method for concurrent determination of sildenafil and rosiglitazone in rat plasma” Patients with pulmonary arterial hypertension (PAH) are currently treated with more than one drug. Sildenafil, a phosphodiesterase type 5 (PDE-5) inhibitor, and rosiglitazone, a peroxisome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ ) activator, is one of those combinations that could be used in PAH. To monitor the pharmacokinetics of sildenafil in the presence of rosiglitazone, we have developed and

validated a sensitive, specific and rapid liquid chromatography-tandem mass spectrometric (LC-MS/MS) method. We have used this validated method to study the pharmacokinetics of sildenafil and rosiglitazone after intravenous administration of sildenafil alone or a combination of sildenafil plus rosiglitazone to adult male Sprague-Dawley rats. Sildenafil and rosiglitazone were extracted from plasma by protein precipitation with methanol. With an octadeuterated sildenafil as the internal standard, the drugs were separated via gradient elution using a C18 column and formic acid in methanol or in water as the mobile phase with a flow rate of 0.25 mL/min. Both sildenafil and rosiglitazone samples in rat plasma produced linear response, when the concentration ranged between 5 and 1000 ng/mL ( $r^2 > 0.99$ ). The pharmacokinetics study suggests that intravenous co-administration rosiglitazone plus sildenafil increases the plasma concentration of sildenafil and extends the drug's elimination half-life.<sup>[8]</sup>

### 2.9 Linagliptine

Parsha et al. reported LC-MS/MS and NMR analysis developed for the identification and characterization of key impurities of Linagliptin and Pramipexole with the title "LC-MS/MS and NMR Characterization of Key Impurities in Linagliptin and Pramipexole" is described as follows : A sensitive, rugged, and robust LC-MS/MS and NMR analysis has been developed for the identification and characterization of key impurities of Linagliptin and Pramipexole. Linagliptin is used in the treatment of Type-2 diabetes. Linagliptin is a DPP-4 inhibitor which is an enzyme that degrades the incretin hormones (glucagon like peptide-1 (GLP)) and glucose dependent insulinotropic polypeptide (GIP). Both these hormones increase insulin biosynthesis and secretion from pancreatic beta cells in the presence of normal and elevated blood glucose 20 levels. Pramipexole is a dopamine agonist of the non-ergoline class indicated for the treatment of Parkinson's disease and restless legs Q1 syndrome. Parkinson's disease is a neurodegenerative disease that affects the basal ganglia component, i.e., substantia nigra. Observed one of the key impurities in the analytical HPLC at around 1.30RRT in Linagliptin. To further characterize the impurity, the impurity was synthesized in presence of dibromo methane and was subjected to flash chromatography for further isolation. Thus isolated impurity was subjected to NMR and mass analysis for structure identification. Similarly observed another key impurity in Pramipexole at around 0.96RRT. This impurity was enriched in presence of formaldehyde and was subjected to preparative HPLC for isolation and further characterized by LC-MS and NMR.<sup>[9]</sup>

### 2.10 Sitagliptine

Salim et al. reported spectrofluorimetric method for determination of sitagliptin and identification of potential alkaline degradation products using LC-MS with the title "Micelle-enhanced spectrofluorimetric method for determination of sitagliptin and identification of potential

alkaline degradation products using LC-MS" is described as follows : A novel, quick, simple and highly sensitive spectrofluorimetric method was developed and validated for the determination of sitagliptin (SG) in its pharmaceutical formulations. The proposed method is based on investigation of the fluorescence spectral behavior of sitagliptin in an SDS micellar system. In an aqueous solution of phosphate buffer pH 4.0, the fluorescence intensity of SG in the presence of SDS was greatly enhanced, by 200%, i.e. twofold enhancement. The fluorescence intensity of SG was measured at 300 nm after excitation at 270 nm. The method showed good linearity in the range 0.03–10.0 µg/mL with a good correlation coefficient ( $r = 0.9998$ ). The limits of detection and quantitation values were 5.31 and 16.1 ng/mL, respectively. The proposed method was successfully applied to the analysis of SG in its single and co-formulated commercial tablets; the results were in good agreement with those obtained using a reference method. Application of the proposed method was extended to stability studies of SG after exposure to different forced degradation conditions according to the ICH guidelines, such as acidic, alkaline, thermal, photo- and oxidative stress. The chemical structure of certain potential degradation products (DPs) were investigated using LC-MS.<sup>[10]</sup>

### 2.11 Sitagliptin & Simvastatin

Burugula et al. reported liquid chromatography-tandem mass spectrometric (LC-MS/MS) assay method for simultaneous quantification of sitagliptin and simvastatin in human plasma with the title "Simultaneous determination of sitagliptin and simvastatin in human plasma by LC-MS/MS and its application to a human pharmacokinetic study" is described as follows : A simple, rapid and sensitive liquid chromatography-tandem mass spectrometric (LC-MS/MS) assay method has been developed and validated for simultaneous quantification of sitagliptin and simvastatin in human plasma. Carbamazepine was used as an internal standard (IS). The analytes and IS were extracted from the human plasma by liquid-liquid extraction technique. The reconstituted samples were chromatographed on an Alltima HP C<sub>18</sub> column using an isocratic solvent mixture [acetonitrile–5 mm ammonium acetate (pH 4.5), 85:15 (v/v)] at a flow rate of 1.0 mL/min. Method validation was performed as per Food and Drug Administration guidelines and the results met the acceptance criteria. The calibration curves obtained were linear ( $r^2 \geq 0.99$ ) over the concentration range of 0.10–501 and 0.05–105 ng/mL for sitagliptin and simvastatin, respectively. The results of the intra- and inter-day precision and accuracy studies were well within the acceptable limits. Both the analytes were found to be stable in a battery of stability studies. The method is precise and sensitive enough for its intended purpose. A run time of 3.0 min for each sample made it possible to analyze more than 300 plasma samples per day. The developed assay was successfully applied to a pharmacokinetic study in human volunteers.<sup>[11]</sup>

### 2.12 Dasatinib & Sitagliptin

Patel P *et al.* reported simultaneous determination of Dasatinib and Sitagliptin in rat plasma with the title "Validated LC-MS/MS method for simultaneous determination of Dasatinib and Sitagliptin in rat plasma and its application to pharmacokinetic study" Dasatinib (DST), a tyrosine kinase inhibitor, is a novel anticancer agent and Sitagliptin (STG) is an antidiabetic agent of dipeptidyl peptidase-IV inhibitor class. A novel, sensitive and specific liquid chromatography tandem mass spectrometry (LC-MS/MS) based method has been developed for simultaneous monitoring of plasma levels of STG and DST in rat plasma. Both analytes and an internal standard (tolbutamide) were chromatographed on YMC-Pack ODS-AM (50 mm × 4.6 mm i.d., 3 μm) using a methanol:2 mM ammonium acetate binary gradient mobile phase at a flow rate of 1 ml min<sup>-1</sup> with a splitter (1:1) over a 5 min run time. Detection of analytes was performed on a LC-MS/MS system in multiple reaction monitoring (MRM) mode. The transitions monitored were 488.1 → 401.0, 408.1 → 174.0 and 271.1 → 155.0 for DST, STG and IS, respectively. The method was validated over a concentration range of 5.41–1029.60 ng ml<sup>-1</sup> for DST and 5.64–1073.56 ng ml<sup>-1</sup> for STG. The lower limit of quantification was 5.41 ng ml<sup>-1</sup> and 5.64 ng ml<sup>-1</sup> for DST and STG, respectively. Recoveries from spiked controls were >82% for the analytes and the internal standard at all QC levels. The intra- and inter-batch precision and accuracy across four quality control levels met established criteria of US Food and Drug Administration guidelines. This method was successfully applied to monitor the pharmacokinetic profile of both STG and DST in Wistar rats. This method can be applicable for pharmacokinetic drug–drug interaction studies.<sup>[12]</sup>

### 2.13 Alogliptine

Mowaka *et al.* reported Enhanced LC-MS/MS determination of alogliptin and metformin in plasma with the title "Enhanced LC-MS/MS determination of alogliptin and metformin in plasma: Application to a pharmacokinetic study" A fast, sensitive and specific LC-MS/MS method was developed for estimation of alogliptin (AG) and metformin (MF) using sitagliptin (SG) as an internal standard (IS). Hypersil Gold column (50 mm × 2.1 mm, 1.9 μm) was used applying gradient elution of acetonitrile and 0.2% formic acid solution. Selected reaction monitoring (SRM), with transitions of 340.33 → 116.32 (*m/z*), 130.12 → 71.32 (*m/z*) and 408.12 → 235.24 (*m/z*), was used to quantify AG, MF and SG (IS), respectively on a triple quadrupole mass spectrometer in positive ion mode. Enhanced sample preparation involved direct precipitation and an evaporation technique. As per FDA guidelines, a detailed validation of the method was carried out and the standard curves were found to be linear between 5 and 400 ng mL<sup>-1</sup> for AG and 25–2000 ng mL<sup>-1</sup> for MF. Precision and accuracy results were within the acceptable limits. A short run time of

2.5 min for each sample offered the rapid analysis of high number of human plasma samples per day. Application to a pharmacokinetic study was achieved successfully on Egyptian healthy human volunteers after the approval by the ethical committee. Pharmacokinetic parameters can vary between different races, so the present analysis considered the Egyptian African volunteers to be compared with the results obtained from the other ethnic populations. The applied pharmacokinetic study is considered as a mandatory prerequisite for new drugs registration and approval according to Egyptian Ministry of Health regulations.<sup>[13]</sup>

### 2.14 Alogliptine & Pioglitazone

Abdel Ghany *et al.* reported LC-MS/MS analysis of alogliptin and pioglitazone in human plasma with the title "Enhanced LC-MS/MS analysis of alogliptin and pioglitazone in human plasma: Applied to a preliminary pharmacokinetic study" A new fast LC-MS/MS method was developed for determination of alogliptin and pioglitazone in human plasma. Linearity ranges of 10–400 ng mL<sup>-1</sup> for alogliptin and 25–2000 ng mL<sup>-1</sup> for pioglitazone, were found to be suitable for their bioanalysis covering the C<sub>min</sub> and C<sub>max</sub> values of the drugs. Direct precipitation technique was used for simultaneous extraction of the drugs successfully from human plasma samples. Chromatographic separation was achieved on a BEH C<sub>18</sub> column (50 mm × 2.1 mm, 1.7 μm) with 0.1% aqueous formic acid: acetonitrile (40:60, v/v) at a flow rate of 0.3 mL min<sup>-1</sup>. The validated method was applied to a preliminary pharmacokinetic study on human volunteers. Monitoring the transition pairs of *m/z* 340.18 to 116.08 for alogliptin and *m/z* 356.99 to 133.92 for pioglitazone, using triple quadrupole mass spectrometer with multiple reaction monitoring, was achieved in the positive mode. The validated method is accurate and suitable for further clinical applications and possible bioequivalence studies.<sup>[14]</sup>

### 2.15 Empagliflozine

Ayoub *et al.* reported Determination of Empagliflozin and Metformin with the title "LC-MS/MS Determination of Empagliflozin and Metformin" A new LC-MS/MS method was developed for determination of empagliflozin and metformin. Bridged Ethylene Hybrid C<sub>18</sub> column (50 mm × 2.1 mm, 1.7 μm), isocratic elution based on 0.1% aqueous formic acid:acetonitrile (75:25, v/v) as a mobile phase, column temperature at 55°C and flow rate at 0.2 mL min<sup>-1</sup> were used. The mass spectrometer was operated under multiple reaction monitoring mode using electrospray ionization by monitoring the transition pairs (precursor to product ion) of *m/z* 451.04–71.07 for empagliflozin and *m/z* 130.11–71.14 for metformin in the positive mode. The validation parameters were acceptable over concentration ranges of 5–1,000 ng mL<sup>-1</sup> and 50–25,000 ng mL<sup>-1</sup> for empagliflozin and metformin, respectively. The optimized method was validated according to International Conference on Harmonization guidelines.

Regression parameters, limit of detection, limit of quantification, accuracy, precision and pharmaceutical formulation analysis were investigated. The developed method was proved to be accurate for the quality control of recently approved Synjardy® tablets.<sup>[15]</sup>

### 2.16 Canagliflozine

Kobuchi et al. reported LC-MS/MS method for the determination of canagliflozin with the title "A validated LC-MS/MS method for the determination of canagliflozin, a sodium–glucose co-transporter 2 (SGLT-2) inhibitor, in a lower volume of rat plasma: application to pharmacokinetic studies in rats" is described as follows : Canagliflozin is a novel, orally selective inhibitor of sodium-dependent glucose co-transporter-2 (SGLT2) for the treatment of patients with type 2 diabetes mellitus. In this study, a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for the quantitative analysis of canagliflozin in a lower volume of rat plasma (0.1 mL) was established and applied to a pharmacokinetic study in rats. Following liquid–liquid extraction by *tert*-butyl methyl ether, chromatographic separation of canagliflozin was performed on a Quicksorb ODS (2.1 mm i.d. × 150 mm, 5 µm size) using acetonitrile–0.1% formic acid (90:10, v/v) as the mobile phase at a flow rate of 0.2 mL/min. The detection was carried out using an API 3200 triple-quadrupole mass spectrometer operating in the positive electrospray ionization mode. Selected ion monitoring transitions of  $m/z = 462.0$   $[M + NH_4]^+ \rightarrow 191.0$  for canagliflozin and  $m/z = 451.2$   $[M + H]^+ \rightarrow 71.0$  for empagliflozin (internal standard) were obtained. The validation of the method was investigated, and it was found to be of sufficient specificity, accuracy and precision. Canagliflozin in rat plasma was stable under the analytical conditions used. This validated method was successfully applied to assess the pharmacokinetics of canagliflozin in rats using 0.1 mL rat plasma.<sup>[1]</sup>

### 3. CONCLUSION

The various extraction procedures used for estimation various anti diabetic drugs is varied from drug to drug, many of the methods, authors used precipitation method. In simultaneous quantification of Metformin and Repaglinide, simple protein precipitation was used to extract MFN and RGN from rat plasma. In the determination of the concentrations of Atorvastatin calcium and Pioglitazone hydrochlorid, the drugs were extracted by precipitating protein, with diazepam as an internal standard. For simultaneous quantification of pioglitazone and candesartan in human plasma, the analytes were extracted from human plasma samples by solid-phase extraction technique using a Strata-X 33Å 1¼m polymeric sorbent.

### 4. ACKNOWLEDGEMENT

Authors are thankful to Sultan Ul Uloom college of pharmacy, for providing facilities to carry out this work.

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